Studies on polymorphism in some provenance collections of *Azadirachta indica* A. Juss.

by

Sudhir Kumar Kaura

Dissertation submitted to the CCS Haryana Agricultural University Hisar, Haryana, India in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Genetics

The College of Basic Sciences & Humanities,
Chaudhary Charan Singh Haryana Agricultural University Hisar-125 004,
Haryana, India

August 2, 2001

Certificate I

This is to certify that this dissertation entitled 'Studies on polymorphism in some provenance collections of *Azadirachta indica* A. Juss.', submitted for the degree of Doctor of Philosophy in the subject of Genetics of CCS Haryana Agricultural University, Hisar, India, is a bonafide research work carried out by Mr Sudhir Kumar Kaura under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of study have been fully acknowledged.

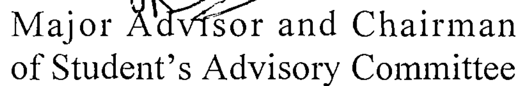
(J. B. Chowdhury)

Major Advisor and Chairman
of Student's Advisory Committee

Dated: August 2, 2001

Certificate II

This is to certify that this dissertation entitled 'Studies on polymorphism in some provenance collections of *Azadirachta indica* A. Juss.', submitted by Mr Sudhir Kumar Kaura to the CCS Haryana Agricultural University, Hisar, India, in partial fulfilment of the requirements for the degree of Doctor of Philosophy in subject of Genetics has been approved by Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.



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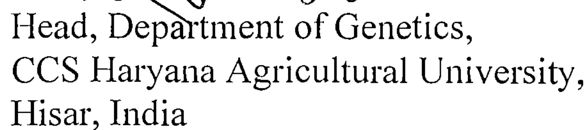

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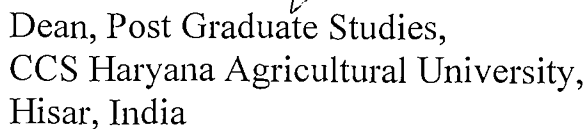

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Studies on polymorphism in some provenance collections of *Azadirachta indica* A. Juss.

by

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(An abstract from the thesis submitted to the
CCS Haryana Agricultural University Hisar, Haryana, India
in partial fulfilment of the requirements for the degree of
Doctor of Philosophy in the subject of Genetics)

Title of thesis	:	Studies on polymorphism in some provenance collections of *Azadirachta indica* A. Juss.
Full name of degree holder	:	Sudhir Kumar Kaura
Title of degree	:	Doctor of Philosophy
Name and address of major advisor	:	Prof. J. B. Chowdhury, Vice-Chancellor, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttaranchal Pradesh
Degree awarding university/institute	:	Chaudhary Charan Singh Haryana Agricultural University, Hisar-125 004, India
Year of award of degree	:	2001 A. D.
Major subject	:	Genetics
Total number of pages in thesis	:	412
Number of words in abstract	:	652

Keywords: neem, *Azadirachta indica*, azadirachtin, diversity, conservation, callus, fingerprinting, heritability, oil, provenance, RAPD, stability, viability

Two hundred and twenty one germplasm accessions of neem (*Azadirachta indica*) from nine states of India (Haryana, Rajasthan, Delhi, Himachal Pradesh, Uttar Pradesh, Panjab, Karnataka, Tamil Nadu and Maharashtra) were studied for all or some of the following aspects viz. tree architecture, endocarp morphology, seed viability, provenance evaluation through growth of seedlings in nursery conditions; and oil content, fatty acid profile, protein content and azadirachtin content of seeds. In addition, thirteen local trees were taken up for studying leaf morphology and tissue culture experiments. Statistically significant differences (both within and between populations) were observed for most of the morphological, biochemical and growth characteristics under study.

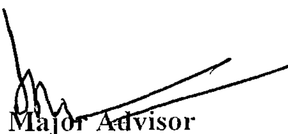
Considerable variation was also recorded within and between provenances in trees surveyed in Haryana and adjoining areas with respect to tree architectural parameters and their linear associations. Of 136 trees studied, 58 candidate plus trees (CPTs) for agroforestry, 45 CPTs for seed production as well as agroforestry and 47 CPTs for seed production were marked. Multiple regression equations (with very high level of goodness of fit of the regression line) based on the available tree architectural parameters, could be developed for predicting values of clear bole, unforked height and ratio of unforked height and height. Highly divergent trees could be

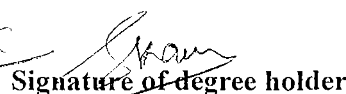
identified for combination of important tree architectural parameters with the help of principal component analysis.

Genetic parameters were studied for endocarp morphological characteristics. Moderate to high estimates of genotypic coefficient of variation, broad sense heritability and genetic advance were obtained for endocarp weight and seed weight, indicating high level of genetic variability and potential of improvement for these traits through selection. Statistically significant genotypic correlations were observed between characteristics like endocarp length, endocarp weight, seed weight in some of the populations suggesting the possibility of enhanced correlated response upon indirect selection for these morphological characteristics. Separation of the genotypic correlation coefficient into components of direct and indirect effects was done using path coefficient analysis for seed weight and ratio of seed weight and endocarp weight. Seed weight was directly and indirectly affected by both endocarp weight and endocarp coat weight in most of the provenance collections. Ratio of seed weight and kernel weight was directly and indirectly affected by all the morphological characteristics of endocarp in all the provenance collections under study. Using multivariate analyses (D^2 analysis; hierarchical cluster analysis; and principal component and principal factor analysis) it was possible to delineate highly divergent samples with respect to endocarp morphological characteristics. Clustering pattern found to have partial dependence on geographical location of the germplasm accessions. Some similarity was observed in clustering pattern obtained by different multivariate techniques. Temporal stability for the endocarp morphological characteristics was quite high except 20 endocarp weight, for which stable genotypes could be identified. Likewise, for seed oil content significant genotype-environment interaction was observed and differences were observed with respect to temporal stability.

Fatty acid profile (stearic acid and oleic acid) of seeds showed a strong association with geographical position and agroclimate of the sampling sites while no geographic association was found for seed oil content. Viability of seeds appeared to be affected by the oil content of seeds rather than fatty acid profile. Callus cultures could be raised from axillary bud, shoot tip, leaf, and mature seed explants. *In vitro* production of azadirachtin was recorded in callus cultures raised from axillary bud explants. Shoot regeneration could be obtained from callus cultures developed from axillary buds and mature seed. Protocol for fingerprinting the neem genotypes using RAPD (Random Amplified polymorphic DNA) technique has been standardised and preliminary investigations with 15 genotypes and pooled DNA samples of 5 populations indicated within and between population genetic variability.

Morphological, physiological and molecular marker data revealed ample genetic diversity in neem at population and individual levels. Clinal variation observed in seed biochemical characteristics like stearic acid and oleic acid suggests that for germplasm conservation, attention should be paid to populations according to latitudinal and longitudinal gradients.





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Acknowledgements

I am grateful to my Major advisor, Prof. J B Chowdhury for providing me an opportunity to work under his able guidance. I am thankful to members of my advisory committee Dr V P Singh, Prof. Randhir Singh, Dr H S Nainawatee, Prof. V K Chowdhury, Dr J Naresh Dr O P Toky, and Dr Veena Jain for always taking keen interest in my research project. Thanks are due to Dr R K Jain, Dr K S Boora, Dr S K Gupta, Dr R K Behl, Dr U N Joshi, Dr K S Bangarwa, Dr Senrayan[1], Mr C M Ketkar[2], Dr S S Bisla, Dr P K Sareen, Dr D S Tonk and Ms. Promila Thakkar[3] for providing me valuable advice on technical matters and academic support. All other colleagues in the laboratory and university are hereby formally thanked for all the camaraderie. I am thankful to Council of Scientific and Industrial Research, New Delhi for the Senior Research Fellowship.

I am thankful to the present Head, Department of Genetics, Dr R K Vashisht and former Heads of the Department, Dr P K Sareen, Dr K P Singh, and Dr O. P. Luthra for providing me all the requisite facilities and infrastrucutre for the doctoral research.

I thank Dr Rajesh Yadav and Dr Jaivir Singh for very kindly guiding me through the statistical analyses and critically reviewing the methodology.

Dated: July 10, 2001 (Sudhir Kumar Kaura)

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List of Abbreviations

*τ	Kendall's rank correlation coefficient
*π	22\7
*ANOVA	analysis of variance
*CD	critical difference
*CLINK	Complete Linkage Clustering Method
*CV	coefficient of variation
*df	degrees of freedom
*G x E	genotype into environment interaction
*GA	Genetic Advance
*GCV	genotypic coefficient of variation
*GDF	Genotype Discriminating Efficiency
*h^2	broad sense heritability
*HCA	Hierarchical Cluster Analysis
*PC	Principal Component
*PCA	Principal Component Analysis
*PCV	phenotypic coefficient of variation
*r	Karl Pearson Correlation Coefficient
*R^2	coefficient of determination
*SD	standard deviation
*SE	standard error
*SLINK	Single Linkage Clustering Method
*UPGMA	unweighted pair-group method using arithmetic averages
A	adenine
AC	activated charcoal
AFLP	Amplified Fragment Length Polymorphism
Anon.	Anonymous
ASA	American Standards Association
Aza	azadirachtin
B5	B5 medium
BA / BAP	6-benzyl adenine
C	cytosine
CIMAP	Central Institute of Medicinal and Aromatic Plants, Lucknow, New Delhi, India
CPT	candidate plus tree
dbh / DBH	diameter at breast height
DNA	deoxyribonucleic acid
dNTPs	deoxynucleoside 5' - triphosphate
DSIR	Department of Scientific and Industrial Research, New Delhi, India
DW	distilled water
EPA	Environment Protection Agency
EPO	European Patent Office
g	grams
G	guanine
gbh / GBH	girth at breast height
GLC	Gas Liquid Chromatography
ha	hectares
HPLC	High Performance Liquid Chromatography
hr / Hr	hours
ht / Ht	height
IAA	indole acetic acid
IBA	indole butyric acid
J&K	Jammu and Kashmir

Kin / Kn	kinetin
mc / MC	moisture content
MS	Murashige and Skoog (1962)
NAA	naphthalene acetic acid
NCL	National Chemicals Laboratory, Pune, India
Nic. Acid	nicotinic acid
NKM	Neem kernel meal
NMR	Nuclear Magnetic Resonance
OD	optical density
PCR	Polymerase Chain Reaction
PF	principal factor
PFA	principal factor analysis
PGR	plant growth regulator
PVP	polyvinyl pyrollidone
Py. HCl	pyridoxine HCl
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
rh / RH	relative humidity
RNA	ribonucleic acid
rpm / RPM	rotations per minute
SLR	single lens reflex
SPSS	Statistical Package for Social Scientists
T	thymine
Taq	*Thermus aquaticus*
TDZ	thidiazuron
TLC	Thin Layer Chromatography
TN	Tamil Nadu
UP	Uttar Pradesh
UV	ultra violet
V	volts
v/v	volume by volume
VIS	visible
w.r.t.	with respect to
w/v	weight by volume
wk	week
yr	year
zea	zeatin

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*statistical and mathematical terminology

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1. Introduction

The neem tree belonging to mahogany family, Meliaceae is a multipurpose tree native to Southeast Asia. It is known as neem in Hindi, *nimba* in Sanskrit *and Azadirachta indica* A. Juss. to the botanists. Now it is widely distributed throughout tropical and subtropical Asia, Africa, Middle East, West Indies, Australia and central and south America and has adapted to diverse climatic and edaphic conditions. (Schmutterer, 1990a; Ascher, 1993; Hegde, 1996).

In India, neem is hailed as "village pharmacy". Its leaves, bark, oil, seeds and twigs were well cited in Sanskrit writings like *Charaka Samhita* and *Susruta Samhita*; and is widely used in Ayurveda, *Unani tibb* (Greco-Persian system of medicine), Homeopathy, and indigenous systems of medicine (Medhi 1991; Strzok, 1991). Millions of people brush their teeth with twigs of this tree. Its antiseptic, antibacterial, antiviral, antifungal, analgesic, antipyretic, antiarthritic, anti-inflammatory, and anti-hyperglycaemic properties are very well documented (Jacobson, 1989; Koul *et al.*, 1990; Tewari, 1992; Puri, 1999).

Decision makers pondering agricultural development strategies are often confronted with a dilemma: on the one hand there cannot be adequate agricultural production without pest control; on the other, the environment may not be harmed by injudicious use of pesticides. Even with the widespread use of pesticides, more than one-third of global food and agricultural production is reportedly destroyed annually by over 20,000 species of insects, mites, nematodes, weeds, plant pathogens and other pests (McEwen, 1978).

Approximately, 2400 plant species reportedly possess pest control properties (Jacobson *et al.*, 1983; Grainge and Ahmed, 1988). However, not all the plants can be readily used for pest control purposes by marginal farmers. Few plant species may meet all these characteristics. Ahmed *et al.* (1984) identified 25 species that come close, the neem tree appears to possess all. Neem holds out the promise of providing a highly effective, economical, non-toxic, and environmentally harmless means of controlling or eliminating insect pests that inflict losses in agricultural production (Govindachari, 1992). Neem extracts are known to affect more than 200 species of insects (many of which are resistant to or inherently difficult to control with conventional pesticides), mites and nematodes, including destructive species such as locust, borers, weevils and termites (Warthen, 1979; Grainge and Ahmed, 1988; Wulf, 1991). In recent years, several reviews have been published which outline the use of the neem tree, as a botanical insecticide (Jacobson, 1989; Koul *et al.* 1990; Schmutterer, 1990b; Ascher, 1993; Coventry and Allan 1997). The pesticidal properties of *A. indica* have primarily been attributed to a general class of natural products called limonoids of which the best known are nimbin, nimbidin, salannin and azadirone; and several types of azadirachtins.

Pesticides based on these compounds are biodegradable and are active in very minute concentrations against a variety of pests, therefore, are potential candidates as broad spectrum pesticides and good substitutes for synthetic pesticides (National Research Council, 1992).

The major active principle of neem, the highly oxidized tetranortriterpenoid, azadirachtin, was isolated for the first time in the year 1968 (Butterworth and Morgan 1968) and it exhibits complex biological activity in various pests of crops (Nakanishi, 1975; Srimannarayana, 1989) Unlike many synthetic insecticides, low doses of azadirachtin were found to have little or no mammalian toxicity, and insects showed little resistance to the compound even through several generations. Azadirachtin has generated wide academic and industrial interests as a potentially new potent insect control molecule. The mode of action of azadirachtin, the principal insecticidal constituent of neem oil has only recently been elucidated (Mordue & Blackwell, 1993). Schmutterer (1990b) suggested that azadirachtin modifies the programs of insects by influencing hormonal systems, especially that of ecdysone.

Oil extracted from kernels of this tree has commercial importance, as it is put to a variety of uses ranging from insecticides to pharmaceuticals. In composition, cold pressed neem oil is similar to other vegetable oils, composed primarily of triglycerides of oleic, stearic, linoleic and palmitic acids (Anon., 1985; Sankaram *et al.* 1987; Kaura et al., 1998b). It is generally yellow to dark in color and bitter in flavor. Unlike most vegetable oils, it contains sulfur compounds, which give the oil a pungent smell. Oil obtained after extraction with non-polar solvents is free from unpleasant color, and is comparable to groundnut oil (Sankaram *et al.* 1987). A process has been developed to produce colorless and debittered neem oil that can be used for edible purposes (Rukmini, 1987). Combustion of neem oil in combination with diesel oil has been successfully demonstrated and advocated, especially in countries devoid of combustion oil sources (Randhawa and Parmar, 1993).

Forest tree populations, compared to agricultural crops, have been little influenced by human activities until now. In fact, trees like neem have not been subjected to any genetic improvement effort and, therefore, are considered to possess greater variability compared to other plants (Hamrick *et al.* 1979b; 1979a; 1992). Moreover, it is an established fact that tree species with a wide geographical distribution exhibit considerable variation in anatomy, physiology, morphology and genetics to survive and reproduce under varying environmental conditions over generations (Antonovics 1971; Nienstaedt, 1975).

During the course of evolution, different populations of tree species have become adapted to climatic conditions, soil type and other environmental factors. With the increasing pressure on land it has become essential to choose particular provenance that

fits best in particular environmental conditions to obtain highest yield per unit area (Rawat et al., 1987). Genetic diversity found in tree species is a part of nature's strategy for defence and survival against all types of risks met with in the long life spans of forest trees (Heybrock, 1978; Zobel and Talbert 1984). The existing genetic variability differs in different species and populations. The success of genetic improvement programmes, thus, depends upon the location, nature and exploitation of genetic diversity present in the species.

The understanding of genetic architecture of the germplasm is obligatory for the breeders to develop high yielding genotypes through hybridization from the available plant material. For this purpose it is essential to quantify the genetic diversity among populations. High productivity and stability of performance are two most desirable features of a cultivar. The stability in production is particularly important in tree crops, as these are most extensively grown under varied range of agro-climatic conditions. Identification of such genotypes will have a considerable significance in tree improvement. Therefore, there is a need to identify stable and high yielding genotypes; to determine the magnitude of genetic diversity present within and between populations; and to choose or suggest promising cross combinations that can be exploited for breeding purposes. Besides, immediate genetic gain, breeding strategies have several objectives like, genetic conservation, maintenance of continued genetic gain over several generations and supply of genetically improved reproductive material. The effective exploration, identification, documentation and use of genetic resources of neem are a prerequisite for its efficient use (Kundu, 1999).

Tree breeding through the application of genetic principles is basically directed towards modifying the heredity of tree populations so that the trees are able to meet the needs of the forest based industries. Serious attention to selective tree breeding has been paid only in the twentieth century, mainly since 1950, and primarily for industrial wood products (Burley, 1987). To a tree breeder, the most important sources of genetic variation are natural populations, and it is this natural variation that serves as the raw material for genetic gain (Lantz, 1975). Provenance variation and tree to tree differences generally account for the bulk of the genetic variation found within a tree species. These two components may account for as much as 90% of the within-species variation, leading to the fastest and cheapest gains in most tree improvement programs being obtained by selection from the best seed source within the appropriate species (Zobel and Talbert, 1984). Additional gains are then made by selecting the best individuals. Tropical hardwoods (which include neem as well) are more likely to show greater inter-population genetic variation (Bawa, 1976).

Although, the neem tree is widely recognised as an important multi-purpose forest tree, most the published research on this species has dealt with isolation, identification,

formulation and field testing of pest control agents derived from the kernels and leaves. Exhaustive studies related to selection of superior trees, provenance evaluation and genetic improvement are lacking. In the recent past, some efforts have been made to collect and study diverse neem germplasm for its provenance variation and genetic architecture with respect to variation in physiology (Kundu and Tigerstedt, 1999); seed characters, seed viability and seedling growth of germplasm collected from different geographic sources (Oo, 1987; Tewari, 1992; Visvanathan *et al.*, 1996; Kundu and Tigerstedt, 1997; Kaura *et al.*, 1998a, 1998b; Jindal *et al.*, 1999a)reproductive biology (Kundu 1999; Solanki *et al.*,1999;); isozymes and DNA profile (Nuzhat Farooqui, 1998; Kundu, 1999; Singh *et al.*; 1999); oil content and its key chemical constituents (fatty acids) (Jitendra Kumar *et al.*, 1997; Kadir *et al.* 1998; Kaura *et al.*, 1998b, 1998c; Kaushik and Vir; 2000; Ali *et al.*, 1996); azadirachtin (Butterworth and Morgan, 1971; Zanno *et al.*, 1975; Uebel *et al.*, 1979; Schumutterer and Zebitz, 1984; Ermel *et al.*; 1984, 1987, 1998; Locke and Lawson, 1990; Schmutterer, 1990b;Gruber, 1991; Bally *et al.*, 1999). One approach for increasing yield of pesticidal compounds in short time is to select trees whose seeds and leaves contain highest proportion of these compounds and to correlate it with some visual feature or readily identifiable molecular marker of the tree or seedling. Second approach is to select trees that yield maximum numbers of large fruits (National Research Council, 1992).

Recommendations have been made for genetic improvement and conservation of neem through development of higher seed yielding types; rapid multiplication through tissue culture; development of high-oil and superior quality types; development of different canopy architecture; development of types suitable to different situations (industrial areas, abandoned mines, etc.) and environments; collection and augmentation, evaluation and maintenance of different genetic stocks; and use in afforestation of waste lands, problem soils, forestry and environment programmes (Prasad *et al.*, 1996; Kaura *et al.*, 1998a; Kundu, 1999).

Considering the aforementioned facts the present investigation was undertaken with the following objective:

To study polymorphism for morphological, cytological and biochemical characteristics in some provenance collections of *A. indica*.

2. Review of Literature

Tree breeding through the application of genetic principles is basically directed towards modifying the heredity of tree populations so that the trees are able to meet the better needs of the forester and the wood based industries. Serious attention to selective tree breeding has been paid only in the present century, mainly since 1950, and primarily for industrial wood products (Burley, 1987). Initially, work was concentrated in Europe and North America, then in Australia, Japan and Brazil, lately in Africa and Indian sub-continent.

Tree improvement work in India was initiated by Prof. Champion who realized the importance of geographic variation and conducted a seed origin trial of teak during 1930 (Emmanuel *et al.*, 1992). Realizing the importance of this subject, Forest Research Institute, Dehradun established a Forest Genetics Section during 1959-60 attached to Botany branch under the then Directorate of Biological Research.

There is considerable urgency to study the mechanisms by which forest trees track environmental change, given the prospect of possible rapid climatic changes. Environmental tracking is achieved through three basic processes: (1) expression of phenotypic plasticity at the level of the individual; and (2) evolutionary change and (3) migration, both expressed at the level of the population over generations. The current distribution and genetic architecture of a species reflect how these processes interacted in response to past climatic changes during and after the last glaciation. Part of the record is encoded in the DNA of the current generation of trees and, as a result of existing field tests, is accessible for study. These field tests include, in ascending order of genetic resolution: (1) provenance tests; (2) progeny tests; and (3) three-generation clonal tests; as well as (4) clone tests, with or without genetic structure. These field tests represent an important information resource, which deserves to be more effectively used by the scientific community (Stettler and Bradshaw, 1994).

Ledig (1992) published a review, organized in relation to human activities that influence genetic diversity and the structure of genetic diversity. Humans have converted forest to agricultural and urban uses, exploited species, fragmented wild lands, changed the demographic structure of forests, altered habitat, degraded the environment with atmospheric and soil pollutants, introduced exotic pests and competitors, and domesticated favoured species. All have affected genetic diversity by their influence on the evolutionary processes of extinction, selection, drift, gene flow, and mutation, sometimes increasing diversity, as in the case of domestication, but often reducing it. Demographic changes influenced selection by increasing incidence of disease. Deforestation has operated on a vast scale to reduce diversity by direct elimination of locally-adapted populations. Atmospheric pollution and global warming will be a major

threat in the near future, particularly because forests are fragmented and migration is impeded. Baselines are needed to quantify future impacts and provide an early warning of problems. Genetic inventories of indicator species can provide the baselines against which to measure changes in diversity.

Woody species maintained more variation within species and within populations than species with other life forms but had less variation among populations. Although life history and ecological traits explain a significant proportion (34%) of the variation among species for the genetic parameters measured, a large proportion of the interspecific variation is unexplained. The specific evolutionary history of each species must play an important role in determining the level and distribution of genetic diversity (Hamrick *et al.*, 1992).

The literature available on neem and some important tree spp., relevant to the present investigation is reviewed as follows:

2.1. Taxonomy and nomenclature:

The genus *Azadirachta* belongs to the family Meliaceae. In 1830, Adrien Henri Laurent De Jussieu classified the neem tree as *Azadirachta indica.* Its systematic position is as follows:

Order: Rutales; Suborder: Rutineae; Family: Meliaceae; Tribe: Melieae;

Genus: *Azadirachta*

Species: *indica*

The Meliaceae comprises 50 genera and about 800 species (Styles and White, 1991). There are three synonyms of *Neem*, namely: *Melia azadirachta* L., M. indica (A. Juss.) Brandis and *Antelaea azadirachta* (L.) Adelb. The last synonym is not internationally acknowledged. The close relatives of this species with similar bioactive properties are the Thai neem (*A. siamensis* VAL.), the Sentang or Marrango (*A. excelsa* JACK), the Persian lilac or Chinaberry (*Melia azedarach* L.), M. toosendan SIEB. & ZUCC., M., and *volkensii* GÜRKE. Other genera of this family such as Aglaia, Cedrela, Khaya, Sandoricum, Trichilia, Chisocheton, Toona and Turraea also posses noteworthy biological activity against insects. Among these genera, the extracts from the seeds and bark of *Azadirachta* and *Melia* have outstanding bioactive properties (Isman *et al.*, 1995).

The present popular name "neem", also spelled earlier as "nim", has been derived from the Sanskrit word "*nimba*" which means sprinkler, which is the short term for "sprinkler of nectar (ambrosia)".

The meaning of the generic name *Azadirachta* does not appear to be interpreted properly in most of the literature. It is often said that it is from the Persian words *azad* – free, and *drakhat* – tree, i.e. free tree, and when the specific name *indica* is added to it,

the meaning for the botanical name becomes the free tree from India, which does not convey any specific significance of the name.

Two differing chromosome numbers have been recorded in neem i.e. 2n=30 and 2n= 28, have also been reported from the root tip mitosis (Pathak and Singh, 1949; Mukherji, 1952; Styles and Vosa, 1971; Mehra *et al.*, 1972). According to Gill *et al.* (1993), this species has small-sized chromosomes but a high chromosome number. The loss in combination due to a low chiasmata index is compensated by an increase in the number of linkage groups and the allogamous nature.

2.2. Origin, distribution, habitat and domestication of neem:

The exact region of origin of neem is unknown but the species occurs widely in Thailand, Myanmar and the Indian subcontinent. According to Gamble (1902), the centre of origin of *A. indica* is in the forests of Karnataka (south India) or the dried inland forests of Burma (Myanmar) (Troup , 1921; ~~Vartak and Ghate, 1990~~). Other authors were of the opinion that this tree originated in the forests of the Shivalik hills (foothills of the western Himalayas) or on east coast of south India. *A. indica* is widely distributed by introduction, mainly in the drier tropical and subtropical zone of islands. Neem has been introduced mainly in the arid tropical and subtropical zones of Asia, Africa, the Americas and the South Pacific Islands.

Neem tree is most competitive in seasonal climate with long dry season and annual rainfall of 450-1150 mm. Most common at 0-700 m altitude but can grow up to 1500 m provided the temperature remains moderate. It is widely used as a shade tree in many areas because it tolerates a wide variety of field conditions (Koul, *et al.*, 1990; Schmutterer, 1990a). The tree tolerates heat up to 50° C, and poor, shallow, even saline soils (Koul *et al.*, 1990; Schmutterer, 1990a; Ascher, 1993). *A. indica* grows rapidly; 4-7 meters in its first five years of growth and 5-11m for the following five years. It will bear fruit within three years and reach a maximum fruiting yield of 50 kg seed/year ten years after planting (Jacobson,1989; Koul *et al.*, 1990; Ascher, 1993). *A. indica* is sensitive to injury at temperatures around 0° C, which limits its distribution in temperate regions of the world (Jacobson, 1989; Koul *et al.*, 1990; Ascher, 1993).

The neem tree is well known for its drought resistance (Schmutterer, 1995). It thrives well in low rainfall (130 mm/year) areas. In high rainfall (3,000 mm/year to 4,000 mm/year) areas, it has failed completely (Schmutterer, 1995). Neem performs well on a wide range of soil types. In its native environment, neem is found growing in mixed forests in association with *Acacia* and *Dalbergia* spp. (Benge, 1989).

Neem was introduced to Malaysia in 1940s by the migrating Indians as a shade tree with medicinal uses in home gardens. Neem was introduced from India to Africa in early 1920s by the Indian migrants. It reached Nigeria around 1928 and to Senegal in 1944. Now it is widely distributed throughout tropical and subtropical Asia, Africa, Middle

East, West Indies, Australia and central and south America and has adapted to diverse climatic and edaphic conditions (Schmutterer, 1990a; Ascher, 1993).

In India it is grown from the southern tip of Kerala to Himalayan hills, from tropical to sub-tropical regions, from semi-arid to wet tropical regions and from sea level to about 700m elevation. In India, it is found naturally occurring on the Shivalik hills of Uttar Pradesh and hillocks of Deccan, in Karnataka and adjoining states. In Myanmar, neem grows in the natural forests of the central dry zone. Excepting these locations, neem is generally found growing around backyards, along roads and field bunds, in and around villages, than in the forests. This is an important reason why it was neglected by the foresters in the past. It is a fairly fast growing and sturdy tree that can be established without irrigation in hot and dry regions of the world where it grows well on poor, shallow, stony or sandy soils (Jacobson, 1984). It is very efficient in restoring soil productivity and simultaneously providing fodder, fuelwood and other products to meet basic requirements of rural population.

Chiu Shin Foon and Zhang Ye Guang (1989) reported successful introduction of neem in south-east part of Hainan province, China. Troup (1921) reported neem to be common in the open shrub forests of the dry zone of Myanmar, and it is found in some dry parts of India. In the Indian subcontinent and Myanmar, the neem has been domesticated by the people as homestead and avenue trees. Nowadays, cultivation of neem has been spread to most of the warmer parts of the world including Australia and USA. Despite its high commercial potential, little work has been carried out on neem genetics to date. Provenance research on neem started in India and Thailand with the local material in 1992.

A program on provenance trials for the International neem Network has initiated the evaluation and improvement of genetic resources of the species in 1996, co-ordinated by the FAO. Twenty five seed sources from 11 countries are included and 30 trial are established in 16 Asian and African countries under this program (Anon.,1998).

2.3. Economic importance of neem:

Both in its native lands and elsewhere the neem is one of the most useful of all trees. It has a great economic potential to help farmers in addressing requirements for organic manure, organic pesticides, generation of income and empoyment opportunities.

The leaves, fruits and bark of neem have been used traditionally in ayurvedic medicine in the Indian subcontinent since ancient times. The various pharmacological properties of seed oil, leaves, and bark of the tree have been reviewed by many authors (Ketkar and Ketkar, 1995; Koul *et al.*, 1990). The seed oil has been used for anti-malaria, febrifuge, anti-helminthic, vermifuge, antiseptic, and anti-microbial purposes, and as wound-healing agent. A number of commercial neem based products with the reported

activities are now available in India (Ketkar and Ketkar, 1995). The efficacy of neem seed extract is reported against biting midge, a nuisance pest of coast of Australia.

In Assam, the leaves are boiled in water and the cooled water is then used to bathe patients after measles or chicken pox. The plant itself acts as an air purifier (Medhi, 1991). Neem leaf extract has been wound to have wormicidal effect against the eggs of animal parasitic nematodes (*Trichuris ovis*, *Ascaridia galli*, and *Parahetrakis gubernaculi*) and plant parasitic nematodes (*Meloidogyne incognita*) and the tape worm (*Hymenolepsis nana*)(Bhatnagar and Nama,1990).

Integration and exploitation of neem in traditional farming systems have been well documented (Jain *et al.*, 1998a).Traditionally neem has been used for protecting the stored grains (Gupta and Patel, 1991; Kaura *et al.* (1999a); for protecting a variety of crops from insect-pests (Gupta *et al.*, 1995, Vivekanandan, 1996, 1997a, 1999, 2000; Gupta and Patel, 1991a, 1991c; 1992b) in human/animal medicine (Pradhan, 1999; Vivekanandan, 1995; Anonymous, 2000; Gupta, and Patel, 1993; Prakash, 1997; Anonymous, 1999; Vivekanandan, 1996b); manure supplement (Vivekanandan, 1996a, 1997; Prakash, 2000); fodder supplement in dry climate (Gupta and Patel, 1991b). Infact, the traditional and indigenous use of neem has inspired the present day research to a great extent. There is a need for a systematic investigation of renewable resources for sustainable agricultural development to increase the resiliency of traditional farming systems and reduce their vulnerability to the uncertainties of, and dependence on, imported high-technology agriculture (Ahmed and Tang, 1991).

The EPO has received 51 patent applications for 'inventions' based on the neem tree, and has so far granted 11 patents. Around 90 patents exploiting the tree have been granted worldwide till date (Hellerer Ulrike and Jarayaman, 2000). There has been a widespread debate on the patents on neem and in the recent past in India, there have been initiatives on the part of government and semi-governmental bodies (Indian Council of Agricultural Research, National Innovation Foundation, Department of Science and Technology, etc.) and non governmental bodies (Society for Research and Initiatives for Sustainable Technologies, Ahmedabad) to systematically document the prevalent and extinct indigenous uses of neem so that the novelty of patents on neem by industry can be challenged in a systematic manner and intellectual property rights (IPRs) of indigenous people could be given due recognition and legal protection (Gupta, 1995, 1995a; 1995b; 1995c; Anonymous,2000a;).

Neem is regarded as a first class durable timber. The heavy wood has a specific gravity of 0.56-0.86 (mean 0.68) (Anon., 1980). The yield of the timber varies according to the site quality. McComb (1967) reported yields for plantations in Samura (Nigeria) of 2.55-19.67 m^3/ha^{-1}) for the first 8-year rotation crop and similar yields for 8-year coppice crop.

The ripe neem berries are dried and solvent extracted to yield neem oil that has a variety of uses in the cosmetics, pharmaceuticals and soap manufacturing industries. However, the byproduct, neem kernel meal (NKM) available in sizeable quantities (approx. 0.9 million tonnes annually) awaits appropriate usage other than for coating nitrogenous fertilizers or to be used as such as an organic manure (Verma *et al.,* 1997).

Chemical examination of the commercially available NKM in the laboratory revealed the presence of 38.5% crude protein, 11.5% fibre, 8.0% total minerals, 3.0% ether extract, 0.78% calcium and 0.5% total phosphorus. The protein in NKM has been reported to have a favourable amino acid composition (Singh, 1993).

Neem coated urea reduces volatilisation of ammonia by delaying rapid urea hydrolysis and nitrification. Thus controlled supply of NH^{+}_{4} to soil for longer period is assured. In fact neem when coated on urea reduces fertilizer nitrogen loss through leaching and denitrification by 30-35%, saves urea by 25-40% and increases crop yield by up to 25%.

Neem's efficacy to non-target and beneficial organisms has been documented (Jacobson, 1989; Schmutterer, 1990b; Ascher, 1993; Mordue & Blackwell, 1993). Neem is widely utilized in the tropics by humans for medicinal purposes, and is assumed to have no detrimental effects to humans (Jacobson, 1989; Schmutterer, 1990b). The first commercial neem insecticide, Margosan-O, was registered by the EPA for non-crop use in the United States in July 1985 (Jacobson, 1989). Since that time, the EPA has exempted Margosan-O from food crop tolerances and several other commercial neem insecticides have been developed worldwide (Ascher, 1993).

2.4. Habit:

Neem trees are attractive broad-leaved evergreens (sometimes deciduous) that can grow up to 30 m tall and 2.5 m in girth. Thus pollard well and have coppicing properties. The seedlings are shade lovers but the mature trees are demanders of strong light in nature pure stands are rare. Leaves imparipinnately compound with 7-17 pairs of leaflets, which are ovate or lanceolate, falcate with uneven base and dentate margins, 6-8cm long, 1-3 cm wide. Inflorescence a 10-30 cm long panicle with many, small white to cream coloured flowers (Puri, 1999).

Fruit is ellipsoid drupe, 1.2-2 cm long, green/yellow when ripe, with a thin hard cuticle and juicy fruit pulp. The pyrene contains one, rarely two, seeds. Seed weight varies with location and seed source. Available information indicates from 1700 seeds per kg in the Sahel to 3500-9000 seeds per kg in India.

2.5.1. Phenology:

Neem flowers are hermaphroditic or male. Pollination by insects. The tree starts flowering and fruiting at about 5 years of age. Flowering generally occurs in the dry

season and fruit ripening during the early part of the rainy season. Season and duration of reproductive phenoperiods vary according to location and climate. In bi-modal climates there are sometimes two flowering and fruiting seasons.

In India flowering occurs 2-5 weeks earlier in the southern than in the northern part of the country, with an approx. delay of reproductive season of 4.5 days for each 1^0 increase in latitude between 20^0 and 30^0 N. Duration of period from flowering to mature seeds is 10-12 weeks. Individual fruits have a development and ripening period for 1-2 months.

Trees growing in areas with a warm winter, bloom first, followed by areas where the winter is comparatively colder. Depending on the locality, flowering may range from January to May (Gupta *et al.,* 1995). Sporadic flowering in September-October has been observed quite often, in addition to that in February-March. Shanthi *et al.* (1996b)reported abnormal seedlings in December from these trees and suggested that these trees may be used as germplasm. In the Murshidabad area of West bengal (India), the tree flowered throughout the year (Guhabakshi, 1984). Generally the tree starts flowering at three to five years of age and becomes fully productive at the age of about ten years.

Shanthi *et al.* (1996b)reported about late flowering forms in neem. Neem normally flowers in April [in Tamil Nadu] and the fruits are ready for harvesting by July, although the fruiting season sometimes extends to August. The seeds have a short period of viability (only a few months after harvest) so late flowering forms would be useful for solving seed procurement problems for later planting. Such a genotype was identified in Tirupur. As well as flowering and fruiting at the normal times this tree flowered in September and produced fruits in December. Seed germination and seedling characteristics were compared from the 2 periods; germination was 83% in the early flowering type and 67% in the later flowering type. Seedling growth was similar, but there were more abnormal seedlings (12 vs. 2%) in the later flowering type, probably due to selfing. Late flowering types would be useful for breeding studies.

Kadir *et al.*(1998): The distribution of neem trees in Peninsular Malaysia and their phenological characteristics were determined. The greatest abundance of neem trees was found in northern Peninsular Malaysia which also produced higher yield of fruits compared with that of southern and east-coast of Peninsular Malaysia.

Use of genetically improved seed or germplasm is not a standard practice for tree planting in India and many other countries because the reproductive materials are not genetically identified, developed, produced, certified or released in any systematic tree breeding program for use of tree growers. Many indigenous tree species are harvested commercially but not genetically developed and multiplied for use in operational plantations. Seeds used are mostly unselected and without any provenance or seed source control. Knowledge of breeding systems of indigenous trees, particularly of tropical tree species, is scarce (Dogra and Dhiman, 1998).

2.5.2. Reproductive Biology of neem:

Reproduction normally begins after five years. The timing of flowering and fruiting of neem varies from place to place. In India, neem flowers from January to April, and fruits mature from June to August. Bisexual and male flowers occur on the same individual i.e., the species is 'andromonoecious'. The pollen matures before the stigma becomes receptive (protandrous). The anthers start to dehisce around 8 a.m. in the closed flower (Gupta *et al.*, 1996a,b) Pollination is performed by insects. There are 10 glabrous anthers, which are inserted at the base on the flower.

The ovary is trilocular having two ovules in each chamber, thus each ovary contains six ovules. The endocarp encloses one, sometimes two and rarely three seeds (Schmutterer,1995; Singh *et al.,* 1995). This phenomenon may be termed as 'polycarpy'. The exocarp of the fruit is thin and the mesocarp (0.3-0.5 cm thick) consists of a bittersweet pulp. The seeds are recalcitrant or of the orthodox type. There is great variation in germination rate (Poulsen, 1995). Viability drops by 50% after 8 weeks and is completely lost after 12-16 weeks. Germination rate can be improved by removing the endocarp.

Garudamma (1956), Rangaswamy and Promila (1972) have studied the embryology in detail. The ovary is trilocular at base, becoming uni-locular at the ovule bearing region.

Twin embryos occur commonly (Nair and Kanta, 1961) ; the number of seed per embryo may be 1-3 (Gill *et al.*, 1993; Vijayan and Rehill, 1987). The gametophyte develops in the usual way; the embryo sac is of polygonum type. Study of premature fallen fruits indicated that embryo abortion is common (Gill *et al.*, 1993).

The phenomenon of twin seedlings has also been observed, which may be as high as 11.27 percent (Vijayan and Rehill, 1987; Gurudev Singh *et al.*, 1995). This may be due to the development of one or more than one ovules, out of five of the ovaries (penta-carpellary), giving rise to more than one seed under the same endocarp, but according to Pushpakar and Babekey (1995) it is oue to polyembryony, with a frequency of 1 out of 800.

2.6. Tree architecture

While reviewing variation in Indian tree species, Dogra (1981) emphasized on survey of phenotypic variation of silvicultural characteristics of tree species in their naturally distributed range. Devender Singh (1994) reported phenotypic variation in neem from natural populations in states of Haryana, Rajasthan, Panjab and UP covering 12 provenances. Substantial variability was observed for straightness and morphological characters. Stem form ranged from crooked to completely straight. Diameter at breast height and total height of the trees in the natural population were observed up to 148 cm and 22.5m respectively. The measurements of crown spread were almost equal to that of

total height. Completely straight neem treeswere observed at Sirsa, Agra and Sadalpur. The highest mean value of straightness was observed at Agra, with lowest coefficient of variation. In total, ten trees were depicted on the basis of straightness, higher proportion of main stem height to clear bole height and relatively smaller crown size.

Individual selection on the basis phenotypic superiority forms the basis of any tree improvement programme. The success of any individual tree selection largely depends on the magnitude of genetic variation. Where the magnitude of variation is high, the higher selection differential thus obtained are indicative of higher gain. The choice of characters are need based and may depend upon their genetic control (heritability), their variability and also on the economic value. Here the stem quality was taken into consideration, which consists of plant height clear bole length (cbl) and diameter at breast height (dbh) (Bagchi, 1995). These characters are highly variable and reported to have sufficient genetic control (White, 1991).

Based on analysis of variance, Devender Singh (1994) reported significant variation from site to site for total height : dbh, clear bole height : total height, crown spread : dbh, crown spread : total height and non-significant variation with in site for total height : dbh, clear bole height : total height, main stem height : total height, crown spread : dbh and crown spread : total height. Index score analysis clearly indicated the superiority of Agra, Sadulpur and Yamunanagar for the selection of superior trees of *Neem* for agroforestry purpose. Index score analysis also suggested the consideration of Hisar, Karnal , Sirsa and Patiala for the selection of plus trees in order to have a broader genetic base.

Gupta *et al.* (1992) found large amount of phenotypic variation in natural population of *Dalbergia sissoo* and selected plus trees based on stem straightness. Bangarwa *et al.* (1993) collected data on diameter at breast height, total height, main stem height, clear bole height, straightness and crown spread from 20 diverse sites of northern India in order to study the extent of phenotypic variation in natural population of *Dalbergia sissoo* Roxb. Substantial variability was observed in natural population for straightness and other morphological characters. Straightness ranged from crooked or forked to completely straight. Main stem height to total height ratio and clear bole to total height ratio were observed to vary from 0.16 to 0.94 and 0.06 to 0.71 respectively.

Jatasara (1982) found a wide range of variability for tree height, dbh, stem height, canopy diameter of *P. cineraria* in Thar desert during May-June 1981. Continuing this study, Jatasra and Paroda (1983) observed that in natural stands of *P. cineraria* tree height varied from 4-22m with mean values of 9.19m; stem height and canopy height ranged from 1-8m and 1-20m respectively. Maximum variation was observed for canopy volume and least variation was observed for stem height. From main stem height : Canopy ratio (0.56) they concluded that canopy grew almost double the height of stem.

Kackar (1986a) reported variation for morphological characters of *P. cineraria* in natural stands from various edaphic sites and rainfall zones of western Rajasthan. The height of trees was found variable in all the eleven provenances. The minimum and maximum mean heights of 8.8 and 16.3m were observed in Tonk provenance and Barmer provenance, respectively. The mean values of forking height varied from 2.04 to 4.28m in Tonk and Barmer provenances. Average forking height was lower than 3m in all the provenances except Barmer and Nagaur. The value of dbh varied from 0.26 to 2.52m as representative of Tonk and Jallore provenances, respectively.

Singh and Bangarwa (1996) proposed model architecture for trees in agroforestry and emphasized that a model tree for agroforestry should be characterized by fast vertical growth, small crown, few branches, with a smaller angle, self-pruning habit, straight and clean bole with aggressive apical dominance, higher proportion of main stem, deep root system, deciduous nature and loose canopy so that it does not make dense shade.

Kumar (1986) observed significant variation in natural stands of *Pinus gerardiana* for about all the characters studied viz. dbh, height, clear bole height, branch angle, cone character, needle character, etc. Khan (1983) revealed wide ranged of variability in *Pinus wallichiana* occurring under different environmental conditions. Population from J&K gave maximum average height. Whereas from HP (Kullu and Shimla) the plants were shorter in height indicating that population from northern latitude is superior to that from southern latitude.

A neem seedling can be grown straight at least to a height of 10-11 feet. Usually farmers remove all side branches leaving the top leaf bunch. Ganapathi Raman, a farmer in Masarpatti village, observed that certain neem bio-types have a habit of growing straight and these trees so not require pruning. Instead of pruning the side branches he advises nipping the internode bud i.e. bud emerging between the main stem and side stalk (bearing leaflets) alone can be cut or nipped. If the bud is allowed to emerge then this will grow into a strong branch which will affect the value of the neem tree. It increases the vigour of the tree growth. The leaves become much broader and have enlarged leaf surface. It is a very simple method to follow without much hardship (Vivekanandan, 1999a).

Jatasra (1982) collected 250 strains of *Prosopis cineraria* by the 'random biased' sampling from Thar desert during May-June, 1981. The quantitative data recorded on 223 trees indicated a wide range of variability for tree height, dbh (diameter at breast height), stem height, canopy height and canopy diameter. Ranking for leaf fodder and seed yield revealed vast exploitable variations.

In order to assess genetic variability, Srivastava et al. *(1993a)* measured height, number of branches/plant, length of branches, number of leaves/branch, length and breadth of leaves and leaf yield/plant in 15 randomly selected *T. arjuna* (an important multipurpose tree

species used in sericulture) from each of 3 plots in 8-yr-old plantations [in Bihar] consisting of 2 varieties of the species (var. *angustifolia* and var. *Arjuna*) and at 4x4 [ft.] spacing. Maximum range of variation was recorded in leaf yield/plant followed by breadth of leaves, while the genotypic coefficient of variation was highest for leaf yield followed by number of leaves/branches,- and the highest phenotypic coefficient of variation was for leaf yield/plant followed by breadth of leaves. The highest heritability was observed for leaf yield/plant followed by number of leaves/branch. The highest genetic advance was observed for plant height followed by number of leaves/branch. Leaf yield/plant, number of leaves/branch and height should, therefore, be considered as effective parameters for selection in *T. arjuna.*

Gwaze *et al.* (1997) calculated genetic and phenotypic parameters for height and stem straightness from data in 140 full-sib families from an incomplete factorial mating design involving 8 male and 15 female parents of *Pinus taeda*, planted in Zimbabwe during 1972. Measurements were made at 1.5, 9.5, 13.5 and 22.5 years of age. Dominance variance tended to be lower than additive variance for both traits, except for straightness at 1.5 years. Heritability estimates for height were moderate to high (h^2 = 0.14 to 0.73) and peaked at 9.5 years, whilst those for straightness were low at 1.5 years and increased to moderate levels with age (h^2 = 0.01 to 0.33). Individual site heritability estimates were higher than those using data pooled across sites, indicating presence of genotype × environment interactions and/or heterogeneity of variances. It is suggested that combining data as in the pooled analyses was not appropriate for this study. Genetic correlations were higher than their phenotypic counterparts in both traits. Age-age genetic correlations for height were high, indicating opportunity for early selection (0.76 to 0.97). Age-age genetic correlations for straightness ranged from –00.05 to 0.94. Genetic correlations between height and straightness were low.

Carson and Hayes (1998) reported diameter and height distributions for *Pinus* radiata trees grown from seed lots representing a range of genetic improvement were compared at mid-rotation (age 14 or 16) in seven large-plot trials at six sites throughout New Zealand. In one of the trials, comparisons were made at year 5 and annually from age 8 to 16. Differences among seed lots for quadratic mean diameter and mean height were statistically significant and generally reflected the expected level of genetic improvement. Standard deviation, skewness, and kurtosis were not significantly different among seed lots. However, diameter distributions of higher rated seed lots sometimes appeared very slightly more skewed to the right and flatter than the lower rated seed lots, a similar tendency observed as stands age. Models used to predict diameter distribution from stand parameters are not likely to require modification for genetically improved seed lots.

Han *et al.* (1987) studied genetic parameteres for tree architectural characteristics (height, dbh, stem straightness, crown width and branch diameter, angle and length) estimated from 16-year old-open-pollinated progenies of *Pinus koraiensis* S. et Z. in Korea. Estimates of family heritability for branch angle were lower than other characteristics.

Ahn *et al.* (1987) conducted study on characteristics of natural populations of *Pinus densiflora* et Z. in Korea. Simple correlations were studied among characteristics of tree growth and form at Hapch'on and Puan populations. In both the populations, growth characteristics (radius at breast height and height) tended to be positively associated with tree form (crown width)

Kwon *et al.* (1987) made estimates of broad sense heritability and genetic gain w.r.t. height, dbh, stem straightness, crown width and branch diameter, angle and number in 59 plus trees of Pinus densiflora. Significant differences among trees were observed for all characters except stem straightness and branch number. Broad sense heritabilities ranged from 0.31 to 0.50 for different characters. Expected genetic gains from selected 15 clones were 3.3-9.5% in these characteristics.

Doede and Adams (1998): Genetic parameters for stem volume, stem form, and branching traits, along with genetic interrelationships among these traits, were estimated from measurements on 60 open-pollinated families of noble fir (Abies procera) planted on 3 progeny test sites in South-west Washington (USA). Large family-by-site interactions were evident when all 3 sites (<1200m) were analysed separately from the high-elevation site (1402 m). Significant family variation was present in at least one environment for 10 of the 13 traits examined. Estimates of narrow-sense heritabilities were low to moderate(0.09-0.49) for all traits. With the exception of branch number and stem sinuosity, genetic correlations among stem growth, stem form, and branch traits were weak or favourable effects. Amounts of genetic and phenotypic variation present indicated that moderate gains from selection and breeding programmes are possible for stem growth traits, while lesser gains can be achieved for stem form and branching traits.

Tree improvement is increasingly considered as an important research direction for development of more productive agroforestry systems. In systems, trees have multiple economic uses and ecological functions that should be taken into account in tree improvement programmes in order, for these ensure that improved species is adapted to agroforestry. The key issue here may be for tree improvement specialists involved in agroforestry programmes to be able to differentiate, from the range of characters that could be selected for improvement of a given species, characters that are absolutely necessary to improve whatever agro-ecosystem the tree may be grown in (the primary targets) and characters for which improvement would only be appropriate for particular agro-ecosystem (the secondary targets) (Foresta and Michon, 1996).

Genetic parameters were estimated by Arregui *et al.* (1999)for growth, form and *Diplodia pinea* susceptibility in a six-year-old progeny trial containing 30 open pollinated families of *Pinus* radiata selected plus trees. The trial was established in November 1990 at Orozko, Spain. Individual heritabilities were moderate for height, diameter and susceptibility to *Diplodia pinea*, but low for stem straightness and branch diameter. Branch angle showed moderate to low heritability. Height and diameter were positively correlated. Growth traits were negatively correlated with susceptibility to *Diplodia pinea*, so it is possible to achieve simultaneous improvements in both growth and *Diplodia* resistance.

2.7. Plus tree selection:

Plus-tree is an outstanding individual which occurs in natural stands, or in even-aged stands, combining as many desirable traits as possible, such as good stem form, good height and dbh, good bole length, less of taper, narrow crown, resistance to pests and diseases, etc.,thus the approach to tree improvement involves the selection of trees from wild populations based on their apparent superiority in one or more traits. Such superior trees occur in very low frequency and a keen eye is needed to locate such trees. Once a right base population has been identified then the plus-tree selection begins. It has to be a continuous process (Puri, 1998).

By ocular judgement, candidate trees can first be identified for their overall superiority and then marked with a band of yellow paint at breast height and suitably numbered. Then its superiority can be assessed by actual measurements for certain traits and compared with similar measurements made on five check trees which should be dominants and co-dominant in the immediate vicinity of the candidate trees. Then for each trait assessed the superiority of the candidate tree over the average of the five check trees can be compared. Sometimes a total scoring system also called the grading system is followed. Here each character is given a score according to its expression in the individual tree. Each character is assigned a score ranging from usually 0 to 5 with 5 as the most desirable expression. If the total score for all the characters judged exceeds a given minimum then the tree is accepted a plus tree and a second band with yellow paint is given to it (Puri, 1998).

Brown and Goddard (1961) expressed the idea that plus-tree selection should not identify the largest tree, but should identify the tree that has utilized the growing space most efficiently. Crown measurements are made and related to basal area and volume to identify plus-tree.

Stanton and Canavera (1983) selected white birch phenotypes on the basis of three techniques : (i) mean annual volume increment after adjustment by comparison trees, (ii) without this adjustment, and (ii) on the basis of differences between actual and predicted basal area increments regressed on crown surface area. Results indicated significant

differences between techniques but none of them produced progenies significantly taller than the controls. Another study by Van Damme and Parker (1987) applied to black spruce used: (i) regression of basal-area increment of crown-surface area, (ii) stem volume on crown radius, and (iii) height on age. Open pollinated progenies from the selected trees at 2 years of age were significantly different but there were no differences among selection methods. In a study of various competition indices applied to improve plus-tree selection, Thomas (1980) also found that this approach is not helpful (Puri, 1998).

There are other reasons why selection based on crown characteristics may not be effective. Although correlations of growth and leaf characters in young trees and in experiments are often strong, there are reasons why these relationships may not exist in mature stands or may not be easy to assess (Assmann, 1970). Crown measurement may not accurately reflect leaf surface area; there may be differences in leaf arrangement and proportion of sun and shade leaves, and differences in phenology. Also, selection based on crown measurements cannot eliminate or account for differences in micro-site. These reasons indicate that selection by this technique may not be promising (Puri, 1998).

It has been found that in *Pinus roxburghii, P. wallichiana, Tectona grandis, Dalbergia sissoo, Acacia arabica,* seed origin makes big differences in growth and quality of plantations (Suri and Seth, 1959; Champion and Seth, 1968).

Khurana and Khosla (1982) made a range wide trial on *Populus ciliata* with 84 clones and subsequently, the number of clones were increased to 134. Nine promising provenances were singled out for genotypic site interaction trial. Sagwal (1985) also studied the clonal performance of these promising clones from Himachal Pradesh.

Chauhan (1987), while studying twenty eight clones of *Populus ciliata* collected from their geographical distribution in the western Himalaya, found high range of diversity with regard to survival percentage, height, trunk diameter, fresh and dry weight, leaf area and over all growth among different provenances.

Neil (1990) conducted provenance trial of *Dalbergia sissoo* in Nepal after collecting seed material from Nepal and Pakistan. He observed small differences among seven provenances of Nepal and large differences between provenances of Nepal vis-à-vis that of Pakistan.

Mathur *et al.* (1984) conducted extensive study on the germination behaviour of provenances of *A. nilotica.* A study based on 18 treatments comprising 12 provenances of variety "Jaquemontii", three provenances of variety "Vediana", and three provenances of sub species cupressiformis was conducted in the seed testing laboratory at Forest Research Institute, Dehradun. The morphological variation and physiological differences, and their effect on germination behaviour of seeds of different varieties and provenances were recorded. They found that out of the various provenances of *A. nilotica* var.

jaquemontil viz.; Fazilka, Paratwara, Rohtak and Kurukshetra provenances have been proved to be better.

Selection of Indian tree species for genetic improvement can be carried out from natural populations on the basis of desired traits. For fast growth, selection is for the tallest tree. Working criteria can, however, differ greatly depending upon the uses to which the species is put. Stem straightness is related to wood quality, easy handling in processing and to subsequent use. Simple selection for tree form can improve bole form and straightness considerably and thus improve the quality, easy handling in processing and to subsequent use. Simple selection for tree form can improve bole form and thus improve the quality and quantity of produce (Faulkner, 1969; Shelbourne, 1969). Straight and persistent axis in teak, clear cylindrical bole; light and spreading branches with knot angles approaching 90 degrees, are some of the desired morphological characters used for plus tree selection(Keidings,1966).

It is essential to know the selection differential and genetic concepts before exercising selection in commercial tree species; and genetic gain is simply the product of selection differential and heritability. Therefore, the rate of tree improvement can be increased or decreased by influencing the selection differential or heritability, or by reducing the total variance. The gain thus achieved from plus trees can immediately be realized through mass scale clonal propagation by vegetative means for general plantation purposes and further gains can be achieved subsequently by progeny selection and selective breeding. In a study on teak, Bagchi (1995) concluded (1) the stem quality character variabilities were high;(2) heritabilities of individual traits were high, and with a selection intensity of 20%, the genetic gain as estimated, is expected to be realized on selection.

Vidakovic and Ahsan (1970) described the methodology for the measurement of straightness. Ahsan (1970) mentioned the following criteria for a plus tree of *Dalbergia sissoo*: Vigorous growth, Straight stem, no forking, thin fine branches, Symmetrical crown, Cylindrical stem with 2/3 clear bole of total height of tree, resistance to biological and physical injuries, capable of producing seed.

Plus trees are the superior phenotypes with most desired features, selected from natural forests or plantations. This is the first step for initiating any long term tree improvement programme to produce genetically superior seeds on mass scale. Based on the assessment of commercially important forest tree species for their end use, selection criteria were prepared for candidate trees and their approval as plus trees (Mathews, 1961; Muniswamy, 1978; Bagchi, 1983) for a number of species viz., Teak, Semul, *Shisham*, Chir, Kail, Deodar, Fir, Spruce, *Prosopis*, *Acacia* etc.

In collaboration with state forest departments approximately 1200 plus trees have been selected (Kedharnath, 1967; 1982a; Rai, 1986; Emmanuel and Bagchi, 1988) for different species, prominent among them were *Tectona grandis, Bombax ceiba,*

Dalbergia sissoo, Gmelina arborea, Pinus roxburghii, Santalum album and *Prerocarpus santalinus*.

Khosla (1985) introduced the possibility of genetic improvement of agroforestry trees. Sheikh (1988) raised a large tree form of *P. juliflora* by collecting seed from plus trees. He further proposed to use the straight tree form for planting in arid and semi-arid areas.

Considerable variability with regard to crooked or straight bole and growth rate exists in *Dalbergia sissoo* (Vidakovic and Siddiqui, 1968; Vidakovic and Ahsan, 1970). Such variation occurs even in one year old trees so far as the crookedness in stem is concerned, as was evident from a high coefficient of variation for 23 one year old open pollinated progenies. Selection of *shisham* trees based upon stem form would be profitable as environment has relatively little effect on this trait as compared to growth. Teak seedlings showed no branching up to 3 years (Champion and Seth, 1968). Variability studies and selection for superior tree form can be made on species showing inherent tendencies to form straight bole such as *Tectona, Shorea* etc. (Dogra, 1981).

A study was made by Vidakovic and Siddiqui (1968) about heritability of height and diameter growth in *shisham* using parent progeny test. A number of plus trees of *shisham* with an apparent higher growth rate of diameter and height, were selected in 1963. The seeds from these trees were collected in 1966. The plants were grown in rows so that progeny of every mother tree was represented by one or two rows of plants. About 30 to 35 plants were selected at random from progeny of each mother tree. Measurements of progenies were undertaken at the end of the first vegetation period. Calculations of heritability for diameter, height and crookedness were carried out by using regression for parent progeny test. Heritability for height and diameter was very low whereas for crookedness, the heritability was high.

It was suggested to raise progenies from a large number of parent trees under the same environmental conditions and subsequently to carry out intensive selection within and between progenies (Sheikh, 1989).

Surendran and Chandrasekharan (1984) studied heritable variation and genetic gain estimates in half-sib progenies of *Eucalyptus tereticornis*. Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability estimates and genetic advance as percentage of mean were worked out for eight characters studies in 35 plus trees. The heritability estimates for girth at base, number of branches, leaf breadth and leaf length: breadth ratio were consistent at different stages of growth.

Gupta and Patil (1988) made an investigation of the variation in fodder and fuelwood yield with different plant characteristics in 40 accessions of *L. leucocephala*. The analysis of variance indicated significant differences among the accessions for all the

characters. Moderate to high estimates of broad sense heritability were observed for most characters.

Volker *et al.* (1990) estimated genetic parameters for growth, stem form and branch size from measurements made at around six years in seedling seed orchard of *Eucalyptus globulus*. Individual heritabilities for volume and stem form were moderate.

Jindal *et al.* (1991) studied variability and changes in genetic parameters of height in juvenile progenies of *Tecomella undulata*. Significant difference, among progenies were observed. Heritability and genetic advance showed decreasing trend with increasing age. They also reported that correlation of juvenile height at different stage with mean height of one-year-old progenies in field was non-significant suggesting that selection for height at juvenile stages in nursery may not be effective.

Sidhu (1997a) reported that *Dalbergia sissoo* is a very good timber species but has the drawback of a crooked and low forking stem which reduces lumber quality. Variability in these traits was surveyed in 1400 km long strip plantations in the Indian Punjab to select superior trees for stem form. Sixteen candidate trees were marked and observations on tree height, clear bole height, diameter, fork diameter and number of crooks were recorded on candidate tree report proformas. Data on these traits were also recorded on random trees and used as a baseline for the selection of plus trees. Twelve plus trees were selected. Mean values for total height, clear bole height and apical dominance were significantly more in plus trees than baseline trees. However, the diameter, fork diameter ratio and number of crooks per metre height of clear bole were more in baseline trees. There was no difference between the 2 groups in stem roundness. The results are discussed in terms of genetic gain in the light of heritability of traits for stem form (taken as 0.53). A high genetic gain for number of crooks is predicted from these selections in the next generation – crooks/m clear bole height in plus trees and baseline trees were 0.070 and 0.272, respectively, giving a calculated genetic gain of –0.107 (selection differential × heritability), thus reducing crooks/m bole to 0.165 in the next generation.

Vakshasya *et al.* (1988) state that until now the emphasis on teak plus tree selection has been vigour, good form and crown characteristics. Three different types of selections are reported here.

Sidhu (1996) gave an outline of the steps involved in plus tree selection for a tree breeding programme. The first step is to select plus trees based on the desired objectives. Such trees may be selected from base populations consisting of either uneven-aged or even-aged stands/plantations, but even-aged stands, preferably plantations, are to be preferred. A plus tree is selected either by comparing the phenotypic values of a candidate tree with those of the base population (comparison methods) or by evaluating a candidate tree on the basis of its score values (individual tree methods). The comparison

methods used include the comparison tree method, the base value method and the regression method. Under individual tree methods either the sum-total of scores, independent culling or selection indices are used to select a plus tree. The comparison tree method is an easy and effective method of plus tree selection.

Goel *et al.* (1997) selected 21 plus trees were on the basis phenotypic superiority in growth, tree form and disease resistance. The minimum selection standard in terms of superiority percentage was 20% for heights and 35% for diameter. Stem form was also considered.

2.8. Leaf morphology:

Soni et al. (1981) observed the flattening of twigs and crowding of leaves with prominent ridges and furrows with a disturbed phyllotaxy. The leaflets are 2-7 cm long and 1-4cm broad, imparipinnate, lanceolate, upper side bigger than the lower but it may vary within a population, often alternate, obliquely falcate, coarsely and bluntly serrate. The breadth of lamina and the degree of dentation on the margin of leaflets vary from locality to locality. In general, leaflets from dry areas have narrow lamina and sharp teeth along the sides (Puri, 1999).

A study of the herbarium at New York Botanical Gardens and Panjab University revealed that the neem trees from arid areas at the foothills of the Himalayas in general have narrow leaflets with sharp dentation in their serrate margin, as compared to the trees from a humid climate which have comparatively broader leaflets with a less sharp serrate margin (Puri, 1999).

Bobowicz and Korczyk *(1994a)*: Two-years old needles were collected from 272 standing trees of *Pinus sylvestris* representing 8 Polish populations. The needles were studied with respect to 15 morphological and anatomical traits. The data obtained were subjected to multivariate statistical analysis in order to delineate inter-populational variability. Discriminant analysis was also conducted. Mahalanobis distances were calculated between each of population in pairs, and their significance was estimated using Hotelling T^2 statistics. On the basis of the shortest Mahalanobis distances a minimum spanning tree was constructed and on the basis of Euclidean distances hierarchy grouping was performed. A large majority of the populations was found to differ significantly from the remaining populations. The population from Bolewice proved to be most divergent. The principal variables which proved capable of discriminating between populations included needle length, the number of stomata on the flat side of the needle and the number of resin canals.

Cordell *et al.* (1998) reported that *Metrosideros polymorpha*, a dominant tree species in Hawaiian ecosystems, occupies a wide range of habitats. Complementary field and common-garden studies of *M. polymorpha* populations were conducted across an altitudinal gradient at two different substrate ages to ascertain if the large phenotypic

variation of this species is determined by genetic differences or by phenotypic modifications resulting from environmental conditions. Morphological characteristics such as leaf size, petiole length, and internode length decreased with increasing altitude in the field and were retained when grown in the common garden, suggesting a potential genetic basis for these traits. The combination of environmentally induced variability in physiological and anatomical characteristics and genetically determined variation in morphological traits allows Hawaiian *M. polymorpha* to attain and dominate an extremely wide ecological distribution not observed in other tree species.

Rawat *et al.* (1998) published data on leaf morphological parameters for 21 teak (*Tectona grandis*) provenances from South and South East Asia (including many Indian provenances) grown from seed in the nursery at the Forest Research Institute, Dehradun, Uttar Pradesh. A study of these revealed certain diagnostic characters in plant > 2 yr old, on the basis of which different provenances/sources could easily be identified.

Magini *et al.* (1982) studied phenotypic, genotypic and environmental correlations between length and width of the leaf blade in 2 provenances of Italian alder (*Alnus cordata*).

Daehler (1999): Substantial phenotypic variation in Acacia koa has been reported in the Hawaiian Islands. Seventy-two families of A. koa were grown in two common garden plots on O'ahu to determine whether phenotypic differences in phyllode morphology, extra-floral nectary morphology, and other characteristics (seed morphology, growth characteristics including height growth, presence of juvenile leaves at 1 yr. old, stem form, branch coloration) have a genetic basis. Significant differences among islands and families were observed for phyllode width, curvature, and pubescence, as well as extra-floral nectary size and pigmentation, retention of juvenile leaves, and branch bark colour. Seed shape also differed significantly among islands. Discriminant analysis revealed that families from the island of Hawai'i were distinct from O'ahu and Kaua'i families. The O'shu and Kaua'i families, however, could not be reliably distinguished based on sapling morphology or growth characters.

Higginbotham *et al.* (1989) reported in variation in leaf, fruit, bract and catkin morphology of *Betula alleghaniensis* and *B. lenta* studied along the altitudinal gradient in the southern Appalachians, in order to characterize the regional structure of the 2 species. Populations were sampled at 52 sites in North Carolina. Weak to moderate linear relations with altitude were found in most size attributes for both species. A few shape characters were also correlated with altitude for one species.

2.9. Seed viability:

For several wild species whose seedlings establish in spring, seed populations show relevant variation that can be correlated with habitat conditions. Populations from severe winter sites, where major risk is frost, tend to have a long chilling requirements or to

germinate very slowly at low temperatures. Populations from warmer sites, where the major risk is drought, are non-dormant and germinate very rapidly under these same conditions. Seed populations from intermediate sites exhibit variation dormancy levels, both among and within plants, which spreads germination across a considerable time period (Allen and Meyer, 1998).

The ultimate fate of seeds under natural (wild) conditions depends on their ability to time germination so that survival of seedlings is maximized. Mechanisms that regulate germination timing through seed dormancy are an important aspect of species life history and ecology (Venable, 1989; Meyer *et al.*, 1995, 1997). Many plants have a wide distribution range encompassing a variety of ecological habitats. For such species, contrasting selection pressures associated with different sites can result in highly variable dormancy patterns (Allen and Meyer, 1998).

Results from common garden experiments, wherein seeds collected from wild populations produce offsprings exhibit dormancy patterns similar to parental plants, suggest that germination differences have a strong genetic basis both among populations and among plants in a population (Allen and Meyer, 1998). This type of data clearly illustrates the need to consider variation in germination strategy in restoration seedings (Allen and Meyer, 1998).

Neem seed is tropical recalcitrant in nature and the viability of the seed falls off rapidly after two weeks (Dent, 1948). Tropical recalcitrant seeds can be stored for comparatively longer duration by maintaining high seed moisture content and a certain amount of gas exchange. But these seeds are sensitive to low temperatures. In considering seed storage as a mean of conserving germplasm, both the characteristics of tree seed and the technology of storage conditions needs to be understood. Seed moisture content and storage temperature are the major environmental factors affecting the preservation of stored seeds, with seed moisture content usually more critical than temperature (Justice and Bass, 1988). Chaisurisri *et al.* (1986) obtained success in storing *Neem* seeds in a cotton bag at 15°C. Seeds were dried down to a moisture content of 46.18 per cent before storing. Under these conditions seeds retained viability for more than 4 months. Germination of seeds stored for 16 weeks was 62 per cent.

Maithani *et al.* (1989) stored *Neem* seeds at room temperature and at 15 or 5°C in sealed and perforated polythene bags, perforated cardboard boxes and over silica gel in desiccators. Both sealed containers and low temperature (5°C) caused rapid deterioration of seeds (complete loss of germinative capacity in 1-4 months), while use of aerated containers at room temperature of 15°C inhibited deterioration so that some seeds still germinated after 6 months storage. The seeds deteriorated much earlier in sealed containers as compared to perforated containers. This is due to the fact that seeds with relatively high moisture content require high O_2 concentration for maximum retention of

viability. Perforated containers maintained low CO_2/O_2 ratio, thereby prolonging the viability of seeds.

Neem seed is reputed to have limited tolerance to desiccation, to be sensitive to chilling and imbibitional stress, and to display intermediate storage behaviour. To understand this behaviour the properties of water in seed tissues were studied. Water sorption isotherms showed that at similar relative humidity (RH), the water content was consistently higher in axes than in cotyledons, mainly due to the elevated lipid content (51%) in the cotyledons. Using differential scanning calorimetry, melting transitions of water were observed at water contents higher than 0.14 g H_2O g^{-1} DW (distilled water) in the cotyledons and 0.23g H_2O g^{-1} DW in the axes. Beside melting transitions of lipid, as verified by infrared spectroscopy, changes in heat capacity were observed which shifted with water content, indicative of glass-to-liquid transitions. State diagrams are given on the basis of the water content of seed tissues, and also on the basis of the RH at 20 degrees C. Longevity was considerably improved, and the sensitivity to chilling/subzero temperatures was reduced when axis and cotyledons were dehydrated to moisture contents < or = of approximately 0.05 g H_2O g^{-1} DW. However, longevity during storage at very low water contents was limited. A possible mechanism for the loss of sensitivity to chilling/subzero temperatures at low water contents is discussed. The results suggest that dry neem seeds in the glassy state have great potential for extended storability, also at subzero temperatures(Sacande *et al.,* 2000).

Mishra *et al..* (1998) studied effect of substratum, storage temperature and orientation on percentage seed germination of *Neem* Fresh seed of neem were collected from 25 morphologically superior trees in Jodhpur district of Rajasthan. In germination tests, the top of filter paper was the best substrate, followed by vermiculite and sand. The germination percentage was higher in seeds stored at low temperature (4° C) as compared with room temperature (35° C). Orientation of seed and removal of endocarp showed no appreciable change in the percentage germination. However, endocarp removal decreased the energy period (time to peak germination).

Chaturvedi *et al.* (1999) conducted studies on germination and storage of neem seeds and growth of seedlings. Neem fruits were collected in September 1991 from 5 trees selected on the Pusa campus of Rajendra Agricultural University in Bihar, India. the fruits were peeled, washed in running water, and the seeds given 5 treatments: none (fresh seeds), air drying in the shade for 6 days, sun drying for 1 or 2 days, and storing in the refrigerator for 6 days. The seeds were then sown in petri dishes in the laboratory or in nursery beds. Fresh seeds exhibited maximum germination (92.8% in the laboratory and 94.1% in the nursery), followed by seeds sun dried for 1 day, seeds dried for 6 days, seeds sun dried for 2 days, and seeds cold stored for 6 days (the latter giving 63.5% germination in the laboratory and 76.2% in the nursery). Germination energy and

germinable seeds within energy period were maximum in seeds sun dried for 1 day and germinated under laboratory conditions, whereas under nursery conditions these values were maximum for the seeds air dried for 6 days. Fresh seeds stored in air tight containers were viable for a longer period than seeds stored in cold storage, although germination decreases with time in both cases. The highest values in respect of growth and biomass were recorded in seedlings germinated from fresh seeds, with seedlings growth decreasing in the other treatments in the same order as for germination percentage.

Gamene *et al.* (1996) studied storage behaviour of neem seeds from Burkina Faso. The storage behaviour of a neem seed lot from Burkina Faso, West Africa, was studied by varying both seed moisture content and temperature during storage. The initial germination capacity, expressed as percentage normal seedlings, was 87%. To determine the effect of moisture content, seeds with intact endocarp were stored in drums with air of a constant temperature (20° C) and different relative humidities. During 9 weeks of storage, the germination capacity remained highest (70% or more) at relative humidities of 55% (moisture content about 9% on a fresh weight basis) and 75% (moisture content about 13%) and was markedly lower at either lower (32 and 20-25%) or higher (95%) relative humidities. The effect of storage temperature was examined in 2 experiments. In the first, seeds with 8.9% moisture were stored at 3, 20, 30 or 50° C for 2 weeks. The germination capacity of these seeds was lowered at the highest temperature only. In the second experiment, seeds with 4.5, 9.2 or 12.9% moisture were stored at both 3 and 30° C for up to 5 weeks. Irrespective of seed moisture content, germination capacity was lost most rapidly at the lowest temperature. The results for this neem seed lot are consistent with the intermediate category of seed storage behaviour.

Sacande *et al.*(1998) conducted a multifactorial study of conditions influencing longevity of neem seeds. Seeds of Sri Lanka and 4 Burkina Faso provenances were dried to equilibrium moisture content (MC) at different RH at 20° C, then place in open storage at 20° C or hermetically sealed in packets and stored at temperatures ranging from -20 to +20° C for 2 years. There was hardly any difference in storage behaviour between seed lots, regardless of provenance. Seeds originating from mature yellow fruits had greater longevity than seeds from green or brown fruits. In all storage experiments with seeds having MC ≥ 10%, viability was preserved best at 10-15° C, indicating that neem seed is chilling and freezing sensitive. There was no survival longer than 2 years under these conditions. At MC of 4-8%, seeds were considerably more tolerant of low temperature storage and had 40-60% viability after 2 years at all temperatures tested (-20 to +20° C). However, the seeds were sensitive to imbibitional stress, which could be alleviated by imbibition at temperature of 25-30° C or above.

Hong and Ellis (1998) Seeds of six species of Meliaceae were tested for germination following desiccation and subsequent hermetic storage for up to 26 months in different

environments. Seeds of both Aglaia clarkii and Sandoricum koetjape were very sensitive to desiccation; no seeds survived desiccation to 20% moisture content or below. In contrast, stones of Melia azedarach survived desiccation to 3.5% moisture content and viability was maintained during 26 months' subsequent hermetic storage in 14 of the 15 environments which combined factorially five stone moisture contents between 3.5 and 11.7% with three temperatures between -20° C, and 10°C, the exception being that environment which provided the highest moisture content and temperature (i.e. 10°C with 11.7% moisture content) in which loss in viability was considerable. Seeds of *Azadirachta indica* tolerated desiccation to moisture contents in equilibrium at 20° C with 30-55% r.h. depending on seed lot, and a considerable proportion of seeds survived further desiccation to moisture contents in equilibrium with 6.5 to 30% r.h. Seeds of Swietenia macrophylla and Khaya senegalensis were more tolerant of desiccation than those of *Azadirachta indica*, but some loss in viability was detected on desiccation to moisture contents in equilibrium with 17% r.h. Moreover, the longevity of all three species in hermetic air-dry storage was shorter at cooler (-20° C and 0° C) than at warmer (10°C) temperatures. It is concluded that Aglaia clarkii and Sandoricum koetjape show recalcitrant seed storage behaviour, while Melia azedarach shows orthodox seed storage behaviour, but that *Azadirachta indica*, Khaya senegalensis and Swietenia macrophylla show intermediate seed storage behaviour. It is suggested that combining information on four criteria (seed weight, shape, moisture content at maturity, and plant ecology) may provide a guide to likely seed storage behaviour in Meliaceae.

Solanki *et al.* (1999) reported that seed viability was increased by 10-15 days through depulping of seeds in neem. Fornos Reyes (1997) studied influence of drying on the viability of seeds of neem. Eeswara *et al.* (1998) studied The influence of stage of seed maturity, moisture content and storage temperature on the survival of neem seed in storage. Neem seed extracted from fruit harvested at two stages of maturity (referred to as ripe and fully mature) were stored at three seed moisture contents (mc) (undried, <15% mc, <10% mc) and in three storage conditions; an air-conditioned room (22°C, 76% relative humidity), refrigerator (3° C), and freezer (-20° C) in sealed thick polythene bags. The germination of the undried ripe seed was high (86%) and remained above 80% when seeds were dried to 13.2 and 7.1% mc whereas the low germination (46%) of the fully mature seed fell to around 30% after drying to 14.2 and 8.1% mc. At all seed mcs the loss of germination during storage occurred most slowly in the refrigerator followed by the air-conditioned room. With the exception of seed having <10% mc, storage in the freezer was highly damaging. Thus after 5 weeks storage at 13.2% mc the ripe seeds had germinations of 65%, 52% and 0% when stored in the refrigerator, air-conditioned room and freezer respectively. Reducing the seed mc prolonged the storage life of both ripe and fully mature seeds in all three storage conditions with the result that the germination

of ripe seed remained above 50% for 24 weeks when the seeds were stored at 7.1% mc. These results are discussed in relation to the classification of neem as a recalcitrant, intermediate or orthodox seed.

Berjak *et al.* (1995) studied responses of seeds of *Azadirachta indica* neem to short-term storage under ambient or chilled conditions. Neem seeds show curious post-shredding behaviour and have been variously documented as orthodox, intermediate or recalcitrant, apparently relating to provenance. In addition, even in the air-dry state, hermetically stored seeds rapidly lose viability. In the present case, putatively recalcitrant, hydrated neem seeds from a Kenyan coastal provenance (Mombasa) were stored in loosely-closed polythene bags to ascertain: (1) whether or not the axes showed metabolic enhancement indicative of the onset of germination under ambient storage conditions for 10 d; and (2) whether chilling ($4 \pm 2^{\circ}C$ for 10 d) had an adverse effect on the axis cells. There was a slight germination lag relative to the control material in the ambient-stored seeds, which is ascribed to their partial enclosure, but the totality of germination was unaffected, while chilling had markedly adverse effects on both rate and totality. The ultrastructure of control material, sampled prior to storage, was typical of embryonic axes in the stage of late development or early stages of germination. During ambient storage, there was considerable enhancement of ultrastructural features indicating that germination (even though slightly delayed) was indeed underway. In contrast, a regression of subcellular development accompanied chilling, many axis cells showing degenerative changes. In terms of this behaviour, seeds of *A. indica* from this African provenance conform to the characteristics expected of tropical recalcitrant species.

Rakesh-Kumar *et al.* (1996a)studied seed storability in *Neem* Seeds collected from 10 randomly selected trees growing naturally in Hisar, Haryana. The seeds were stored under 4 conditions; in plastic bottles, polyethylene bags and cloth bags at room temperature, and under cold storage (4° C) in polyethylene bags. Germination was recorded at 15-day intervals for 60 days. Seeds stored in cloth bags germinated best, followed by those stored in plastic bottles and polyethylene bags (which gave similar results) with germination least in cold stored seeds. Germination percentage showed a steady reduction with time from 15 to 60 days. There was also significant variation in germination between seed collections.

Although the normal practice is to collect fresh seeds naturally dropped on the ground for sowing, new techniques have now been developed to extend the seed viability by improved handling and storage practices (Hegde, 1996). Roederer and Bellefontaine (1989) have reported 42% germination of neem seeds which were stored for five years at 4° C. According to Vandenbeldt and Bhumibhamon (1992), collection of just ripe fruits, depulping, quick washing, shade drying and storage of seeds in cloth bag, at low

temperature upto 4° C can improve the seed viability. Surendran *et al.* (1992) have reported that the storage of depulped and shade dried seeds in earthen pots containing wet sand (30 per cent moisture) can retain the viability upto 62 per cent, at the end of three months.

As the seeds contain chemicals which inhibit germination, it has been recommended to soak the seeds in warm water at 65-70° C for 30 minutes for improving the germination (Hegde, 1996). Neem seed quality is reported to vary with location of collection. The quality of neem seed is not only governed by environmental and genetic factors but also by the moisture content of seeds. Tela (1983) reported that the neem seed loses its viability after two weeks, but it can retain viability for 12 months in dry, low-temperature storage. For long the neem seed was considered to be a species with recalcitrant seed characteristics.

Accelerated ageing test for vigour and viability assessment in neem indicated that the loss in dehydrogenase enzyme activity and membrane integrity could be taken as a measure of vigour. It also revealed that loss in vigour precedes the seed viability (Karivaratharaju *et al.*, 1999b).

In a study on the effect of seed moisture and drying methods, Karivaratharaju *et al.* (1999a)reported that drying methods did not significantly influence the germination (%), root length, epicotyl length, hypocotyl length, dry matter production and vigour index of the neem seeds. The study also revealed that the neem seeds can be dried to 10% moisture content by different drying methods namely shade, sun and oven drying (at 40° C) methods without much damage to the seed viability.

Rakesh Kumar and Bangarwa (1999) reported significant differences among progenies at 2 months old for vigour index and growth parameters (germination percentage, seedling length, seedling dry mater, vigour index I and II, collar diameter and number of leaves) in neem.

El-Kassaby and Edwards (1998) studied genetic control of germination parameters in mountain hemlock (*Tsuga mertensiana*) standard germination tests. Strong genetic control was confirmed by the high heritability estimates that ranged from 0.35 to 0.82 (stratified) and from 0.58 to 0.73 (unstratified) for Sooke and from 0.30 to 0.85 (stratified) and from 0.45 to 0.84 (unstratified) for San Juan.

Unfortunately, seed storage technology need to be developed for many forest tree species, especially of tropical trees which possessa major problem because of absence of dormancy or presence of a very short period of dormancy in the seed (Dogra and Dhiman, 1998).

Information on seed ripening periods and dispersal characteristics of a species is important for proper harvesting and storage of fruits and seeds (Dogra and Dhiman, 1998). High quality heavy seed production areas or seed stands must be selected for different tree species from its distributional range. In these, poor phenotypes can be roughed out and outstanding trees maintained to intermate and produce improved seeds. Seeds thus collected will be of known origin and have a better genetic quality (Dogra and Dhiman, 1998).

2.10. Seedling growth:

Srivastava, *et al.* (1993b) conducted study of half sib seedlings raised from seeds of *T. arjuna* . Three leaf characters showed low GCV and genetic gain and high heritability, suggesting intra- and inter-allelic interactions. Seedling height had high heritability, genetic advance and GCV , suggesting additive gene action.

Pires *et al. (1987)* studied genetic variation among provenances detected in ht. up to 2 months old, branch diameter, branch angle and natural pruning (at 1 yr old). Lopez Mata, (1987) studied genecological differentiation in nine provenances of *Brosimum alicastrum* – a tree of moist tropical forests. Seeds were randomly collected from 5-11 individuals of each provenance along a latitudinal gradients. Principal component analysis and linear correlation indicated the existence of ecoclinal variation in seed size (weight and diameter). Seed size strongly influenced initial seedling size and the relative size of seedling roots which increased in response to habitat dryness and geographic origin. The results suggested that *Brosimum alicastrum* employs an adaptive strategy in response to water deficit during dry seasons in initial phase of seedling establishment.

Seeds were collected by Rakesh Kumar and Bangarwa (1999) from 10 randomly selected neem (Azadirachta indica) plus trees growing naturally in Hisar, Haryana, India. The seeds were germinated and seedlings grown in a mist chamber. Significant differences were observed among progenies at 2 months old for vigour index and growth parameters. The average germination percentage, seedling length, seedling dry mater, vigour index I and II, collar diameter and number of leaves.

In *Salvadora oleoides*, Jindal *et al.* (1999b) found substantial variation with respect to seedling growth characteristics. Relationship of 100 -fruit weight with seedling height and nodes per plant, and of 100-seed weight with seed oil content and germination percentage were positive and non-significant. Significant and positive association was noticed between seedling height and nodes/plant.

Solanki *et al.* (1999): at the National Research Centre for Agroforestry, studies on neem since 1988 indicated that it has good adaptability in semi-arid tropics with an annual increment of 55 cm for plant height and 1.38 cm for collar diameter in red soil. Seed viability was increased by 10-15 days through depulping of seeds. Substantial variability was present for all characters under study. Results of provenance trails showed that seedlings from Riva, Katni, Bhopal, Guna, Shivpuri and Karera districts were excellent. Studies on reproductive biology over 3 years (1993-96) suggest that neem is predominantly a self-pollinated species.

The wide variation observed in the seed germination and seedling growth of different provenances, may be mainly genetical as all the plants were growing in nursery under similar environmental conditions and received similar silvicultural practices. An earlier study (Arya *et al.*, 1993) on *T. undulata* provenances had shown a significant variation in the pod length and seed weight. Others working with *Acacia mangium* (Salazar, 1989), *Acacia albida*(Sniezki and Stewart, 1989), *Albizia lebbeck* (Kumar and Toky, 1996) and *Prosopis cineraria* (Arya *et al.*, 1995a) have reported variation in seed germination and seedling growth among provenances.

Daehler (1999): Substantial phenotypic variation in Acacia koa has been reported in the Hawaiian Islands. Seventy-two families of A. koa were grown in two common garden plots on O'ahu to determine whether phenotypic differences in phyllode morphology, extra-floral nectary morphology, and other characteristics (seed morphology, growth characteristics including height growth, presence of juvenile leaves at 1 yr. Old, stem form, branch coloration) have a genetic basis. Significant differences among islands and families were observed for phyllode width, curvature, and pubescence, as well as extra-floral nectary size and pigmentation, retention of juvenile leaves, and branch bark colour. Seed shape also differed significantly among islands. Discriminant analysis revealed that families from the island of Hawai'i were distinct from O'ahu and Kaua'i families. The O'shu and Kaua'i families, however, could not be reliably distinguished based on sapling morphology or growth characters.

2.11. Fruit and Seed morphology:

Veerendra (1995) conducted study on variation studies in provenances of *Azadirachta indica* (the neem tree). An exploratory survey was undertaken with 10 provenances of neem collected from Karnataka and Andhra Pradesh in June, 1993. Analysis of three characters (seed length, width and weight) indicated the presence of genetic variation among the provenances. The provenance Ramanagar 'H' from Karnataka gave the highest range and lowest of coefficient of variation (C.V.) for all 3 characters studied. The highest C.V. for seed length was recorded in Ramanagar 'Y' (73%), and for seed width and seed weight, in Hoskote provenance (35.7 and 25.1%, respectively), both from Karnataka.

Jindal *et al.* (1999a) studied variability and associations for seed yield, oil content and tree morphological traits in neem (*Azadirachta indica*). The *ex situ* variability of various tree morphological traits (height, collar diameter, diameter at breast height (dbh), clear bole length, canopy diameter), seed yield related parameters (inflorescence length, fruits/inflorescence, fruit yield/tree, 100-fruit, 100-seed and 100 kernel weights, number of kernel/100 seeds), and seed (kernel) oil percentage, and other associations were assessed in 25 neem trees randomly selected from about 200 trees (13 yr old, grown from seed sources from throughout Rajasthan, India) at the Central Research Farm, Jodhpur, Rajasthan. Correlation matrices are given showing the various relationships. Tree height was positively and significantly associated with collar diameter, dbh, canopy diameter and fruit yield/tree. Fruit yield was also significantly positively correlated with collar diameter, dbh and canopy diameter. Number of fruits per inflorescence was positively and significantly correlated with inflorescence length. Seed oil content showed positive, small but significant correlations with fruits/inflorescence, fruit yield/tree and number of kernels /100 seeds, and negative but not significant correlations with 100-fruit, 100-seed and 100-kernel weights. Associations among fruit, seed and kernel weights were positive and highly significant, and the correlation between fruit yield and weight was also positive and significant.

Burley (1965) assessed variability for seed weight in thirty provenances of Sitka spruce. He did not find any apparent relationship of seed weight with latitude. However, there was a trend for northern provenances to have comparatively heavier seeds.

Solanki *et al.* (1985) reported phenotypic variation in pod and seed characters of *Acacia senegal* in the natural stands of western Rajasthan. From their studies of 52 individual trees, they reported substantial variability for various characters like pod length, pod breadth, seeds per pod and 100-seed weight.

An exploratory survey and germplasm collection from 140 trees was undertaken by Kacker *et al.* (1986b) from various places of Indian Thar Desert viz., Ajmer, Barmer, Bikaner, Churu, Jaipur, Jaisalmer, Jalore, Jhunjhunu, Jodhpur, Nagaur, Sikar and Tonk districts of Rajasthan. Analysis of three characters i.e. seed weight, pod length and number of seeds per pod revealed ample variation. Maximum range of variability for pod length was observed in Jodhpur provenance (8.6-26.0 cm with mean 13.46 ± 1.12) whereas for the number of seeds per pod, range was maximum (2.0-17.0 with mean 9.60 ± 1.48) in Nagaur provenance and for seed weight maximum range was observed in Churu provenance (4.0-7.4 with mean 5.34 ± 0.25 g). For all the three characters studied, maximum variation was observed in the provenance of Barmer district as shown by the high values of coefficient of variation.

Salazar (1986) assessed the various provenances of *Gliricidia sepium* for seed weight, length, width and thickness and considerable genetic variation was found in seed traits, with a clear increase in seed weight with increasing altitude.

Shiv Kumar and Banerjee (1986) found a considerable variation in external seed characters, germination, viability, plumule and radicle ratio of *Acacia nilotica.*

Huang (1989) reported significant differences between provenances of *Acacia auriculiformis* in seed weight and seedling traits.

Maley and Parker (1993) on the basis of nested analyses of variance indicated that most of the variation was expressed between and within trees, with relatively little (1.6-18.9% depending on the trait) expressed between sampled populations. Discriminant analysis of cone and needle data indicated that the 64 populations generally varied longitudinally with a steep cline apparent in the Nipigon area (about 88°15' W) for cone data. A corresponding irregularity in the pattern of needle variation suggested that the modern pattern may be the result of separate lineages, i.e. descendants from two migration routes along eastern and western shores of Lake Superior or from two refugia. However, temperature data follow a similar pattern in this area of Ontario, and multiple regression of the discriminant analysis for the 64 sampled populations against altitude, climatic, and soil data indicated that much of the cone and needle variation expressed between population could be accounted for by the regressions. Thus, the observed patterns of phenotypic variation may simply be the result of local adaptations to a variable environment.

Lopez Mata (1987) studied genecological differentiation in nine provenances of *Brosimum alicastrum* – a tree of moist tropical forests. Seeds were randomly collected from 5-11 individuals of each provenance along a latitudinal gradients. Principal component analysis and linear correlation indicated the existence of ecoclinal variation in seed size (weight and diameter). Seed size strongly influenced initial seedling size and the relative size of seedling roots which increased in response to habitat dryness and geographic origin. The results suggested that *Brosimum alicastrum* employs an adaptive strategy in response to water deficit during dry seasons in initial phase of seedling establishment.

Jindal *et al.* (1999b) selected the trees of *Salvadora oleoides* on the basis of large canopy, profuse fruiting, and absence of diseases and pests. There were large variations in fruit colour, 100-fruit weight and 100-seed weight. Mean values for seed oil content, seed germination, seedling mortality due to damping off and seedling height were 37.2%, 79.3%, 18.2% and 6.67 cm, respectively. Relationship of 100-fruit weight with seedling height and nodes per plant, and of 100-seed weight with seed oil content and germination percentage were positive and non-significant.

Tyagi (1999) reported differences in seed morphological traits (length, width, thickness, volume, 100-seed weight) and germination were compared for 7 provenances of *Grewia optiva* from different parts of the Tehri-Garhwal District of Uttar Pradesh and the Solan District of Himachal Pradesh (India). The analysis demonstrated genetic variation between the provenances, and highly significant correlations between different pairs of characters. A multiple regression analysis showed that seed length and 100-seed weight might be used as the predictors of germination in G. optiva.

Sindhuveerendra *et al.* (1999) reported that Sandal (Santalum album) seeds from various seed sources show significant variation morphologically and physiologically. This paper reports data on the seed parameters (seed length, seed width and seed weight) of 9 provenances from Karnataka and Kerala (India), which demonstrate a significant amount of variation among the different sources. The coefficients of variation (CV) were calculated for each seed parameter and source to aid comparison between them. It is suggested that outstanding trees may be selected from those provenances which showed highest CV for different seed characteristics and could be incorporated as base population for establishing second generation seed orchards of S. album at different sites as a prerequisite for formulating a multiple population breeding strategy.

Daehler (1999) found substantial phenotypic variation in Acacia koa in the Hawaiian Islands. Seventy-two families of A. koa were grown in two common garden plots on O'ahu to determine whether phenotypic differences in phyllode morphology, extra-floral nectary morphology, and other characteristics (seed morphology, growth characteristics including height growth, presence of juvenile leaves at 1 yr. old, stem form, branch coloration) have a genetic basis. Significant differences among islands and families were observed for phyllode width, curvature, and pubescence, as well as extra-floral nectary size and pigmentation, retention of juvenile leaves, and branch bark colour. Seed shape also differed significantly among islands. Discriminant analysis revealed that families from the island of Hawai'i were distinct from O'ahu and Kaua'i families. The O'shu and Kaua'i families, however, could not be reliably distinguished based on sapling morphology or growth characters.

Correlations between different fruit parameters (weight, length, diameter, volume and pulp weight) were found to be positive and significant in 31 genotypes of jamun (*Syzygium cuminii*) from the Shahdol and Jabalpur areas of Madhya Pradesh, India (Jadon *et al.,* 1999).

Sharma *et al.* (1999) studied the genetic parameters of the cone and seed characteristics of *Pinus* roxburghii, estimated from a study of cones and seeds collected from 33 phenotypically superior trees selected in 1997-98 in different areas of Himachal Pradesh (India). Significant variations were observed for all 5 characters studied (cone and seed weight, cone width and length and number of seeds per cone). The difference between

phenotypic and genotypic coefficients of variation were low for all the characters. The high broad sense heritability and genetic gain obtained for cone weight and number of seeds per cone indicated additive gene action. Genetic correlations were positive and significant for seed weight, cone length and cone width.

2.12. Biochemical composition of seeds:

2.12.1. Seed oil content:

Neem oil has been widely employed in manufacturing of soaps, oils, skin ointments, waxes, lubricants, epoxy compounds and as a fuel for lighting. It has been reported to have insecticidal, antifertility, antifungal and antiseptic properties (Kumar *et al.* , 1990, Tewari, 1992). A new process has been recently developed to produce colourless and debittered neem oil that can be used for edible purposes (Rukmini, 1987).

Neem oil is used for making soap, shampoo, cream, hand and body lotion and toothpaste, waxes and lubricant as well as fuel for lighting and heating. India currently produces over 80,000 metric tonnes of neem oil annually, which is used primarily in the manufacture of soap. Gum extracted from the bark is used as a soil fertilizer and soil amendment. ~~and is for tannins and dental care.~~ A neem twig is commonly used as a disposable toothbrush for maintaining healthy teeth and gums. The leaves are used for preparing vegetable for human food and also for livestock feed. Honey from the nectar of the flower is popular in Bangladesh and India.

Jaryum (1991) reported the in Borno State of Nigeria, oil yield per Kg of seeds ranges from 400-500 ml. Neem oil has a variety of uses *viz.* insecticides, pharmaceuticals, soaps, oils, waxes, lubricants, epoxy compounds, and as fuel for lighting. As many as 14 million trees are estimated to be growing in the country with total potential of 4,14,000 tonnes of oil.

Combustion of neem oil in combination with diesel oil has been successfully demonstrated and advocated, especially in countries devoid of combustion oil sources (Randhawa and Parmar, 1993). ~~Barrett and Walter Preparation of neem oil with lower phytotoxicity and improved stability.~~ A new method for preparing a neem oil with an acceptably low foliar phytotoxicity involves crude neem oil treatment with dilute aq. alkali to precipitate a solid, separating the precipitate, and determining residual phytotoxicity. Alternatively, the crude neem oil may be treated with a lipase (EC-3.1.1.3) to degrade a suitable percentage of lipid, followed by enzyme deactivation and determining residual phytotoxicity. The alkali-treated neem oil has a cloud point of 10-13 deg or lower, and a foliar phytotoxicity rating of 2.3 or lower. The resulting product is a shelf-stable, low-phytotoxicity insecticide and fungicide, useful in controlling Colorado potato beetle, diamond-backed moth, whitefly, mealybug, aphid, hornworm, lacebug, flea, mosquito, fly, mildew, rust, dollar spot, brown patch, black spot, Botrytis, mite, lice, tick or scabies. The oil may also be used to treat symptoms of eczema or dermatitis. The

processes reduce the cloud point of the neem oil, eliminating the waxy waste stream produced by previous methods, and giving an active product with improved stability.

Jacobson (1984) reported that the neem seeds contain 42-50% of oil that has potential use in production of waxes, lubricants and aromatic chemicals. Azadirachtin, a complex sesquiterpenoid and the major insecticidal component of the seed, repels and prevents or drastically reduces feeding by at least 40 spp.of pest insects and nematodes. It is effective at concentrations as low as 0.1 ppm, and appeared to be safe to beneficial insects, fish, animals and most crop plants.

Jindal *et al.* (1999a) studied variability and associations for seed yield, oil content and tree morphological traits in neem (*Azadirachta indica*). The *ex situ* variability of various tree morphological traits (height, collar diameter, diameter at breast height (dbh), clear bole length, canopy diameter), seed yield related parameters (inflorescence length, fruits/inflorescence, fruit yield/tree, 100-fruit, 100-seed and 100 kernel weights, number of kernel/100 seeds), and seed (kernel) oil percentage, and other associations were assessed in 25 neem trees randomly selected from about 200 trees (13 yr old, grown from seed sources from throughout Rajasthan, India) at the Central Research Farm, Jodhpur, Rajasthan. Seed oil content showed positive, small but significant correlations with fruits/inflorescence, fruit yield/tree and number of kernels /100 seeds, and negative but not significant correlations with 100-fruit. 100-seed and 100-kernel weights.

In trees grown in Nicaragua, seeds grown with high azadirachtin content (10.07 mg g^{-1} seed) did not produce the same amount in offsprings (Gruber, 1991).

Kadir *et al.*(1998) studied the oil content of seeds of neem trees in Peninsular Malaysia ~~and their phenological~~. The sample from Jalan Sungai Batu Pahat, Perlis, northern Peninsular Malaysia contained the highest percentage of neem oil (63.8%).

Sridharan and Venugopal (1998) worked on the effect of environmental conditions on the yield of oil in neem. A study of variation in the levels of oil in neem seeds was conducted at the agricultural College and Research Institute at Madurai, Tamil Nadu, during 1993-95. Seed samples from individual trees were collected from 12 different locations in Tamil Nadu, and oil contents determined. Seeds from different locations had significant variations in their oil content. Climatic factors at the different locations were correlated with oil contents of seeds, with a significant positive correlation of sunshine hours during September-March with seed oil content.

Jitendra-Kumar *et al.* (1997) reported neem oil content in relation to the agro-ecological factors and regions of India. The variation in yield of neem oil in relation to the ecosystem, tree growth period, soil type, and precipitation in India, is described. A moderate climate with a moderate tree growth period, Indo-Gangetic alluvium soil and 400-1600 mm precipitation were the most suitable conditions for a high oil yield.

Schiller and Grunwald (1987) studied xylem resin monoterpene composition as related to provenance in 472 trees of *Pinus halepenis* from 22 provenances from Greece, Spain Morocco, Algeria, Tunisia and Israel. Except for the Greek and Israeli provenances monoterpene composition between provenances was low; however, 3 district groupings (Greek, West European and North America) could be distinguished. Susceptibility to *Matsucoccus josephi* of 7 provenances was related to cortex resin monoterpene composition, which differed significantly between provenances.

2.12.2. Fatty acid profile of seeds:

The current commercial production of vegetable oils is primarily targeted towards their utilization as an edible component of the human diet. The world wide market for vegetable oils has increased by 300% since 1960, not only due to increases in world population but also to increases in living standards (Hills & Murphy, 1991). A further interesting factor is that the market share supplied by animal - derived fats fell from 39% of the total in 1960 to 26% in 1990 , in some part due to an increased awareness of dietary factors (Murphy, 1992). Though the primary market for vegetable oils is for human consumption, up to 33% of the total production is designated for a range of non-edible applications, such as lubricants, plasticisers and detergent ingredients. The potential for such diversification is an area of considerable recent interest (for reviews, see : Hills & Murphy, 1991 ; Robbelen, 1991 ; Murphy, 1992). There are a number of approaches for obtaining sources of oils containing the fatty acid profiles required by industry. One approach is to search for wild undomesticated species that contain oils of interest and attempt to domesticate them.

Vegetable oils are comprised of glycero-lipids containing predominately 16-and 18-carbon fatty acids. Together the number of carbon atoms, and the frequency and position of carbon double-bonds, in fatty acids affect the physical and chemical characteristics of the oil (Somerville & Browse, 1991).

The qualitative manipulation of seed oils involves the modification of their fatty acid compositions. It is the fatty acid profile which determines the chemical properties responsible for the particular end use of the seed oil. Oil-bearing plants produce many hundreds of fatty acids that can serve as actual or potential industrial raw materials. ~~In attempting to demonstrate the effectiveness of biotechnology for producing new industrial oil crops.~~ It is therefore, important to decide initially upon a relatively limited number of target fatty acids to be produced. (Murphy, 1993b).

The selection of target fatty acids for designer rapeseed varieties has been largely determined by the availability or the ease of isolation of the relevant gene clones. It has proved relatively straightforward to purify many of the soluble enzymes associated with fatty acid formation de novo and therefore to clone their genes. (Murphy, 1995).

Stearic acid is a C18 saturated fatty acid that serves as a major ingredient in margarines and cocoa-butter substitutes. The Δ_9 stearoyl-ACP desaturase gene which normally converts stearic to oleic acid was partially inactivated in rapeseed using antisense methods, resulting in the accumulation of a seed oil containing up to 40% stearic acid, in contrast to levels of <5% stearic acid in conventional rapeseed varieties (Knutzon *et al.*, 1992).

Studies of the inheritance of oil quality in other oilseed crops have indicated that several genes are involved, each with a small effect. However, major genes affecting fatty acid composition have been reported in two other oilseeds, rapeseed (*Brassica napus* L. ssp. *Oleifera*) (Harvey, 1961) and safflower (*Carthamus tinctorius* L.) (Knowles and Hill, 1964) . Widstrom and Jellum (1984) found the existence of a major dominant gene that influenced germ oil composition for oleic and linoleic acids in an inbred line of maize (*Zea mays* L.). They also reported that a recessive gene located on another chromosome influenced the control of oleic and linoleic acid levels.

Concern over high saturates in human diets has prompted the development of soybean [*Glycine max* (L.) Merr.] lines producing oil with reduced saturated fatty acid concentration. To better understand those factors that influence phenotypic expression for palmitic and stearic acid content in soybean, thirty soybean lines random for saturated fatty acid content were grown in eight field environments contrasting for mean temperature during seed-filling. Palmitic and stearic acid content varied significantly ($P<0.01$) both among genotypes and across environments, while genotype x environment interactions were reflected in changes in line variance and ranking for both traits. Therefore selection of a superior genotype for saturated fatty acid composition may not correlate well from one environment to another. In general, early-maturing lines were less sensitive than later-maturing lines in their response to changes in mean daily temperature for palmitic concentration. However, factors in addition to temperature appeared to influence genotype response for stearic acid content. It appears that genetic systems conditioning palmitic and stearic acids are independent, and that separate breeding strategies need be adopted to make simultaneous improvement for these two oil traits. In summary, development of soybean lines with low or high saturated fatty acid content may be accomplished through evaluation and selection in a few environments contrasting for temperature (Rebetzke, 1996).

For both palmitic and stearic acid, stronger linear relationships with mean temp. for late-maturing lines suggest that lines are essentially linear in their fatty acid response to increases in mean temperature and that there is genetic variation in soybean for physiological sensitivity to changes in temperature (Rebetzke, 1996). In that regard, temperature effects upon membrane fluidity are well documented (e.g. Sommerville &

Browse, 1991), but there has been little consideration of a genetic basis for genotypic differences within crop species.

The compositions of seed oils can be varied by selective breeding with little effect on the agronomic characteristics of the plant. For example, a high oleate peanut cultivar called SunOleic 95R has been developed by the University of Florida, in which the polyunsaturated fatty acyl content of the seed oil was greatly reduced. This high oleate peanut originated from a naturally occurring variety and contained 80.6% oleate and 2.8% linoleate (Gorbert and Knauft, 1997). The reduced polyunsaturates mean that the oil, now much like olive oil in composition, has a much longer shelf-life than the usual peanut varieties before exhibiting rancidity (O'Keefe *et al.*, 1993). Additionally, high oleate, whole peanuts remain fresh longer, an important property for increasing the shelf life.

Linoleate is biosynthesized in a single enzymatic step from oleate by a desaturase that inserts a double bond into a monounsaturated fatty acid like oleate one methylene group removed from the existing double bond. One class of desaturases functions in chloroplasts. But the major desaturase involved in the formation of seed oil during seed development in the oleoyl-PC desaturase, found in microsomes, this desaturase acts on fatty acyl residues present in phospholipids, chiefly phosphatidylcholine (PC) in cell membranes form higher plants like peanut. Biochemical comparison of developing seeds from mutant and parental varieties of peanut showed that the high oleate phenotypes was correlated with reduced activity of the microsomal oleoyl-PC desaturase (Ray *et al.*, 1993).The oleoyl-PC desaturase was the target of the gene suppression providing the high oleate varieties of soybean(Glycine max (L.) Merr.) and canola (Brassica ssp.) (Mazur *et al.*, 1999 and references therein).

Dodd and Rafii (1995) studied eco-geographic variation in seed fatty acids of 104 trees from 13 natural populations of *Austrocedrus chilensis* in Andean Chile and Argentina. Multivariate analyses indicated a separation of xeric zone populations from mesic populations. These differences were associated with a greater abundance of C20 unsaturated acids in the mesic group. Differentiation among mediterranean Chilean populations was relatively high, perhaps as a result of their disjunct distribution. There was no evidence for lower level of within -population variation for isolated populations.

Mean temperature appeared relatively unimportant in discriminating among early-maturing genotypes for stearic acid, and to a lesser extent, palmitic acid response. Therefore, genotype sensitivity to environment for stearic acid appeared to be conditioned by environmental factors additional to temperature. For example, reductions in soil-water potential have been reported to affect saturated fatty acid content (Dornbos & Mullen, 1992), while duration and spectral quality of light can alter soybean oil for saturate composition.

The inheritance of the high oleic acid phenotype in sunflower has been widely studied with crosses between high oleic stocks originating from Pervenets and low oleic stocks originating from various sources. Although results have varied in different genetic backgrounds, a dominant mutation (*Ol*) with pronounced effect on oleic acid content has segregated in every genetic background (Fick, 1984; Urie, 1985; Miller *et al.,* 1987 ; Fernandez-Martinez *et al.*, 1989).

The first OLD gene was cloned from *A. thaliana* with a t-DNA tagged line with higher 18 : 1 content in seeds, roots, and leaves than the wild-type line (Okuley *et al.*, 1994). The *Arabidopsis* OLD gene was expressed in developing seeds and various other tissues in wild-type lines. Two OLD cDNAs were subsequently isolated from soybean (*G. max* (L,) Merr.) with the Arabidopsis OLD gene as a heterologous probe (Hepppard *et al.*, 1996). One was expressed in developing seeds, whereas the other was expressed in several tissues (leaves, roots, stems) in addition to developing seeds.

The sequence flanking the first methionine codon differed by two base pairs (AACATATGGG) from the proposed consensus sequence (AACAATGGC) for translation initiation in plants (Latcke *et al.*, 1987). The 1137-bp open reading frame of OLD-7 encodes a 378 amino acid sequence which is five amino acids shorter than the *Arabidopsis* sequence (Okuley *et al.*, 1994). The nucleotide and deduced amino acid sequence identities of OLD-7 and the *Arabidopsis* OLD ORFs were 68.8 and 70.3%, respectively. The amino acid identities between the sunflower OLD-7 cDNA and other plant OLD sequence were 68.4% for *Brassica juncea* L. 70.4 and 71.3% for Glycine max L., and 72.7% *Solanum commersonii* L. Analysis of genomic sequences produced by PCR revealed no interns in the sunflower OLD-7 coding region. OLD-7 was strongly expressed in developing seeds, but was not expressed in leaves, mature seeds, 5-d old cotyledons, flower buds, roots, and stems. Oleic and linoleic acid comprise more than 70% of the fatty acids in leaf cells and 55 to 70% of the fatty acids in roots and other non-photosynthetic tissues in sunflower and most higher plants (Harwood, 1980).

Neem seed samples from different provenances of the Rajasthan state in India were collected. These samples were analysed by GLC to study the variability of fatty acid composition. Significant variability in individual fatty acids was observed. The palmitic acid ranged from 16 to 34%, stearic acid from 6 to 24%, oleic acid from 25 to 58% and linoleic acid from 6 to 17%. This variability can be exploited for selection of trees and for studying the genetic variability in neem. These selections can also be utilized for genetic improvement of the tree (Kaushik and Vir, 2000).

Ali *et al.* (1996) conducted studies on the fatty acids and glyceride compositions of Nim (Melia *Azadirachta indica*) seed oil. ~~The fatty acid and glyceride compositions of neem [*Azadirachta indica*] seed oil were studied.~~ It was observed that neem seed grown under the soil and climatic conditions of Bangladesh contains 40% of acrid bitter

greenish yellow to brown oil with a strong disagreeable garlic-like odour. The oil was fractionated into mono-, di- and triglycerides by silicic acid column chromatography. The content of triglycerides varied from 89.0 to 89.9%, diglycerides from 2.7 to 3.2% and monoglycerides from 2.6 to 3.0% depending on the soil texture in the areas where the plants were grown. The fatty acid composition of oil was analysed by GLC. Oleic, palmitic, stearic, linoleic, arachidic and behenic acids made up 55.8, 16.5, 2.5, 20.5, 2.4 and 2.2%, respectively, of the total fatty acids.

Jitendra Kumar *et al.* (1997) reported neem oil content and its key chemical constituents in relation to the agro-ecological factors and regions of India. The variation in yield of key fatty acids (oleic, stearic and palmitic) in relation to the ecosystem, tree growth period, soil type, and precipitation in India, is described. Coastal areas, coastal alluvium and red soils, trees with a growth period of >210 days and 2000-3200 mm precipitation favoured a high oleic, stearic, palmitic and total fatty acid content of oils. *Azadirachta* excelsa seeds were extracted with hexane at room temperature. It showed yields of approximate 35% based on seed kernel weight. Physical properties of seed oils were determined by the method of the American Oil Chemists' Society (AOCS) and the American Society for Testing Materials (ASTM). It exhibited the following physical properties: density at 29°C 0.878, refractive index at 29°C 1.460, specific gravity at 23°C 0.1987, acid value 16.35, and saponification number 203.38. The fatty acid composition of seed oil were analyzed by Gas chromatography (Shimadzu GC-9A). They were composed of Caprylic acid 0.30%, n-Capric acid 0.96%, Palmitic acid 9.8%, Stearic acid 4.7%, Heneicosanoic acid 0.72%, Behenic acid 2.76%, and Tricosanoic acid 0.75% etc.

2.12.3. Azadirachtin content of seeds:

Sridharan and Venugopal (1998) worked on the effect of environmental conditions on the yield of azadirachtin and oil in neem. The levels of azadirachtin and oil in seeds influence the effectiveness of neem seed derivatives in pest management. A study of variation in the levels of azadirachtin and oil in neem seeds was conducted at the agricultural College and Research Institute at Madurai, Tamil Nadu, during 1993-95. Seed samples from individual trees were collected from 12 different locations in Tamil Nadu, and azadirachtin and oil contents determined. Seeds from different locations had significant variations in their azadirachtin and oil content; climatic factors at the different locations were correlated with azadirachtin and oil contents of seeds, with a significant positive correlation of sunshine hours during September-March with seed oil content and a negative correlation between the total number of rainy days during the fruiting season (April-August) with seed azadirachtin content.

Eeswara *et al.* (1997a) studied azadirachtin, salannin and nimbin contents of seeds of neem (*Azadirachta indica* A. Juss.). Azadirachtin, salannin and nimbin contents were determined of neem seeds from trees at 3 sites in the Dry Zone and 1 site in the Intermediate Zones of Sri Lanka. All neem seed kernels examined contained azadirachtin, nimbin and salannin. The azadirachtin contents of seeds ranged from 2000 to 6500 $\mu g\ g^{-1}$ of seed kernels. The nimbin and salannin contents of the seeds were very low. The variation in meliacin content between trees was greater than that between sites.

Sidhu and Behl (1996) reported seasonal variation in azadirachtins in seeds of Azadirachta indica. *A. indica* produces seeds during the rainy season in India. Phenotypes that produced seeds out-of-season in November-December were identified. Qualitative and quantitative variations in azadirachtin content were determined in seeds produced in the rainy season (July) and in winter. Seed produced during July contained higher concentrations of crude azadirachtins (1.53%) than those produced in the winter (1.26%). Concentrations of azadirachtins A, B and F were determined by HPLC using an improved solvent-eluting system for better resolution. Azadirachtin A was the major metabolite in July seeds. Azadirachtins A and B were in nearly equal proportions in winter seeds. The concentration of azadirachtin F was >2-fold higher in winter seeds than in July seeds. The sum of the peak area of azadirachtins A, B and F was higher in seeds produced in winter. Winter stress appeared to favour synthesis of azadirachtins B and F. Such phenotypes could be a material of choice for clonal multiplication of *A. indica*.

Many researchers, (Butterworth and Morgan, 1971; Zanno *et al.*, 1975; Uebel *et al.*, 1979; Ermel *et al.*, 1984, 1987, and Schmutterer and Zebitz, 1984), have reported seed azadirachtin levels in trees from all over the neem-growing world, quoting levels between 0.02% and 0.66%. However, it is not always clear whether the values quoted are percentage of fruit, seed or kernel and at what moisture content the assay was done (Bally *et al.*, 1999).

Rengasamy and Parmar (1994) reported azadirachtin content at different states of flowering and fruiting in neem. The fresh flowers and fruits of a 10-year-old neem tree in India were analysed for their azadirachtin-A concn. The compound was not detectable in the flowers and in green fruits collected at 20, 30 and 40 days after anthesis (DAA); green fruits analysed at 50 DAA and yellow fruits analysed at 60 DAA contained 0.051 ± 0.001 and 0.112 ± 0.002% azadirachtin-A, respectively.

Kadir *et al.*(1998) studied the azadirachtin content of neem seeds in Peninsular Malaysia. The greatest abundance of neem trees was found in northern Peninsular Malaysia which also produced higher yield of fruits compared with that of southern and east-coast of Peninsular Malaysia. Quantitative analysis of azadirachtin (potent insect antifeedant and growth-regulating agent) content in the neem seed kernel extract was

carried out by High Performance Liquid Chromatography (HPLC). The sample from Tanjung Rhu, Langkawi contained the highest content of azadirachtin (0.40%).

Seeni Rengasamy and Parmar (1995) studied azadirachtin-A content of seeds of neem ecotypes in relation to the agroecological regions of India. The azadirachtin-A content of seed kernels of neem (*Azadirachta indica*) ecotypes from 11 of the 21 agroecological regions of India was investigated in relation to agroecological factors such as ecosystem and climate, growth period (the period of continuous plant growth in a year) and soil. The azadirachtin-A content, to which the pest control properties of neem are attributed, ranged from 0.14 to 2.02% w/w of kernel with an average of 0.68%. The ecotypes growing in the regions with coastal, arid and semiarid ecosystems showed high (>0.72%) and those from the regions with subhumid ecosystems showed very low (0.27%) average azadirachtin-A contents. The growth period up to 150 days appeared to be more conducive for azadirachtin-A content under arid and semiarid ecosystems. The ecotypes growing in the agroecological regions with red soil had higher azadirachtin-A contents than those growing in other soil types, the highest azadirachtin-A content being recorded in the samples from red soil falling under the rain-shadow areas of the south-west monsoon.

Marked differences in the yield of azadirachtin from neem seeds collected from various sources in Asia, Africa and South America have been found(Morgan, 1981; Ermel *et al.*, 1984; Schumutterer and Zebitz, 1984; Ermel *et al.*, 1987). According to a study conducted in India, extracts of seed kernels, seed coats and fallen leaves of neem near the desert possessed higher anti-feedant activity than those from coastal areas (Singh, 1987).

Bally *et al.* (1999)studied 145 neem trees of six ecotypes from different countries and grown at one place for their azadirachtin content in the seed. The seed azadirachtin levels ranged between 0.35 and 0.89% of the dried kernel, with an average of 0.58%. When the azadirachtin levels were looked at on an ecotype basis, the average azadirachtin levels for each ecotype were not significantly different. However, the variation of individual trees within an ecotype was large. Azadirachtin levels of individual trees differed over the three years. This indicated that the azadirachtin yield of an individual tree or a plantation will fluctuate from year to year. The reason for this fluctuation is unclear at present, but likely to be influenced by climatic factors and cultural practices such as irrigation and nutrition.

There are indications that not only genetic, but also environmental factors play an important role in the production of azadirachtin. Trees derived from seeds, obtained from high azadirachtin yielding plantations in Togo, yielded less azadirachtin in the Dominican Republic. Similarly, several thousand neem trees planted in Ecuador from sees obtained in Nigeria produced top yields of nuts with high azadirachtin content every

second year (Schmutterer, 1990a).The location in Ecuador is characterised by high rainfall, followed by a long dry season (Locke and Lawson, 1990).

A detailed investigation was carried out by CIMAP to document the variation in azadirachtin, nimbin, nimbidin and salanin contents during growth of neem seeds in different agro-climatic zones of India depending upon temperature, humidity and altitude. It has been observed that the percentage of all these limonoids in the seeds keep on increasing right from the formation of fruit till the maturity of the seeds and biosynthesis of these limonoids is very fast while the fruit is being converted from green to the ripe, thereafter the increase is slow, and slightly declines when the fruit is about to fall or has just fallen from the tree suggesting that harvesting at the time of maturity is beneficial (DSIR, 1999).

Samiayyan *et al.* (1995) studied azadirachtin content in 112 neem ecotypes of Tamil Nadu, India. Ecotypes from Bannari, Tuticorin town, Thiruppathur, Pudukkottai town, Dharampuri and MYD038 recorded highest concentration of azadirachtin i.e., 1000, 8832, 8105, 7952, 7875 and 7322 mg kg^{-1} respectively. Crown collected berries during initial periods of harvest at Mettupalayam also recorded higher azadirachtin content (8096 mg kg^{-1}). Seeds of Chidambaranar and Ramnad region viz., CB77 recorded the lowest content of azadirachtin (293 mg kg^{-1}) among the types tested. Other samples from different regions which recorded lower contents were: Thanjavur - TVR 13 (548 mg kg^{-1}), South Arcot - VPM 68 (559 mg kg^{-1}) VPM 42 (644 mg kg^{-1}), Periyar KA 25 (783 mg kg^{-1}), and Trichy - TRI 037 (976 mg kg^{-1}).

Venkateswarlu and Mukhopadhyaya (1999) studied azadirachtin content in the seeds of micropropagated neem plants in relation to its mother tree. Two neem (*Azadirachta indica*) plus trees were selected from Anantpur and Ranga Reddy districts in Andhra Pradesh (India) and micropropagated using an already established *in vitro* culture technique. Some of the micropropagated plats were established at the research farm at Hyderabad in May 1996 and in 1997. They were irrigated in the summer and grew well, non-irrigated plants did not grow well. In the 1996 plated saplings, flowering and fruiting were observed after 25 months in the irrigated plants)but not in the non-irrigated plants), and comparisons were made in 1998 of the azadirachtin content and of other seed related parameters in these 25-months-old progeny and the mother trees. The azadirachtin content and chemical composition (content and ratio of azadirachtin A and B) of the seeds was comparable to that of the mother trees in both accessions

Yakkundi *et al.* (1995) Variation of azadirachtin content during growth and storage of neem (*Azadirachta indica*) seeds. Variation of azadirachtin content during the growth and storage of neem seeds was studied by reversed-phase high-performance liquid chromatography using anisole as internal standard. Samples were collected in Bangalore,

Karnataka, every fortnight from the initial bud stage to the ripened fruit stage and analysed using an efficient sample preparation protocol developed for this purpose. Azadirachtin appeared only after the 9th week, gradually reaching a maximum of 0.38±0.06% (w/w, on a dry weight basis) around the 17th week, and decreasing to 0.29±0.03% (w/w) by the 19th week. Thus, the fruits can be profitably harvested in the 17th week of development (when the neem fruit turns from green to yellow) for better yield of azadirachtin. Application of the method for storage studies of neem seeds indicated that azadirachtin was unstable under normal conditions of storage of the seeds, with azadirachtin content reducing to about 68% of the original level over a period of 4 months in the dark and to 55% in daylight.

Sridharan and Venugopal (1998) worked on the effect of environmental conditions on the yield of azadirachtin and oil in neem. The levels of azadirachtin and oil in seeds influence the effectiveness of neem seed derivatives in pest management. A study of variation in the levels of azadirachtin and oil in neem seeds was conducted at the agricultural College and Research Institute at Madurai, Tamil Nadu, during 1993-95. Seed samples from individual trees were collected from 12 different locations in Tamil Nadu, and azadirachtin and old contents determined. Seeds from different locations had significant variations in their azadirachtin and oil content. climatic factors at the different locations were correlated with azadirachtin and oil contents of seeds, with a significant positive correlation of sunshine hours during September-March with seed oil content and a negative correlation between the total number of rainy days during the fruiting season (April-August) with seed azadirachtin content.

2.13. Stability analysis:

Measuring genotype environment interactions helps to determine an optimum breeding strategy of either to breed for specific or wide adaptation which depends on the expression of stability under a limited or wide range (Romagosa & Fox, 1993; Annicchiarico, 1997b; Yue *et al.*, 1997). Moreover, genotype location interaction allows the grouping of relatively similar sites in relation to genotypic performance within which the interaction is minimum (Eberhart & Russell, 1966; Romagosa & Fox, 1993; Annicchiarico, 1997b). Genotype environment interactions are classified into two types; quantitative (without changes in ranks) and qualitative (with changes in ranks) and it is the latter type that presents a challenge to breeders (Romagosa & Fox, 1993). In the presence of interaction, mean yield is inadequate as it does not sufficiently indicate consistency of performance (Francis & Kannenberg, 1978).

In subsistence agriculture maximum yield per se may not be as important as the possibility of achieving a certain minimum yield level or stability (Romagosa & Fox, 1993). In Ethiopia high variation in climatic and edaphic conditions leads to significant genotype environment interactions even within a small geographic area, making cultivar

development and recommendation more difficult. On the other hand, the benefits accruing from agroecological diversity could be the opportunities it offers to screen and select for stress resistance (Romagosa & Fox, 1993).

In natural populations many random effects (e.g. maternal effects,) as well as fixed effects (e.g., sex, or year of birth) may have considerable influence on quantitative traits (Thomas *et al.*, 2000). Measures of stability of use in plant breeding can be obtained from estimates of the genotype x environment interaction (Horner & Frey, 1957; Plaisted and Peterson, (1959), but these techniques are difficult to use when a large number of genotypes is being evaluated. Finlay and Wilkinson (1963) used the average yield of a large group of genotypes to describe the value of an environment, circumventing the complexities involved in the analytical study of the edaphic and seasonal factors. They measured stability of a genotype in terms of the linear regression of genotype means on environmental means. Eberhart & Russel (1966) included deviation from linear regression as an additional parameter to describe stability. They suggested that deviations from regression should be given special consideration, since the variety x environment (Linear) sums of squares were only a small portion of the variety x environment interaction sums of squares.

High productivity and stability of performance are two most desirable feature of a crop variety. In fact, the stability in production is particularly important in tree crops, as it is most extensively grown under varied range of agro-climatic conditions. Identification of such genotypes will have a considerable significance in crop improvement.

It is commonly observed that the relative performance of different genotypes alter in different environments, i.e., that there exists a genotype-environment interaction. It has been further observed by various authors viz., Yates and Cochran (1938), Finlay and Wilkinson (1963), Rowe and Andrew (1964), Eberhart and Russel (1966), Perkins and Jinks (1968a), Breese (1969) and Baker (1969), that the relation between the performance of different genotypes in the various environments and some measure of these environments is frequently linear , or nearly so. For this purpose , the statistical theory of regression has been employed, but unfortunately the fundamental statistic assumptions have not been satisfied in any of the work quoted.

The concept of G x E interaction as a departure from linear and independent genotypic and environmental "main effects" assumes that each factor contributes an effect to the phenotype (Falconer, 1981). A phenotype is the result of an interaction between genotype and its environment. The failure of a genotype to give the same phenotype performance when grown under different environments is the reflection of G x E interaction (Verma *et al.*, 1987). Gregorius and Namkoong (1986) reported that G x E interaction is essentially a synonym for undefinability of effect of casual variables.

Quantitative characters are much influenced by the environment and G x E interaction gives an idea of the magnitude of bias in the estimation of genetic parameters . This bias is accounted for by growing the breeding material over a number of environments of breeder's interest because the effect of heterogeneous environment on genetic variability is a classical problem in population genetics (Via and Lande,1987).

Perkins and Jinks (1968b) observed the non-linear component of variation by grouping the varieties into homogeneous groups on the basis of deviation from linear regression and reported a significant and marked reduction in the non-linear component of the interaction as a result of grouping the varieties.

The linear regression could simply be regarded as a measure of response of a particular genotype, whereas, deviation is now considered to be measure of stability, genotype with lowest deviation being the most stable and vice versa (Breese, 1969; Samuel *et al.* 1970; Paroda and Hayes,1971).

It is commonly observed that the relative performances of different genotypes alter in different environments, i.e., there exists a genotype-environment interaction. It has been further observed by various authors e.g., Yates and Cochran (1938); Finlay and Wilkinson (1963); Rowe and Andrew (1964); Eberhart and Russell (1966); Perkins and Jinks (1968a); Breese (1969); Baker (1969), that the relation between the performance of different genotypes in the various environments and some measure of these environments is frequently linear, or nearly so. These observations, which have all been made independently, lead to the conclusion that there may well be some genuine underlying linear relation between performance of particular genotypes and environmental conditions, even though it does not always account for all the observed interaction. Attention has thus been paid to the measurement of the environmental response and also to determination of the difference between the responses for different genotypes. For this purpose, the statistical theory of regression has been employed, but unfortunately the fundamental statistical assumptions have not been satisfied in any of the work quoted.

The most striking feature of provenance tests is the generally low sensitivity of populations to changing environments, even with regard to highly adaptive traits such as height growth. Provenances transferred over large distances into very different environments are able to grow and even compete with the native, local populations. This indicates a very high level of individual homeostasis. As a consequence, the phenotypic stability of populations is usually high. Reaction norms of individual populations are usually not well expressed within considerably large geographic areas, and growth differences between adjacent areas are difficult to detect in absence of steep gradients (e.g. mountain slopes). On geographically not fragmented plains the distance between populations with measurable growth differences may exceed 50 to 100 km along ecological gradients.

Stability differences have also been observed among provenances in some coniferous species. Furthermore, stable provenances, seem to concentrate in certain 'stable' areas. While the value of stable populations is very great, the evolutionary causes for their appearance are not clear. Phenotypic stability per se need not be adaptive. However, various theoretical models agree upon the fact that environmental heterogeneity in time favors the evolution of stability by increasing individual homeostasis (Mitton & Grant, 1984; Parsons, 1987; Powers *et al.*, 1991; Scheiner, 1993b). Indications that high climatic instability may be responsible for the evolution of phenotypically stable populations, has been found for some coniferous species (Matyas, 1986).

A method for estimating the genetic value of an individual tree from multiple measurements is described, based on phenotypic residuals from the analysis of co-variance and the within-tree repeatability of a trait. With-tree variance and heterogeneity produced by the environment in the original data were removed by this procedure in most traits. It is concluded that improvement of timber quality by breeding for a wider branch angle is feasible , judging by a standard deviation of 7% among trees, and within-tree repeatability of 0.22-0.36. Branch length and diameter Appeared to be less promising traits for improvement (Magnussen and Yeatman, 1987).

Plasticity for anatomical traits might have important implications for plant taxonomy (Dube and Morisset, 1996). Plant architecture can also vary in response to the environment, as has been shown for the number and length of sylleptic versus proleptic branches in Populus trees (Wu and Stettler, 1998). In herbaceous plants, shading can alter the plant's architecture as a result of effects on meristem initiation and fate as well as organ size and structure (Huber *et al.*, 1999). Studies of architectural plasticity provide useful insight into the specific developmental components of plastic responses (Diggle, 1994) The timing of plant development, including plastic responses to the environment, can itself be plastic. Developmental plasticity might be limited to early stages of the life cycle or might vary in timing among different genotypes, populations or species (Pigliucci and Schlichting, 1995; Bell and Sultan, 1999; Gedroc *et al.*, 1996).

A plasticity studies have extended beyond simple effects on plant growth, researchers have been found that key life-history traits such as sex expression and breeding system, reproductive allocation and phenology can vary in responses to the environment. For example, the proportions of staminate and hermaphroditic flowers in an andromonoecious Solanum were shown to depend on plant resource status, confirming a long-standing ecological hypothesis (Diggle, 1994).

A more surprising finding was that normally self-incompatible plant can switch to self-fertilization in response to floral age and lack of prior fruit development (Vogler, *et al.*, 1998). This plasticity for self-compatibility results in a 'delayed selfing' strategy that

insures reproduction if out crossing fails. This case makes it clear that a plastic switch can occur in response to a plant's internal environment as well as to resource availability or other external cues (Sultan, 2000).

Plastic reproductive timing and allocation have been documented in several herbaceous species (Geber, 1990; Galloway, 1995 ; Pigliucci, 1997). These changes are likely directly to affect plant fitness and therefore population persistence and response to natural selection or example, Mimulus plants flower early in unfavourable conditions, whereas plants in favorable condition delay flowering to allocate more biomass to vegetative growth (Galloway, 1995). A selection experiment confirmed that these contrasting reproductive patterns reflect different fitness priorities in the two types of environment : in poor sites, plants have shorter life spans and maximizing early flower production is advantageous; in favorable sites, where plants live longer, greater allocation to vegetative growth followed by later flowering maximizes fitness (Galloway, 1995).

Plants can respond to environmental conditions not only by adjusting their own phenotypes but also by altering those of their offspring, through changes in the quantity and quality of seed prodvisioning, and in the structure or biochemistry of the seed coat and fruit tissues. Studies of these cross-generational effects rigorously distinguish environmental from genetic causes of offspring variation and focus on ecologically relevant propagule and seedling traits rather than on propagule mass alone (Mazer and Gorchov , 1996 ; Sultan, 1996 ; Lacey, *et al.* 1997 ; Thiede, 1998). Although the mechanisms are not well understood (Lacey, *et al.* (1997). Offspring structure, development and morphology can be influenced in remarkably specific ways by parent environment. Certain species respond to contrasting growth conditions by changing the structure or thickness of the seed coats or pericarps while maintaining the quantity and quality of the embryo and endosperm tissues that determine initial seedling size (Sultan, 1996 ; Lacey *et al.*, 1997)

. The progeny of nutrient-deprive plants can increase root biomass allocation compared with seedling offspring of plants give ample nutrients (Wulf, and Bazzaz, 1992). Similarly, the offspring of light-deprived plants can reduce root extension relative to shoot growth compared with offspring of genetically identical plants grown at high light (Sultan, 1996)). Such specific plastic changes to seedling growth patterns might allow offspring to maintain critical aspects of function such as root uptake capacity even if the initial seedling biomass is reduced by parental resource deprivation. Seedling offspring or nutrient-deprive *Polygonum* plants produced thinner roots that extended downward more rapidly into the soil, resulting in root systems that were as long as seedlings form nutrient-rich parents in spite of their lower mass (Elmendorf and Sultan, unpublished).

Both positive and negative cross-generational effects can alter offspring quality in ways that affect a population's ability to regenerate. Significant effects of parental CO_2 environment on seedling development were found in inbred lines of *Arabidopsis thaliana* raised at ambient and elevated CO_2 levels : the offspring of plants grown at high CO_2 produced shorter and less branched roots, possibly owing to reduced seed size and nitrogen content (Andalo *et al.*, 1998)

Similar negative effects of elevated CO_2 on offspring mass, carbon : nitrogen ration and relative growth rates were found in a study of the annual grass *Bromus rubens* (Huxman, *et al.,* 1998). Plasticity patterns for ecologically important traits often vary genetically with in natural populations, which indicates that the genetic potential for the evolution of adaptive plasticity can exist in many taxa (Sultan, 2000). However, the selective evolution of plasticity in any given population will depend in part on whether the plastic response has high energetic, functional or genetic costs (DeWitt,. *et al.* 1998).

The genetic mechanisms that underlie plastic response are as yet poorly known (Schlichting, and Pigliucci, 1998 ; Pigliucci, and Schmitt, 1999 ; Via, *et al.*, 1995), although it has become clear that several different mechanisms might be involved in different aspects of plasticity (Scheiner, 1993a; Wu, 1998). These mechanisms are believed to include environmentally dependent regulatory loci as well as non-epistatic loci at which allelic expression varies with the environment (Via *et al.*, 1995)

Because plastic responses involve both environmental perception and the production of the appropriate phenotype (which might ential a suit of anatomical, morphological, physiological and other traits), these responses are likely to be influence d by multiple loci (Wu, 1998).

Population differences in both endogenous production and sensitivity to growth hormones might contribute to different patterns of plastic response. For instance, differences in ethylene production and sensitivity influence the ability of alpine *Stellaria longipes* plants to produce a compact habit in harsh conditions (Emery *et al.*, 1994).

Comparative studies at the population and species level are revealing several important connections between individual plasticity and higher-level ecological and evolutionary patterns. Species that consist of highly plastic genotype might be ecological generalists (Sultan, 1995), whereas species whose constituent individual express limited adaptive plasticity might be restricted to narrower, 'specialist' ecological ranges.

In addition to ecological breadth plasticity, might also contribute to a species' invasiveness.Widespread colonizing species are often characterized by high phenotypic plasticity, which should in theory allow them to inhabit diverse new sites without undergoing local genetic adaptation through natural selection. Work on several colonizing species has confirmed that populations across broad geographic and environmental ranges can show remarkably little genetic or morphological differentiation but instead

consist of genetically similar populations of highly plastic genotypes (Williams *et al.*, 1995; Hermanutz and Weaver, 1996)

Thus plasticity might facilitate the rapid spread of introduced as well as native taxa into new ranges without the evolutionary lag time required to adapt to these unfamiliar habitats through natural selection.

Plasticity might also contribute to the ability of species to with-stand sudden environmental changes, such as those caused by human disturbance (Sultan, 2000). Finally, plasticity can influence patterns of evolutionary diversification. If individual genotypes are sufficiently plastic to produce phenotypes appropriate to different local environments, natural selection will not occur for genetically distinct, locally specialized ecotypes (Sultan, 1987, 1995).

2.14. Correlation studies and path analysis:

Correlations between characters have three main causes, namely pleiotropy, linkage and environmental effects (Falconer, 1989). The genetic correlation arising from pleiotropy expresses therefore the extent to which two characters are influenced by the same genes. In old natural populations, which have mated at random for many generations the genetic cause of correlations is mainly pleiotropy. In such populations, linkage, which causes transient correlations, have been broken by recombination (Falconer, 1989). Environmental correlations reflect a similarity or dissimilarity in the response of traits to a common environment (Falconer, 1989; Aastveit & Aastveit, 1993). Knowledge about the sign and magnitude of genetic correlations are important both for understanding the relationship between a quantitative character and fitness in natural populations, and for predictions of correlated responses to selection in breeding programmes (Falconer, 1989; Hebert *et al.*, 1994).

Correlation and path analysis provide useful information for a breeder and assist him to work on those specific traits which can directly or indirectly enhance the desired yield level of a crop. Path coefficient studies for seed and seedling traits are scanty in Neem.

Seed yield is a quantitative character, which is largely influenced by the environment and hence has a low heritability (Johnson, 1989). As a result, the response to direct selection for seed yield may be unpredictable, unless there is good control of environmental variation. Plant breeders are seldom interested in one character and therefore, there is the need to examine the relationships among various characters, especially between seed yield and other characters.

As the number of independent variables influencing a particular dependent variable is increased a certain amount of interdependence is expected. In such situations, correlations may be insufficient to explain the associations in a manner that will enable breeders to decide on either a direct or an indirect selection strategy (Singh & Singh, 1979).

Path-coefficient analysis provides a method of separating direct and indirect effects and measuring the relative importance of the causal factors involved. Several researchers have used this method to assess the importance of the components of yield.

Correlations between quantitative characters are helpful in determining the components of a complex trait, such as yield, they do not provide an exact picture of the relative importance of the component characters. Path coefficient analysis, developed by Wright (1921, 1923), is a standardized partial regression analysis, and as such it measures the direct influence of one variable upon another and permits the separation of the correlation coefficient into components of direct and indirect effects (Dewey & Lu, 1959).

Path analysis has been used to organize and present the causal relationships between predictor variables and response variables through a path diagram that is based on experimental results or on a priori grounds. The advantage of path analysis is that it permits the partitioning of the correlation coefficient into its components – one component being the path coefficient (or standardized partial regression coefficient) that measures the direct effect of a predictor variable upon its response variable; the second component being the indirect effect(s) of predictor variable on the response variable through other predictor variables (Dewey and Lu, 1959). In agriculture, path analysis has been used by plant breeders to assist in identifying traits that are useful as selection criteria to improve crop yield (Dewey and Lu, 1959).

Ashok Kumar and Gurumurthi (1996) conducted path coefficient studies on morphological traits (Ht, Dbh, Diameter at ground level, number of branches in relation to total biomass) in *Casuarina equisetifolia*. Gupta and Patil (1988) studied Genetic variability and path analysis in white popinac (*Leucaena latisiliqua*) for leaf morphology, yield, seedling morphology.

2.15. Divergence studies:

Temporal variation in the environment is generally thought to be less efficient in maintaining genetic polymorphism than spatial variation. However, if there is delayed germination or diapause, insome situations the conditions for genetic polymorphism are greatly broadened in a temporally variable environment (Hedrick, 1995).

The maintenance of genetic polymorphism in heterogeneous environments continues to be a topic of research interest in evolutionary genetics 40 years after Levene (1953), who first gave the conditions for a polymorphism when there is spatial variation in the environment. One conclusion that has been widely accepted over the years is that the conditions for maintenance of polymorphism when there is temporal variation in selection appear to be much more restrictive than those for maintaining variation from

spatial variation in fitness (e.g. Hedrick *et al.*, 1976). Levene (1953) showed that the conditions for temporally varying environments are based on the geometric mean, the latter giving much more stringent limits.

Some organisms may be able to avoid an environment for which they are not adapted when there is temporal variation in the environment because they can exist in a life stage that does not encounter the effects of the environment. For example, some plants have extensive seed pools and the seed that do not germinate do not experience many of the environmental effects encountered by the seed that do germinate. Recently, El ner & Hairston (1994) have extended these ecological models to show that the conditions for maintenance of genetic variation can be greater in fluctuation environments in which there is the opportunity for genotypes to escape unfavourable environments via the storage mechanism. In their recent study, El ner & Hairston (1994) did show that a general model with overlapping generations can maintain genetic variance, given that the variance of selective fluctuations, the generational overlap, and selection intensity are sufficiently high. The importance of genetic diversity has long been appreciated for selection of parents but the basic constraint has been of recognition and estimation of such diversity without making actual cross (Bhatt, 1970). Peeters and Martinelli (1989) opined that principally because of genotype x environment interactions, univariate approaches to exploit quantitative and qualitative data could lack precision, which can be increased either by replicating estimates for a given variable or by increasing number variables. They suggested use of multivariate statistics under such circumstances.

Multivariate analysis is the analysis of observations on several correlated random variables, for a number of individuals. Such analysis becomes necessary when one deals with several variables simultaneously. A series of univariate statistical analysis carried out separately for each of the variable is, in general, not adequate as it ignores the correlation among the variables and may even be misleading sometimes. On the contrary, multivariate analysis can throw light on relationships, interdependence and relative importance of the characters involved and yield more meaningful information (Kshirsagar, 1974).

2.15.1. D^2 Analysis:

Geographical diversity was earlier considered as a criterion for measure of genetic diversity (Dhawan and Singh, 1961; Moll *et al.*, 1962) but it was overruled by Somayajulu *et al.* (1970), Jayaparkash *et al.* (1974) and Chandra (1977). Hutchinson's polygraph (Hutchinson, 1936) and Metroglyph and Index Score analysis (Anderson, 1957) broadly classified the germplasm but they do not provide numerical estimates for precise comparison. Discriminant function of Fisher (1936) is good for selection but the situation becomes difficult with the increase in number of variables.

Mahalanobis (1925) was first to introduce the concept of generalized distance based on second degree statistics. Mahalanobis (1930, 1936) for the first time applied D^2 statistic on extensive measurements of Sweden population. D^2 statistic is a measure of group distance based on multiple characters and it permits precise comparison among all pairs of population alongwith their classification, before affecting actual crosses, in modelling the clusters in a desired genetic architecture. Rao (1952) described it as a measure of actual divergence between any pair of population that amounts to a measure of genetic divergence.

Murty and Pavate (1962) were first to use this approach for the study of divergence. Murty and Arunachalam (1966) and Govil and Murty (1973) hypothesized that Mahalanobis D^2 statistic could be a useful multivariate tool for effective discrimination among parents on the basis of genetic diversity.

Rao (1960) suggested the use of D^2 statistic in genetic problems and this approach was first used by Murty and Pavate (1962) to the study of genetic divergence. They used this technique to form tentative group of varieties of a particular crop according to their diversities from each other and recommended its use for making selection of the parents on the basis of diversity.

Bagchi (1999) conducted studies at the Forest research Institute, Dehradun (Uttar Pradesh) to assess the magnitude of seed-source variations among 27 seed-sources of *Acacia nilotica*, bulk seeds of which were collected from different Indian states. Ten quantitative characters (plant height, clear-bole length, collar diameter, number of branches, first, second and third inter-branch distances and angles of first, second and third branches with reference to the main stem) were recorded in 8-month-old seedlings raised from the different seed sources. The observations were statistically analysed. Application of the generalized D^2-statistic identified 5 cluster groupings among the 27 seed sources, which were designated A-E. Group A contained 21 seed sources, group B2, group C 2, and groups D and E one each. Group A included seed sources from West Bengal, Andhra Pradesh, Uttar Pradesh Haryana, Rajasthan and Karnataka. Seed sources in groups B, C, D and E were all from Rajasthan, indicating that this state contained the extremes in divergence, suggesting not only maximum variability but also that the divergence pattern is not dependent on the geographical distribution. Group B was distantly related to group D, followed (in order of strength of the relationship) by B with C, B with E, D with E, and A with E. Group B and D were the be best for different reasons. Group B had maximum plant height, superior collar diameter, the highest number of branches, lower inter-branch distances and smaller (less wide) branch angles; group D had better tree form, lower height but with the longest clear bole, more than average collar diameter, a lower number of branches, more than average inter-branch distances and widest branch angle. From these characteristics it would be expected that

group B would produce higher biomass and fodder yields, whereas group D would produce increased timber yield.

Vijayan *et al.* (1999) derived information on genetic variance from data on 11 yield components in 70 genotypes. These were grouped into 8 clusters using Mahalanobis D^2 statistic analysis. More than 53 genotypes exhibited very close genetic affinity with each other while the rest showed substantial divergence.

2.15.2. Hierarchical cluster analysis (HCA): HCA allows one to identify groups of objects of variables that are similar among themselves, especially when they are closely related (Sneath & Sokal, 1973).

Peeters and Martinelli (1989) suggested that a multivariate algorithm such as hierarchical cluster analysis has several advantages. First, it allows mixing of both quantitative and qualitative data and, therefore, all the information available on the sample can be utilized. Secondly, each entry is treated as an individual entity of equal weight in analysis, contrary to a number of other multivariate techniques, which are based on variation of groups of entries. This analysis can also define the degree of relatedness in the gene bank samples and can predict the degree of segregation of given samples thus becoming a powerful tool to classify the population with precision.

A difficulty in using this statistic is the choice of algorithm to be used. Sokal (1986) and Rohlf and Wooten (1988) agreed that UPGMA clustering method generally yields results which are the most accurate for classification purposes, although Lebada and Jendrulek (1987) found in their analysis (based on quantitative data ~~based on quantitative data~~ alone) that, for six of the most commonly used procedures, results were nearly identical.

Since accuracy generally increase with increase in number of characters treated (Rohlf and Wooten, 1988 and Peeters and Martinelli, 1989) and some type of data are known to have more weight in multivariate analysis than others (Thorpe, 1985), the choice of characters in addition to algorithm method, also affect the outcome. Romesburg (1990) suggested use of different algorithm methods and resemblance coefficients for different types of data sets. While reviewing the works on cluster analysis, he found that UPGMA was the most frequently used method because researchers considered that it is based on a middle-of-the-road philosophy cast between the two extremes e.g. Complete Linkage Clustering Method (CLINK) and Single Linkage Clustering Method (SLINK) (Romesburg, 1990).

Through a very few examples of hierarchical cluster analysis exist in crop plants and even fewer in Neem, yet they provide fruitful insight in the diversity studies. Eyde (1976) while studying *Stylosanthes* introduction advocated analysis of agronomic databases in a manner similar to those used commonly in numerical taxonomy. Such an analysis was shown to elucidate patterns of variation in those attributes, which are of

economic importance, irrespective of taxonomic or genetic distance measured by molecular, chemical, of other means.

Smith *et al.* (1985a & 1985b) used cluster analysis to illustrate relative genetic distances and genetic diversity in maize germplasm. Lin *et al.* (1986) have shown advantages of using hierarchical cluster analysis in stability analysis over a range of environments.

In D^2 and hierarchical cluster analyses, most of the workers reported no definite association between geographical diversity and genotypic diversity. However, Ayana and Bekele (1999), while studying 415 sorghum genotypes from Ethiopia and Eritrea,found that a greater proportion of accessions of similar adaptation zones and accessions from regions of origin with similar agroclimatic conditions were grouped together. Ezeaku *et al.* (1999) also reported that geographic diversity, although important, was not the only factor responsible for determining genetic divergence. Because of the mathematical simplicity of cluster analysis, many assume that it may be inferior to other, more complex multivariate methods. However, no scientific study has ever shown that "mathematical simplicity" equates to "inferiority" and that the more complex a method is the better it must be.

2.15.3. Principal component and principal factor analysis:

Franco *et al.* (1997) compared the performance of nine different hierarchical and non-hierarchical clustering strategies for classifying accessions of five Mexican maize races. They compared the formation of the a priori groups using several hierarchical clustering methods with the distance measure developed by Gower (1971) and the application of Normix to these a priori groups. Based on five criteria for evaluating clustering strategies, the authors concluded that the application of Normix to the a priori groups after UPGMA (arithmetic average method) (Sokal and Michener, 1958) or after Ward (1963) allows re-allocation of accessions among groups and therefore the formation of homogeneous, compact, and well characterized and separated groups.

The idea of principal component analysis was initially floated by Pearson(1901) and later developed by Hotelling (1933) for his work in educational psychology. It was further described by many workers to solve the complex data matrices by transferring the original set of variables into a smaller set of linear combinations that accounts for most of the variability of the original set (Anderson, 1972; Morrison, 1978 and Dillon and Goldstein, 1984). Out of the many orthogonal rotation methods, Varimax method of Kaiser (1958) is most popular and is often used to rotate principal component solutions.

Though principal component analysis and principal factor analysis have several advantages yet very limited studies had been carried out in crop plants on these aspects. Hamman (1972) found that multivariate techniques could resolve several phenotypic measurements into fewer, more interpretable and more easily visualized dimensions. The

technique also assists in parameterizing the environment variability for maximising the yield (Goldchild and Boyd, 1975). Zobel *et al.* (1988) illustrated the Additive Main Effects and Multiplicative Interaction (AMMI) method for analysis of genotype x environment interactions, which involves a principal component decomposition of genotype x environment deviations and subsequent biplots of component scores of environments and cultivars. This methods was shown to be a more efficient approach for partitioning genotype x environment sum of squares than methods previously developed and the accompanying biplot gives added insight into the nature of interaction.

Observations were made on growth and survival of twenty provenances in the international provenance trials at three sites (optimum, intermediate and stress) in Bangladesh and India. Significant provenance and site effects were observed in the studied traits. A significant genotype x environment interaction was detected for plant height across the three sites in six common provenances. Latitudinal clines were observed for collar diameters and survival rates at two sites in Bangladesh. In a combined analysis of data from the three sites, latitudinal variations were also detected for collar diameters and production percentage. Three stability indices were used to characterize the six common provenances. The PCA on growth and survival data revealed three groups of provenances based on the variations in heights and survival rates at the individual sites. The results suggested that eco-climate attributes played an important role in population differentiation causing G X E interaction (Kundu, 1999).

Li Peng *et al.* (1997a) conducted study on genetic structure and patterns of genetic variations among populations in eastern white spruce (*Picea glauca*). Data were collected on seeding heights at various ages, 1-yr branch number, and 3 yr bud burst and bud set. Significant differences were found for each trait among provenances and among families within provenances. Provenances and families within provenances accounted for similar amounts of the genetic variability. All traits were moderately to strongly inter-correlated at provenance level. Two Principal components composed 87% of the total variation for all traits. Regression models explained between 19 and 65% (an average of 47 %) Of provenance variation and showed that patterns of provenance variation mainly followed a south-north cline and to a lesser extent a west- east cline.

Romisondo and Malusa (1992) on the basis of data on bole diameter, growth, yield efficiency, ripening index, fruit weight and diameter, and soluble solids collected from more than 90 seedlings of *Prunus serotina,* Principal component analysis identified 12 seedlings promising for yield and fruit quality; 2 of these (accessions 66 and 73) could be used directly for experimental planting.

Lopez Mata (1987) studied genecological differentiation in nine provenances of *Brosimum alicastrum* – a tree of moist tropical forests. Seeds were randomly collected from 5-11 individuals of each provenance along a latitudinal gradients. Principal component analysis and linear correlation indicated the existence of ecoclinal variation in seed size (weight and diameter). Seed size strongly influenced initial seedling size and the relative size of seedling roots which increased in response to habitat dryness and geographic origin. The results suggested that *Brosimum alicastrum* employs an adaptive strategy in response to water deficit during dry seasons in initial phase of seedling establishment.

Parker (1984) collected data on seed cone characters, needle morphology and needle flavanoids collected from 16 natural stands of *Abies lasiocarpa* and analysed the data by principal components analysis and canonical variates analysis. The cone data showed no geographical patterning, but eastern and western populations were segregated by both needle morphology and flavanoid analyses. It was concluded that a variety of traits should be considered when discussing the taxonomy of *Abies*.

2.16. Biochemical and Molecular markers:

The ontogenic and environmental stability of isozymes makes them an ideal tool in tree improvement program. The application of isoenzymes in order to control the efficiency of tree improvement activities is described in the following cases: clone identification; controlling the validity of controlled crosses; controlling the genetic efficiency of seed orchards; and origin reconstruction. On the basis of isozymatic studies, the results indicate high variation within the natural population and low variation within the introduced and ornamental ones in *Cupressus sempervirens* L.. The same tendency was observed for the degree of heterozygosity as well. A genetic distance of 0.214 clearly separated the natural from the artificial group (Papageorgiou, 1998).

The tree breeder commonly needs to check the progress of his breeding strategy and the genetic composition of his breeding populations. Genetic markers are necessary for

this purpose; these may include visible, physiological genetic mutants or they can be biochemical (Burley, 1976).

The seed sources for both seeds ad plantations are difficult to identify from morphological and physiological traits. However, some chemicals that are under rigid genetical control may be useful, particularly terpenes and enzymes(Burley, 1976).

Several techniques are now available for the rapid determination of identities and quantities of chemical compounds. These are often applicable to the evaluation of differences between populations of trees. In many cases, evidence of inter- and intra-population variation may be obtained at an early stage of species and provenance research (Burley, 1976).

Chemical and spectrographic analysis of mineral nutrients in foliage have frequently shown inter-provenance differences in nutrient concentrations. Generally the importance of mineral nutrient analysis lies in the evaluation of deficiencies of soil nutrients. However, in evaluating such soil deficiencies it is essential to remember the possibility of inter-provenance differences in the relationship between foliage nutrients and tree growth parameters (i.e., efficiency) (Burley, 1976).

The ~~are usually~~ compounds with low molecular weights, ~~they~~ take part in fundamental metabolism and are not normally stored. Examples are the protein amino acids and simple sugars. Inter-provenance differences in seeds have been found to be associated with climate of seed source in some species but large variations occur in different parts of the plant(Burley, 1976).

Other compounds also have relatively low molecular weights, but unlike the primary metabolites, they are often accumulated either in living cells or else deposited in specialised ducts (e.g., coniferous resins ducts) or dead tissues (e.g., bark and heartwood). They are frequently termed as "waste products" or "metabolic by-products"; whilst some probably do represent the terminal product of particular metabolic pathways, others ma b intermediates in unknown or poorly understood pathways, others may be intermediates in unknown or poorly understood pathways. Examples include lipids, alkanes, waxes, alkaloids, terpenoids, polyols, and polyphenols. Most work on intra-specific variation of trees has been concentrated on terpenes, particularly coniferous monoterpenes.

Two broad groups of macromolecules can be recognised. The first type (monomer and linkage invariant) is characterised by compounds such as cellulose and chitin in which monomeric components are identical in different organisms. The second type (monomer and/or linkage variable) is represented by proteins and nucleic acids which are polymers of a limited number of monomers that show a considerable range of variation.

Seed and leaf proteins, particularly isoenzymes, differ between populations. Since they give a direct indication of an individual's genotype, they are very promising for studies of generic variation at the population and individual level, and for seed source identification. The description of multiple molecular forms of many enzymes (isoenzymes), reflecting allelic variants of given loci, has been largely responsible for the rapid advance in the use of protein characteristics in taxonomic and genetic investigations. Serological techniques have also been used with tree sp. to assess relatedness between antigenic preparations of different populations.

As a result of widespread deforestation and over exploitation, many tree species have recently become the focus of increasing conservation concern. A recent survey lists around 9000 tree species as threatened with extinction worldwide, including more than half of the world's 600 conifer species (Oldfield *et al.*, 1998). Such concerns have added impetus to the study of genetic variation within tree species. The extent and distribution of genetic variation with a species are of fundamental importance to its evolutionary potential, and determine its chances of survival (Holsinger and Gottlieb, 1991). Assessments of genetic variation are therefore of key importance to the development of effective conservation strategies.

Hamrick *et al.* (1992) highlighted limitations of molecular-marker aided selection in forest tree breeding. He reviewed woody plant electrophoretic data in order to compare genetic diversity in woody species with species representing other life forms, and to investigate whether the levels and distribution of genetic diversity in woody species were related to life history and ecological characteristics. Data from 322 woody taxa were used to measure genetic diversity within species and within and among populations of species. Woody species maintained more variation within species and within populations than species with other life forms but had less variation among populations. Woody species with large geographic ranges, outcrossing breeding systems, and wind or animal-ingested seed dispersal had more genetic diversity within species and populations but less variation among populations than woody species with other combinations of traits. Although life history and ecological traits explained a significant proportion (34%) of the variation among species for the genetic parameters measured, a large proportion of the interspecific variation was unexplained. The specific evolutionary history of each species must play an important role in determining the level and distribution of genetic diversity.

Conkle (1992) reviewed the use of biochemical markers from individual trees as a means to resolve and measure genetic diversity. Identifying variation and interpreting such genotypic information will lead to effective means for managing populations to maintain forest health.

Relatively few studies of intra-specific variation using DNA techniques have been undertaken in tropical tree species. Those investigations that have been completed have again highlighted the existence of pronounced genetic structure within several species. Spanish cedar (*Cedrela odorata*) is a high value timber species native to the American tropics and is now considered to be threatened in many parts of its range. Analysis of ten populations from Costa Rica using RAPDs indicated that 55% of the total variation recorded was maintained between rather than within populations (Gillies *et al.*, 1997). This finding reflected a pronounced difference between populations originating from the Pacific and Atlantic regions of Costa Rica. The reason for this discontinuity between adjacent populations is unknown, but might reflect the colonization of these regions from different source populations, or possibly an adaptive response to contrasting edaphic conditions (Gillies *et al.*, 1997).

Studies of the leguminous tree *Gliricidia sepium*, another meso-American species, have also recorded a relatively high degree of population differentiation. In a subsequent survey of eight populations using RAPDs, 60% of the variation recorded was between rather than within populations (Chalmers *et al.*, 1992). Further RAPD and mtDNA assessments of *G. sepium* indicated that a significant degree of differentiation also existed at the sub-population level (Dawson *et al.*, 1995). In contrast, Schierenbeck *et al.* (1997) recorded very little genetic variation between populations of four tropical tree species assessed at a local scale in the Atlantic region of Costa Rica. In this study, RAPDs were used to assess genetic variation along an elevational gradient in species occurring at contrasting population densities. The lack of differentiation at this scale suggests that pollen and seed dispersal occur over distances of several kilometers in this rain forest area (Schierenbeck *et al.*, 1997).

In natural populations, variance components are also of considerable interest for evolutionary studies (Lande, 1982) and, increasingly, for conservation purposes (Storfer, 1996). In natural populations, however, information on relationships may be unreliable or unavailable. Molecular marker information [restriction fragment length polymorphism (RFLP), minisatellites, microsatellites, RAPDs etc.] from the population of interest provides a means of circumventing this problem, by allowing estimation of relationship on a pair-wise basis (Thompson, 1975; Ritland, 1996a) without the need for pedigree reconstruction. These estimates of relationships may be combined with phenotypic information gathered from the same individuals, allowing inferences to be made about variance components (Ritland, 1996b).

Loveless (1992) reviewed data from 97 isoenzyme studies on the genetics of tropical woody species which demonstrated that cultivated taxa maintain higher percentages of polymorphic loci and higher mean heterozygosities than native tropical species. Levels of within-population variation in tropical taxa are as high or higher than in plants in general.

Levels of genetic variation differ significantly among species with different geographic ranges, life forms, and taxonomic affinities. Levels of population differentiation are significantly different only between species with different seed dispersal modes. Outcrossing rates in 16 tropical tree species showed a preponderance of highly out-crossed breeding systems.

Changtragoon *et al.* (1996) studied genetic diversity of *Azadirachta* spp. by using isozyme analysis. Ten putative isozyme gene loci were identified and used for measuring genetic diversity in three Azadirachta species. The average genetic distance between populations of *A. indica* var. *siamensis* was low (0.90). on the other hand, the three taxa were separated by a very high genetic distance. As expected cluster analysis showed strong genetic divergence among the investigated taxa. These results confirm genetic distinctiveness of *A. siamensis*. However additional studies are needed for evaluation of the taxonomic status of this taxon.

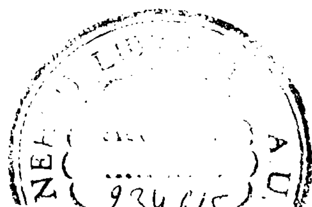
Kundu (1999) performed comparative analysis of seed morphometric and allozyme data among four populations of neem. The objectives of this study were to determine the patterns of variability and to compare genetic distance between seed morphometric and allozyme data for neem. Phenotypic variation from open pollinated seed materials at 12 isoenzyme loci was examined using starch gel electrophoresis for four populations from Thailand, Bangladesh and Korea. All four populations exhibited high levels of variation for seed parameters and isoenzymes. UPGMA cluster analysis of Nei's genetic distance when based on isoenzymes revealed two genetic groups. The population from Kenya showed a close genetic relationship with the Bangladeshi populations. The population from Thailand exhibited a distant relationship with the other three populations. This result suggests that the Kenyan population was introduced from the Indian subcontinent. In contrast, cluster analysis of seed morphometric data indicated three different groups of populations. The population from Kenya showed a distant relationship with all other populations. The study demonstrates that allozyme data may not correspond with morphometric traits when measuring genetic distance. It is suggested that this controversy may depend on environmental influence on morphometric traits, possibly also on the enzyme patterns.

Singh *et al.* (1999) worked on assessment of genetic diversity in *Azadirachta indica* using AFLP markers. Genetic diversity was estimated in 37 neem accessions from different eco-geographic regions of India and four exotic lines from Thailand using AFLP markers. Seven AFLP selective primers combinations generated a total of 422 amplification products. The average number of scorable fragments was 60 per experiment, and a high degree (69.8%) of polymorphism was obtained per assay with values ranging from 58% to 83.8%. Several rare and accession-specific bands were identified which could be effectively used to distinguish the different genotypes.

Nuzhat Farooqui *et al.*(1998) reported RAPD profile variation amongst provenances of neem. Neem (*Azadirachta indica*) is an evergreen, long-lived, multipurpose tree of the tropics with a wide distribution range in India. It is believed to be highly cross-pollinated. Inter-provenance variation has been reported in neem for a range of morphological and physiological characters. Yet no reports about the genetic determinism for this variation are available. RAPD profiles of 34 accessions/provenances of neem were generated with 200 decamer random primers, of which 49 resulted in reproducible amplification products. Based on the presence/absence of bands, a similarity matrix was computed. Dendrogram was constructed by UPGMA method based on the pairwise similarities amongst the profiles. The similarities in RAPD profiles amongst the different accessions was more than that expected due to the outcrossing nature of neem and, furthermore, these more-than-expected similarities were not due to random chance. These results suggest that neem may have a narrow genetic base.

Polymerase chain reaction (PCR) techniques were developed in late 1980's (Saiki *et al.*, 1985) and have been applied widely in genetic identification of biological samples. PCR requires only a small about of DNA, which is amplified to produce a specific marker profile for identification of the sample (Welsh and McClelland, 1990). Random amplified polymorphic DNA (RAPD) assays utilise arbitrary 10-mer oligonucleotide sequences as primers (Williams *et al.*, 1990). Primers hybridise to two nearby sites in the template DNA that are complementary to the primer sequence. Deletions or insertions in the amplified region or base changes alternating primer binding sites will result in polymorphism.

Strauss *et al. (1992)*:The advances to date with quantitative trait locus identification in agronomic crops, which have mostly been with studies of inter- and intra-specific hybrids, are of little relevance to assessing the potential for marker-aided selection in non-hybrid forest tree populations. Although molecular markers provide great opportunities for dissection of quantitative traits in experimental populations, it is suggested that their near-term usefulness in most operational tree breeding programmes will be limited. In addition to cost, this limitation results from quantitative trait locus-marker associations being limited to specific genetic backgrounds as a result of linkage equilibrium, interactions of quantitative trait locus effects with genetic backgrounds, genotype by environment interaction, and changes of quantitative trait locus allele frequencies among generations. Only where large restrictions of genetic base are tolerated, trait heritabilities are low, markers are able to explain much of the additive variance, selection intensities within families are high compared with that among families, and very large numbers of progeny are examined, can marker-aided selection within individually mapped full-sib families substantially aid phenotypic selection. Broad use of marker-aided selection in the longer term will require substantial technical



advances in a number of areas, including: means of precise quantitative trait locus identification; reduction of large-scale mapping and genotyping costs; and changes in breeding and propagation systems.

Kremer (1994): A comparison is made between parameters referring to genetic markers (allelic richness, gene diversity and genetic differentiation coefficient) and those addressing quantitative characters (heritability, coefficient of genetic variation and differentiation). Definitions and relationships between the different parameters are outlined. It is shown that forest trees exhibit higher levels of gene diversity than other plants. Populations are highly differentiated for traits related to growth and phenology, whereas allelic frequencies of gene markers show low variation among populations.

Determinants of genetic structuring in plant population include mating system (Loveless & Hamrick, 1984) natural (and possibility artificial) selection, evolutionary history, life- history characteristics and mechanism of gene flow (Schaal, 1980; Ellstrand *et al.*, 1989; ~~Hamrick & Godt, 1989~~; Hamrick *et al.*, 1992). Together, these factors can lead to complex genetic structuring within populations which is often difficult to resolve. The use of biochemical and molecular markers can enhance under standing of such complexities. Amplified polymorphic fragments are normally inherited in a biparental dominant Mendelian manner (Carlson *et al.*, 1991).

The various statistics used to estimate and partition genetic variation at a detailed level within plant population cannot be applied easily to RAPD data obtained from outcrossing species because of dominance. Codominant products are occasionally revealed (at one locus in the present study), but these are rare (Williams *et al.*, 1990). Despite this, approximate estimators have been devised recently for various population-genetic parameters using RAPDs (Lynch & Milligan, 1994), although these rely on an assumption of Hardy Weinberg equilibrium. As individual *Gliricidia* half-sib families do not approach this equilibrium, further approximations regarding the inter-relationship of RAPD product frequency and underlying genetic variation are required and a relatively large sample of RAPDs must be scored to provide an effective description of population structure. In a group of randomly mating, outcrossing individuals the theoretical expectation for genetic partitioning within and among half- sib families is 75 percent and 25 percent, respectively (Hartl, 1987).

Chalmers *et al.* (1994):RAPD analysis detected intra-and interspecific polymorphism in 29 mahogany accessions from 8 species and 4 genera originating from West Africa and Tropical America. There was a clear separation of *Cedrela odorata* from the other species, with 95% of the variable amplification products differing, whereas *Lovoa trichilioides*, *Khaya* spp. and *Swietenia spp.* were more closely grouped. These results are consistent with current taxonomic evidence based on morphological characteristics.

Although very recently developed and open to such critical appraisal, the RAPD technique has already shown some promise as a tool for the study of genetic diversity within and between populations. The important tropical genus *Gliricidia* has been studied by Chalmers *et al.* (1992), who were able to partition the variation into within- and between-population components for two species. A range of individual accessions of *Theobroma cacao* has been successfully fingerprinted using only a small number of primers (Wilde *et al.*, 1992); this would have been difficult using RFLPs owing to difficulties of DNA extraction with this species. In *Eucalyptus,* where the extraction of high quality DNA is again a major problem for RFLP analysis, RAPD markers can be used for clonal identification (Clerk,1992), even using very young material.

Individuals within a full-sib family of *Picea glauca* were distinguished by Hong *et al.* (1992), who showed that the number of RAPD markers can be maximised by using polyacrylamide gel electrophoresis followed by silver staining of DNA, rather than agarose gels and ethidium bromide. Using a single 10-nucleotide primer, at least 20 polymorphic markers were identified, more than twice the number observed with agarose gels. They demonstrated the application of RAPDs to paternity analysis by comparing mega-gametophyte and embryo from the same seeds and thus identifying the paternally-derived amplification products; for confirmation the genotypes of the paternal trees were also analysed. The same authors are also carrying out a RAPD's-based survey of genetic diversity in British Columbian Douglas-fir populations; between 8 and 20 segregating genetic markers can be identified from each primer screened.

RAPD markers are currently being used in the construction of genome maps in a variety of species of commercial importance in agronomy and forestry, with the advantage over RFLPs of speed and higher genome saturation. They are well suited for mapping haploid genotypes as in conifer mega-gametophyte DNA. In diploid organisms two closely-linked RAPD markers, each amplified from a different parent, may be used as a pair to identify heterozygous regions as described by Williams *et al.* (1992). Alternatively, differences in band intensity can be measured densitometrically to distinguish bands from heterozygous and homozygous regions, although it is pertinent to add that the reliability of this procedure has not yet been adequately substantiated.

Fingerprinting reliability through RAPD has been questioned since comigrating bands from different individuals do not necessarily represent homologous amplification products, but the presence of comigrating bands only reduces the absolute similarities, not the relative similarities nor the relationships among clusters (Adams and Rieseber, 1998). Hence fragment size and frequency can be considered a reliable predictor of homology of closely related individuals (Nicese *et al.*, 1998), provided that they can differentiate between at least two populations.

Lu *et al.* (1997) reported RAPD variation in 10 northern Swedish populations of P. sylvestris was characterized by isolating 10 polymorphic and 10 mono-morphic RAPD fragments for use as probes in Southern analysis of RAPD profiles and genomic DNA digests. Results showed that: (i) individual RAPD bands often contained more than one non-homologous DNA sequence, although one sequence usually predominated; (2) Southern hybridization revealed that the presence/absence polymorphism of RAPD bands was due to the lack of amplification of the corresponding sequence rather than to the absence of the target sequence in the individuals lacking the band; (3) in spite of the large genome in conifers, most of the analysed RAPD fragments did not appear to originate from repetitive sequences. Of the 20 RAPD fragments tested, only one appeared to be of repetitive origin. Implications of the observed molecular properties of RAPD variation for genetic analysis in P. sylvestris are discussed.

Mamta Goswami and Ranade (1999): Genetic variability among accessions of Prosopis was determined using randomly amplified polymorphic DNA (RAPD) profiles. Similarities of profiles were determined using the algorithm of Jaccard and UPGMA and neighbour joining trees were generated from the similarity data.

Kanetani (1997) reported RAPD analysis performed to reveal genetic variation and differentiation within and among 6 populations in Kyushu, Japan. As a result of primers screening, forty primers were selected. Some 92 putative polymorphic loci were obtained.

Skov . (1999): The widespread application of RAPD-markers in diverse organisms has highlighted a need to demonstrate the repeatability of these markers across different laboratories, using deviating protocols, as well as across different genetic material within Picea abies. The present investigation has (1) compared the segregation of RAPD-markers in the haploid mega-gametophytes of the same tree analysed at two different laboratories and (2) compared the homozygosity of two geographical diverse individuals.

2.17. Tissue culture studies:

Neem is a tropical tree native to the Indian sub-continent and Mayanmar. Now it is widely distributed throughout tropical and subtropical Asia, Africa, Australia and central and south America and has adapted to diverse climatic and edaphic conditions. It has been extensively studied and now being exploited commercially for its pesticidal and medicinal properties (Anon., 1985). Conventionally neem is propagated by seeds. Due to their recalcitrant nature, seeds require immediate sowing and generally germplasm exchange and breeding programmes suffer badly. Moreover as reports are available on elite genotypes/ecotypes with higher contents of oil and azadirachtin and superior insecticidal efficiency during bioassays(Singh, 1987; Ermel, 1995; Ermel *et al.*, 1998; Kumar and Parmar, 1996; Kaura *et al.*, 1998c), availability of an efficient

micropropagation tool will be helpful in multiplying the desirable genotypes.

Among the various *in vitro* methods, somatic embryogenesis is the method of choice, because of certain advantages viz., probable single-cell origin of the regenerants, a very high multiplication rate, and possibility of adaptation to system artificial or synthetic seeds.

Currently only three published reports are available on somatic embryogenesis in neem i.e., Shrikhande (1993), Su *et al.* (1998) and Murthy and Saxena (1998). Other reports on *in vitro* regeneration in neem pertain to shoot proliferation from nodal explants (Yaseen 1994), and decapitated seedlings (Rangaswamy and Promila, 1972), Shoot and root formation from anther derived callus (Guatam *et al.*, 1993), and Agrobacterium mediated genetic transformation attempts (Naina *et al.* 1989).

Plant tissue culture techniques are also available for obtaining callus growth and plantlet regeneration from a variety of explants including leaf, cotyledon and stem segments (Rao *et al.*, 1988; Ramesh and Padhya, 1990; Narayan and Jaiswal, 1985; Kearney *et al.*, 1994).

The present study was designed to see the efficiency of mature seeds for shoot regeneration / somatic embryogenesis in different media and also establish callus and suspension cultures to explore the possibility of *in vitro* production of important commercial metabolites.

Mass propagation of neem on a commercial scale has limitations of seed viability. Moreover, vegetative propagation of this tree using conventional methods is not possible (Narayan and Jaiswal, 1985). Tissue culture offers a reliable method for mass production of plants in a shorter time and without seasonal constraints. The present study was designed to see the efficiency mature seeds for shoot regeneration / somatic embryogenesis in different media and also establish callus and suspension cultures to explore the possibility of *in vitro* production of important commercial metabolites.

Tissue culture studies were under taken by National Chemicals Laboratory (NCL), Pune, India for propagation of neem *(Azadirachta indica)* using different pathways like micropropagation, direct and indirect induction of somatic embryogenesis and induction of secondary metabolites from callus cultures. (DSIR, 1999).

Jaiswal and Narayan (1984) reported regeneration of plantlets from stem tissue of *Azadirachta indica* Juss.. Experiments were undertaken to develop a tissue culture method for large-scale clonal multiplication of Neem stem segments. Stem segments,1 cm long,were taken from mature branches of the tree and

inoculated onto Murashige and Skoog medium supplemented with different growth regulators at different concentrations and combinations. Growth regulators used were indoleacetic acid (IAA), naphthaleneacetic acid (NAA), 2,4-D, benzyladenine and kinetin. Cultures were maintained at 25°C with a 12 hr photoperiod. Callus was induced successfully on medium containing 0.5 ppm kinetin or benzyladenine. Shoot buds differentiated when 0.05-0.1 ppm IAA or NAA were added to the same medium. Root induction also occurred on this medium, and in medium without kinetin or benzyladenine. 2,4-D was inhibitory for organogenesis.

Joshi and Thengane (1996) reported *in vitro* propagation of neem by shoot proliferation. Clonal propagation of neem from non-woody branch explants (from 2-5 yr old trees) was achieved on MS medium supplemented with 6-benzylaminopurine [benzyladenine] at 0.5 mg/litre and kinetin at 1.0 mg/litre. Two to three shoots per explant were observed after 20 days. An increase in multiplication frequency of shoots per explant (45-50) and average shoot length (4.7 cm) was observed after 14-16 weeks on medium containing 3/4 dilution of MS macronutrients, full strength MS micronutrients, kinetin (0.05 mg/litre), benzyladenine (0.1 mg/litre), calcium pantothenate and biotin (both 0.05 mg/litre).

Rier and Obasi (1996) reported organogenesis and oil production in neem seed callus. Callus initiated from germinating seeds of *Azadirachta indica* was maintained on a modified Murashige and Skoog medium. Zypman *et al.* (1997) studied tissue culture methods and cloning of the neem tree (*Azadirachta indica*) for bioinsecticide production. Light yellow friable callus and shoots were initiated from hypocotyl explants of *A. indica* on MS (Murashige and Skoog) medium supplemented with 0.5 mg IAA and 1 mg benzyl aminopurine [benzyladenine] (BA)/litre. Shoots were regenerated from nodal buds and calli. Eeswara *et al.* (1997b) developed a standard procedure for the micropropagation of the neem tree (*Azadirachta indica* A. Juss.). Micropropagated shoots were initiated from leaf explants of *Azadirachta indica*, taken from potted plants (6- or 18-months old) grown from seed.

Venkateswarlu *et al.*(1998) worked on micro-propagation of plus neem (*Azadirachta indica* A. Juss.) and evaluation of field transferred plants. A micropropagation protocol for a mature plus tree of neem has been standardized. Nodal explants were collected from actively growing young shoots of a 22 -yr-old plus tree in Andhra Pradesh (India) with a straight stem, 50 kg seed yield/year, 25% oil in seeds and 0.6% azadirachtin in the kernels. Successful shoot bud induction and multiplication could be achieved on MS medium with suitable concentrations of cytokinin.

Yaseen (1994) studied shoot proliferation and plant formation from neem (*Azadirachta indica*) with thidiazuron. Neem tree (*Azadirachta indica*) is one of the most promising plants for producing pesticides, pharmaceuticals and other commonplace materials. A protocol for shoot formation from node and stem explants

was described. Stem nodes and stem segments were obtained from a mature tree and cultured in Murashige-Skoog (MS) medium supplemented with 0.5 µM thidiazuron (TDZ) and 0.5 µM naphthaleneacetic acid (NAA).

Tawfik (1997) reported micropropagation and plant regeneration of neem tree (*Azadirachta indica* Juss.). A micropropagation system was developed to overcome the difficulty in conventional propagation techniques used for the neem tree (*Azadirachta indica*). Four types of explants (shoot-tips, stem nodes, petiole and leaf segments) were used. Shoot tips and stem explants were cultured on woody plant medium (WPM) supplemented with kinetin and benzyladenine (BA) each at 0, 0.5, and 1 mg/litre.

Gautam *et al.* (1993) studied development of shoots and roots in anther-derived callus of *Neem* - a medicinal tree. Anthers from surface-sterilized flower buds of neem tree containing uninucleate microspores were grown on NB (Nitsch) and Murashige-Skoog (MS) culture media containing 3% sucrose, 0.8% agar and various combinations and concentrations of plant growth factors (PGFs). Best callusing occurred on NB medium with 10 µM indoleacetic acid (IAA) plus 1 µM benzyladenine (BA).

Wewetzer (1998) studied callus cultures of *Azadirachta indica* and their potential for the production of azadirachtin. The content of azadirachtin in neem tree (*Azadirachta indica*) seeds is affected by environmental and genetic factors. An *in vitro* culture study investigated if and how azadirachtin contents of callus cultures derived from various cell lines are affected by different nutrient media and carbohydrate concentrations. The azadirachtin contents of the calluses were analysed by TLC and HPLC. Azadirachtin contents of callus cultures varied depending on the cell line, the nutrient medium and the carbohydrate source employed.

Murthy and Saxena (1998) reported somatic embryogenesis and plant regeneration of neem. Somatic embryos were initiated from mature seeds of neem (*Azadirachta indica*) when cultured on Murashige and Skoog's medium supplemented with thidiazuron (TDZ). Regeneration occurred via somatic embryogenesis, direct embryo formation and through an intermediary callus phase.

George and Kulkarni (1997) reported micropropagation of neem. Explants of leaflets, rachis, inflorescence axis and cotyledons from locally growing trees in Maharashtra callused with high frequency in an *in vitro* culture medium of MS + 5% (V/V) coconut water + 2 µM BAP [benzyladenine] in 2-3.5 weeks, and differentiated into shoots in 2.5-5.5 months.

Shrikhande *et al.* (1993) studied somatic embryogenesis and plant regeneration in *Neem*. Culture medium for induction of somatic embryogenesis from immature cotyledonary tissues of neem was optimized. Immature cotyledons were cut in half and grown at 24-26 deg in the dark for 20 days in Murashige-Skoog (MS) medium (pH 5.7) solidified with 0.4% agar with MS vitamins, 1.0 g/l casein hydrolyzate, 5% sucrose and

0.5-5.0 mg/l indoleacetic acid (IAA), alone and in combination with 1.0 mg/l benzyladenine (BA) or 1.0 mg/l kinetin. Callus was initiated on medium containing 0.5 mg/l IAA, 1.0 mg/l BA and 1.0 g/l casein hydrolyzate.

Gautam *et al.* (1991) studied differentiation in long-term callus cultures of *Neem*. Neem tree (*Azadirachta indica*) is a tree of medicinal value, belonging to the Meliaceae, and is a source of insecticides and alkaloids. Its anthers, containing uninucleate pollen grains, developed callus masses when cultured on Murashige-Skoog (MS) culture medium supplemented with the following: 1.86 mg/l naphthaleneacetic acid (NAA) and 0.21 mg/l kinetin (Kn); 1.86 mg/l NAA and 0.22 mg/l benzyladenine (BA); and 1.75 mg/l IAA and 0.22 mg/l BA.

Ramesh and Padhya (1990) studied *in vitro* propagation of neem, *Azadirachta indica* A. Juss., from leaf discs. Excised leaf disks of *Azadirachta indica* A. Juss. were grown on Wood and Braun's culture medium supplemented with various concentrations and combinations of kinetin, benzyladenine, adenine sulfate, indoleacetic acid (IAA), indolebutyric acid, 2,4-D, naphthaleneacetic acid or gibberellin at pH 5.8 and 24-26 deg under a 16 hr photoperiod.

Islam *et al.* (1996) reported high frequency somatic embryogenesis achieved from cotyledon explants in MS medium containing different concentrations of NAA. The embryoids developed into shoots when the embryogenic calli were transferred to cytokinin-supplemented media. However, the frequency of embryogenesis was very low. ~~A high concentration of BA (0.5-2.5 mgl^{-1}) of NAA induced cotyledon and nucellus explants to produce adventitious shoots. Shoot organogenesis best occurred when 2 mgl^{-1} NAA were used.~~

Joarder *et al.* (1996) developed a micropropagation method using nodal explants from field-grown adult *Neem* tree. The explants were stabilised by culturing two weeks in hormone-free MS medium followed by four weeks in cytokinin supplemented-medium. Established explants upon transfer to medium containing low concentration (0.05-0.3 mgl^{-1}) of cytokinin produced shoots.

Preetha *et al.* (1995) studied Peroxidase isoenzymes as markers of organogenesis in *Azadirachta indica*. Isoenzymes of peroxidase were used as biochemical markers for differentiating regenerating callus lines in the early stages of differentiation in *Azadirachta indica*. The methods of callus induction, sample preparation and electrophoresis are given. The isoenzymes characteristic for various types of callus are discussed in detail.

Joshi and Thengane (1996) achieved induction of somatic embryos directly on explant tissue (cotyledons) or from the callus. Type of auxin was found to be a deciding factor for embryo induction pathway. Adventitious multiple shoots (10-15) were induced in excised axillary and terminal buds of neem on medium containing BAP, kinetin and

sucrose. The individual shoots were transferred to sand:soil (1:1) mixture for direct rooting and grown in polyhouse, where 90 per cent survival was observed.

Callus production has been obtained from adult embryos (Rangaswamy and Promila, 1972), young seedlings (Naina *et al.*, 1989), leaves, cotyledons, and petioles (Narayan and Jaiswal, 1985; Rao *et al.*, 1990; Sanyal *et al.*, 1983; Schulz, 1983), stem segments (Jaiswal and Narayan, 1984) and stem bark segments (Sanyal *et al.*, 1981). Occurrence of genetic off-types is well documented in many other species when regenerated from callus cultures (Larkin and Scowcroft, 1981).

Su *et al.* (1997) were able to induce somatic embryogenesis in *Azadirachta indica*. A modified culture protocol was developed for the induction of somatic embryogenesis in *Azadirachta indica*. Embryogenic calli were initiated from cotyledons or hypocotyls using an MS agar medium supplemented with 0.5 mg NAA, 1 mg 6-benzylaminopurine [benzyladenine] (BA), 1 g casein hydrolysate, and 50 g sucrose per litre.

Dumet *et al.* (1997) reported desiccation tolerance and cryopreservation of embryonic axes of recalcitrant species. Because recalcitrant seeds are generally large and show very curtailed longevity even if stored hydrated, cryopreservation of their embryonic axes seems to be the most obvious way to create a gene bank. The aim of this investigation was to determine the minimal water content tolerated by embryos of 5 recalcitrant species and their subsequent survival after cryopreservation.

Tissue culture of neem was first reported in the 1970s (Rangaswamy and Promilla, 1972; Sanyal *et al.*,1981). Since then, several papers have been published on the effect of various growth factors on the differentiation processes in neem tissue cultures and about secondary metabolites, notably nimbin (Schulz, 1983; Sarkar and Datta, 1986; Rao *et al.*, 1988; Sanyal *et al.*, 1988; Ramesh and Padhya, 1988, 1996).

Drew (1996) developed a technique for production of micro-cuttings *in vitro* for clonal propagation of neem line with high yields of azadirachtin. Apically dominant shoots were grown on hormone free MS medium and multiplied by dissection into nodal sections. Roots were initiated on axillary shoots removed from nodal sections when cultured on MS medium plus 10 μM IBA for four days with subsequent root development on hormone-free medium.

Thiagarajan and Murali (1996) reported high frequency regeneration of neem plantlets from excised embryos, with intact cotyledon. The embryos were cultured in basal MS medium supplemented with various combinations of sugars, nitrates, cytokinins and auxins. Nirmalakumari *et al.* (1996) conducted experiments to study the *in vitro* response of various explants viz., leaf, stem, bark, and cotyledon for callus induction; and shoot tip and cotyledons for micropropagation on MS medium with different levels of auxins and cytokinins. The leaf explants produced nodular and friable calli on MS + 2, 4-D (2 mgl^{-1}) + kinetin (0.5mgl^{-1}).

Yousef and Fattah (1999) studied propagation of neem plant by tissue culture. This study was conducted to propagate neem plant *in vitro* using apical and lateral buds. The buds were cultured on a medium supplemented with three concentrations of BA (0.25, 0.5 and 1.0 mg/litre) and two concentrations(0.1 and 0.25 mg/litre) of IBA. The shoots were rooted in a medium supplemented with 0, 0.5 and 1.0 mg IBA/litre.

Zounos *et al.* (1999) worked on bioactive compounds from neem tissue cultures and screening against insects. Hairy root cultures were derived from neem (*Azadirachta indica*) using Agrobacterium rhizogenes and studied for the production of compounds with antifeedant effects on insects. Six-week-old hairy root cultures were extracted, and HPLC yielded fractions ranging from polar tonon-polar compounds.

Naina *et al.* (1989) reported genetic transformation and regeneration of transgenic neem (*Azadirachta indica*) plants using Agrobacterium tumefaciens. The transformation and regeneration of transgenic neem using Agrobacterium tumefaciens is discussed. Seedlings were inoculated using A. tumefaciens strains K12 x 562E and K12 x 167, which contained plasmid pCGN562 and pCGN167, respectively. These are recombinant derivatives of the plasmid pTiA6. Only strain K12 x 562E produced crown galls on the seedlings. The crown galls were formed within 4-5 wk of infection. Gall growth at the base (45-50 mg) was more than at the apex (30-35 mg). 60% of seedlings formed crown galls, which were cultured on Murashige Skoog (MS) medium without phytohormones. The crown galls proliferated and within 2-3 wk weighed 250-300 mg. Strain K12 x 167 gave rise to shoots with teratomas at the base. The transformed shoots were cultured on MS medium without phytohormones. They elongated to 5-8 cm within 4 wk. The transformed calli and shoots also survived on MS medium containing 150 mg/l kanamycin, while the control calli and shoots did not. The kanamycin-resistant plants were then transferred to soil. The presence of octopine was detected.

Verpoorte (1989) reviewed the production of secondary metabolites by plants,

applied to the manufacture of compounds of economic importance, e.g. drugs, toxins, insecticides and additives. This review discusses the importance of studies of the factors regulating secondary metabolism to the development of plant cell and tissue cultures in industrial plant cell biotechnology. The role of secondary metabolites in plant resistance to microorganisms, viruses, insects, nematodes, etc., can lead to important compounds, e.g. the insecticidal azadirachtin produced by an Indian tree, *Azadirachta indica.* The formation of crown galls in plants due to infection by Agrobacterium spp. has provided a means of introducing foreign DNA and hence new genes into plant cells. The development of plant cell cultures in culture vessels has resulted in the successful industrial production of shikonin, berberine and rosmarinic acid. The production of secondary metabolites has been improved by use of elicitors. The culturing of

differentiated plant cells and genetic modifications of cells by targeting key enzymes is also underway for many species now (Verpoorte,1989).

Leaf and bark segments from 2-3-year-old *A. indica* plants form Nicaragua and Togo were subcultured twice and analysed for azadirachtin content. Nicaraguan cell lines had a significantly higher azadirachtin content than those from Togo. Calli from Togo only yielded azadirachtin when derived from leaf explants and cultured on White's medium, though no differentiation was observed on this medium. Azadirachtin content was 3 times higher on media supplemented with 15 g sucrose per litre compared to those with 30 g sucrose per litre (Wewetzer *et al.*,1997).

Eeswara *et al.* (1997a) studied azadirachtin, salannin and nimbin contents of seeds and callus of neem (*Azadirachta indica* A. Juss). Azadirachtin, salannin and nimbin contents were determined of neem seeds and callus cultured from leaf explants from trees at 3 sites in the Dry Zone and 1 site in the Intermediate Zones of Sri Lanka. All callus lines examined contained azadirachtin, nimbin and salannin. The azadirachtin content of callus varied from 4 to 190 $\mu g\ g^{-1}$ freeze-dried callus. The variation in meliacin content between trees was greater than that between sites for both callus and neem seed kernels. No correlation was found between the amount of azadirachtin, nimbin and salannin produced by callus and the same chemical present in seeds collected from the same tree.

The connection between differentiation and secondary metabolism is generally accepted. It was not until the 1990's however, that efforts were started to produce azadirachtin in tissue culture (Wewetzer, 1999).

Tissue culture of neem was first reported in the 1970s (Rangaswamy and Promilla, 1972; Sanyal *et al.*,1981). Since then, several papers have been published on the effect of various growth factors on the differentiation processes in neem tissue cultures and about secondary metabolites, notably nimbin (Schulz, 1983; Sarkar and Datta, 1986; Rao *et al.*, 1988; Sanyal *et al.*, 1988; Ramesh and Padhya, 1988; 1996).

Kuruvilla *et al.* (1999a) studied enhanced secretion of azadirachtin by permeabilized margosa (*Azadirachta indica*) cells. *Azadirachta indica*, commonly known as neem or margosa is well known for its utility as a source of pesticide against a variety of insects. The role of permeabilizing agents on secretion of the pesticide azadirachtin from magosa callus cultures was investigated, under laboratory conditions. Callus cultures of leaf explants were grown on Murashige-Skoog basal culture medium, supplemented with indolebutyric acid (4 mg/l), naphthaleneacetic acid (4 mg/l), benzyladenine (2 mg/l) and 2% sucrose (pH 5.8), and solidified with agar (0.8%).

Lei GuangFu *et al.*(1998) observed the effects of explants from different organs of *A. indica*, culture media and subculture times on callus growth and azadirachtin content in cultures. All calli induced from root, leaf, stem and bark tissue were capable of

synthesizing azadirachtin. Callus growth rate and azadirachtin content were highest in leaf explants. Azadirachtin content was 0.40, 0.50, 0.57 and 0.63 mg/g dry weight for bark, stem, root and leaf explants, respectively.

Srividya *et al.* (1998) reported azadirachtin and nimbin content in *in vitro* cultured shoots and roots of *Neem.* Callus was developed with 100% response from immature embryos of *A. indica* on MS medium supplemented with (a) 2.0 mg NAA + 0.5 mg BA, (b) 2 mg BA and (c) 1.0 mg NAA + 0.5 mg BA/litre. For optimum multiple shoot formation, medium containing (a) 2.0 mg BA or (b) 0.5 mg BA + 0.1 mg NAA was used. These shoots rooted in half-strength MS medium supplemented with 0.5 mg IBA/litre. Azadirachtin and nimbin content were analysed in callus, *in vitro*-cultured roots and shoots. Roots contained 4 ppm azadirachtin and shoots contained 8 ppm azadirachtin and 3 ppm nimbin. Callus had negligible amounts of azadirachtin.

Veeresham *et al.* (1998) was able to produce azadirachtin from callus cultures of *Azadirachta indica.* Callus cultures, initiated from leaves and flowers of *A. indica* (collected from India), were established for the production of azadirachtin. Twelve-week-old flower callus produced 2.46% azadirachtin.

Tissue culture studies were under taken by NCL for propagation of neem *(Azadirachta indica)* using different pathways like micropropagation, direct and indirect induction of somatic embryogenesis and induction of secondary metabolites from callus cultures. (DSIR, 1999).

Murthy and Saxena (1998) studied Somatic embryogenesis and plant regeneration of neem. Somatic embryos were initiated from mature seeds of neem tree when cultured on Murashige and Skoog medium supplemented with thidiazuron (TDZ). Somatic embryos originated directly, and also via an intermediary callus phase, from mature seeds. In both cases, the presence of TDZ alone in the medium was capable of inducing regeneration. TDZ induced somatic embryogenesis across a wide range of concentrations (1-50 µM). However, somatic embryogenesis was accompanied by callus formation at TDZ concentrations of 20 µM and above.

Eeswara *et al.* (1997b)developed a standard procedure for the micropropagation of the neemtree (*Azadirachta indica* A. Juss.). A procedure for the production of complete micropropagated plantlets from neem (*Azadirachta indica* A. Juss.) leaf explants from different ecological regions was developed.

Su *et al.* (1997) were able to induce somatic embryogenesis in *Azadirachta indica.* A modified method for somatic embryogenesis induction in neem (*Azadirachta indica*) is described. Cotyledons and hypocotyls of neem were grown in the dark at 24-26 deg on Murashige-Skoog medium with MS vitamins, 0.5 mg/l naphthaleneacetic acid (NAA), 1 mg/l benzyladenine, 1 g/l casein hydrolyzate and 50 g/l sucrose. Callus formed was transferred to liquid medium similar to the agar medium but with NAA

replaced by 0.5 mg/l indoleacetic acid (IAA). The callus formed globular structures which further developed a rudimentary root, after 4-5 wk incubation.

Kearney *et al.* (1994) studied the antifeedant effects of in-vitro culture extracts of the neemtree, *Azadirachta indica* against the desert locust (Schistocerca gregaria (Forskal)). Callus and micropropagated shoots were initiated from leaf explants of the neemtree. A variety of whole plant and in-vitro cell cultures from neemtree seedlings of Ghanian origin were tested for insect antifeedant compounds using the desert locust (Schistocerca gregaria (Forskal)) as the insect. Feeding suppression occurred when whole extracts of seed, leaf, callus, suspension cells and shoot cultures were tested in no-choice feeding bioassays. Controls of sucrose, carrot (Daucus carota) callus and the plant growth medium showed no antifeedant activity. Azadirachtin, the main component of neemtree seed kernels, was quantified from a seed extract by HPLC, but was not detected in any of the other extracts.

Sanyal and Datta (1984) carried out experiments to find the optimum conditions for the *in vitro* production of a triterpenoid, nimbin, from cultures of neemtree, *Azadirachta indica*. To try to induce calli, explants were taken from bark, leaf, young leaf, epicotyl, and hypocotyl, and plated onto a variety of standard media. The only medium on which callus was induced was new Sanyal and Datta medium comprising basal salts supplemented with vitamins, 30 g/l sucrose, 15% coconut milk, 1.5 mg/l indoleacetic acid and 0.5 mg/l kinetin. Young bark containing cambium tissue responded best. Nimbin gradually decreases during dedifferentiation but a different compound, beta-sitosterol, appears. A combination of high benzyladenine and low naphthaleneacetic acid induces shoots whilst the reverse roots. Indolebutyric acid added to the shoot medium induces roots and shoots together. After organogenesis, nimbin reappears. No propagation was achieved with other members of the Meliaceae family.

Joshi and Thengane (1996) studied *in vitro* propagation of by shoot proliferation. Clonal propagation of neemtree (*Azadirachta indica* A. Juss.) was achieved from node explants from young trees on Murashige-Skoog (MS) culture medium supplemented with 0.5 mg/l benzyladenine and 1.0 mg/l kinetin. 2-3 Shoots per explant were observed after 20 days. Increase in multiplication frequency of shoots per explant (45-50) and average shoot length (4.7 cm) were observed.

Wewetzer (1999) studied effect of different nutrient media and two carbohydrate sources on the azadirachtin content of callus cultures of neem derived from leaf, root, shoot and bark. Level of differentiation was higher on medium supplemented with maltose but overall, average azadirachtin contents were higher when sucrose was used as carbohydrate source.

2.18. Genetic improvement of trees:

The world's forests are retreating rapidly in response to the expansion of human activities. Many species are currently faced with extinction due to declining populations; in many cases, there are only a few populations remaining and just a few individuals in these populations (Kundu, 1999). Genetic diversity found in tree species is a natural strategy for defence and survival against different types of risks encountered in the long life spans of forest trees. (Heybrock, 1978). The largest, cheapest and fastest gain in most forest tree improvement programme can be made by ensuring the use of the preliminary basic information on natural variability for selection. Observation on phenotypic variation is desirable to screen the naturally available genetic variation so as to utilise the best material for afforestation and reforestation in order to get maximum productivity. So it is imperative to understand the pattern of phenotypic variation in the natural population for planning the selection program. Knowledge of the distribution of genetic variability, breeding system of trees and of the evolutionary forces that have shaped them, is a prerequisite for tree improvement (Muona, 1990). Such information is also needed for planning gene conservation and establishing breeding populations.

The use of selection in forest tree crops is certainly not a new or unique idea. Archaeological evidence suggests that plant improvement by selection has been practised as early as 2500 BC early human being simply utilized those individuals which best suited his needs to reproduce the next generation (Blair, 1975). Allard (1960) discussed several crop improvement efforts of the eighteenth century and noted the first reported accounts regarding the effectiveness of selection in England, Belgium and America. Wright (1976): the first experiments in forest trees which provided a basis for selection were provenance (geographic origin) tests, begun about 1820. Since these early efforts, the use of sophisticated selection procedures have become common in forest tree improvement.

To a tree breeder, the most important source of genetic variation is natural populations, and it is this natural variation which serves as the raw material for genetic gain (Lantz, 1975). Provenance variation and tree to tree differences generally account for the bulk of the genetic variation found within a tree species. These two components may account for as much as 90% of the within-species variation, leading to the fastest and cheapest gains in most tree improvement programs being obtained by selection from the best seed source within the appropriate species (Zobel and Talbert, 1984). Additional gains are then made by selecting the best individuals. Tropical hardwoods are more likely to show greater inter-population genetic variation (Bawa, 1976).

In most forest trees, which are long lived, predominantly outcrossed, wind pollinated, and generally widespread in distribution, population variation accounts for most of the genetic variation. Tree improvement programs must place great emphasis on the exploitation of this variation. Knowledge of the genetic structure of the forest populations is important in determining the efficiency of different selection procedures. Every tree improvement program begins with the identification of individual trees which are above average in quality for some particular traits, and are used to establish a base population for future breeding. Plus-tree selection is the very first stage of a breeding program(Puri, 1998).

Tree improvement involves three distinct, but related, phases: conservation, selection and breeding, and propagation. As one moves from one phase to the other decreasing amount of variations are being managed. Therefore, at any one time, more variation is being conserved or selected than is being deployed through propagation (Puri, 1998).

Although, the neem tree is widely recognised as a forest crop with multiple uses including pesticides, medicinal products, oils, tannins, fuelwood and timber, the most published research has dealt with isolation, identification, formulation and field testing of pest control agents derived from the kernels and leaves.

Tree breeding through the application of genetic principles is basically directed towards modifying the heredity of tree populations so that the trees are able to meet the better needs of the forester and wood based industries. Serious attention to selective tree breeding has been paid only in the present century, mainly since 1950, and primarily for industrial wood products (Burley, 1987). Initially, work was concentrated in Europe and North America, then Australia, Japan and Brazil, lately in Africa and Indian sub-continent. The major initial task was to convince the foresters that not only environment, but also the heredity of the trees determines their growth, form and adaptability (Zobel, 1952). A seed orchard has been defined by Zobel *et al.* (1958) as 'an isolated and intensively managed plantation of genetically superior trees, to produce regular and abundant crops of sound and easily harvested seed.'

Besides, immediate genetic gain, breeding strategies have several objectives like, genetic conservation, maintenance of continued genetic gain over several generations and supply of genetically improved reproductive material. Sufficient genetic diversity exists in neem which is useful to attain substantial genetic gains. The greatest potential exists in the selection of desired trees from natural genetic resources. The effective exploration, identification, documentation and use of genetic resources of neem are a prerequisite for its efficient use (Kundu, 1999).

Breeding neem for bioactive compounds or other multiple uses has not yet started systematically. So far, no comprehensive breeding plan is available for neem improvement. Breeding objectives must be set on the basis of present and future needs.

Thereafter, short, medium, and long term breeding programs may be established on the basis of the results of the ongoing international provenance trials. The ongoing international provenance trials would provide a great opportunity to select the best provenance for further improvement and domestication. Recommendations have been made for genetic improvement and conservation of neem through development of different duration types; higher seed yielding types; rapid multiplication through tissue culture; development of high-oil and superior quality types; development of different canopy architecture; development of types suitable to different situations (industrial areas, abandoned mines) and environments; collection/augmentation, evaluation and maintenance of different genetic stocks; and use in afforestation of waste lands, problem soils and forestry and environment programmes (Prasad *et al.*, 1996; Kaura, *et al.*,1998a; Kundu, 1999).

A number of selection methods have been developed for tree improvement programs. The method chosen for a tree species depends on the type of genetic variation present in the population, the type of stands (natural vs. plantation even aged vs. uneven-aged) available for plus tree selection as well as the intensity of the tree improvement effort(Simpson, 1998).

Comparison-tree method: The comparison-tree method is the most widely adopted, method of plus-tree selection. A candidate tree is compared with its nearest neighbours or the average or best dominant trees in a stand; it must be superior in one or more traits in order to be chosen (Morgenstern, 1975) and should not be older than the comparison trees (Brown and Goddard, 1961) this procedure is efficient in plantations and even-aged stands of a single dominant species or when only a few species are co-dominant (Ledig, 1973; Morgenstren, 1975)

The purpose of using comparison trees is to adjust or correct the phenotypic value of the candidate tree for environmental effects common to that stand but distinguishing it from other stands (Ledig, 1973). The use of comparison trees as a correction for environmental effects should increase the genetic gain (Ledig, 1974). One shortcoming is that the comparison trees may be related to the candidate tree and in this case comparison-tree selection becomes within- family selection (Falconer,1981) and could result in a reduction in genetic gain. For six trees in a stand (plus tree and five comparison trees) to be unrelated, 12 parents 24 grandparents and 48 great-grand-parents are required unless a population has been constantly shrinking, comparison and candidate trees will be related. Ledig (1973) concluded that comparison-tree selection should be analyzed as within-family selection. Within-family selection results in higher genetic gain than individual-tree selection only if the family relationship is very low and environmental variation among population is very large relative to genetic and within-population environmental variation (Ledig, 1974)

The fault with comparison-tree selection is not that "bad" trees are chosen but that "good" trees are discarded. The effectiveness of this method can be improved by increasing the number of comparison trees and by using comparison trees not immediately adjacent to the candidate tree. This would reduce the average relationship among trees (Simpson, 1998).

Individual-tree selection: The individual-tree selection procedure is also called ocular or mass selection trees that are vigorous healthy and of good forms,are rapidly identified. No measurements are taken nor are individual traits rated or scored. The procedure can be used for two situations. When the heritability of traits is considered so low that substantial progress can only be made through selection in progeny tests (Morgenstern, 1975) or when the traits are highly heritable in which case the phenotype is a good refection of the genotype (Zobel and Talbert, 1984). It is applicable when only a few traits are being selected (Morgenstern, 1975). It can also be considered when selection crews have gained considerable experience using another method (comparison-tree) and thus increase product selected by eliminating the time required to measure and record information collected from the comparison trees.

Base-line selection: Base-line tree selections can be used when stands are even-aged and when it is difficult to find comparison trees adjacent to the candidate due to high species diversity; individuals are located and their value for traits of interest is compared to the average for the region in which the selections are made .The average called a base-line may take the form of a regression line or equations (Ledig, 1974). Candidate trees must exceed the base-line by a certain amount in order to be incorporated into the breeding population (Morgenstern, 1975).

The base-line procedure was adopted by Brown and Goddard (1961) to enable selection for growth efficiency. It consisted of a regression of basal area increase versus crown length x crown radius. The measurements needed to relate crown size and growth were relatively time consuming so Brown and Goddard (1961) made them only if the candidate tree met minimum requirements in crown and branch characteristics and was free of damage from insects and disease. Rudolf (1956) developed a similar system whereby a regression of diameter at breast height squared x height versus crown diameter squared x crown length was used. He reasoned that if this relationship is expressed as a percentage, then soil and other site difference can be eliminated, to some extent, as variables. Superior trees had to be at least 20 percent greater and plus trees at least 50 percent greater than the average for trees within 20 meters that were of comparable age and species and growing under similar conditions. Ledig (1974) suggested that base-lines could be developed from accumulated comparison-tree data and that estimates of environmental variance indicate that such base-lines could apply over wide areas. With

high heritability for height growth (Teich, 1975) and considerable variations in stem straightness and crown form, a high selection intensity was warranted.

The success of plus-tree selection depends on the variability of the trait(s) being selected, the heritability of the trait(s), and the selection intensity (Morgenstern, 1975) while the method of selection employed depends on the biology of the species being selected (van Buijtenen, 1969).

The steps involved in conducting a plus-tree selection program (Morgenstern, 1975) are given below:

1. Examine the variations pattern of the species to determine at which level to concentrate selection. If experimental evidence indicates stand-to-stand variations is large and tree-to-tree variation within stands is small then selection should be concentrated at the stand level and less effort devoted to selection within stands. It is prudent to select trees from a large number of stands.

2. Decide whether to emphasize high or low-intensity selection. This decision is based on heritability of traits and the breeding strategy to be employed. High heritability of a trait favours high-intensity selection but low heritability would favour low-intensity selection and increased emphasis on progeny testing a large number of selected trees.

3. Choose the traits or characteristics to be improved by considering those of economic importance and that are suspected or known to be inherited. As the number of traits increases it becomes increasingly difficult to find the type of tree desired (van Buijtenen, 1969).

The methodology for seed collection consisted of getting the seeds from a minimum of 25 randomized mother trees spaced at 50-100 m.

The variations in Prosopis pod production and pod characteristics like size and protein contents is large in the region (Lima, 1998). According to Azevedo (1982), the pod production of Prosopis in the semi-arid environment ranges from 2-3 ton/ha/year and in humid zones 8 tons has been recorded in northeastern Brazil.

Population studies of genetic variation cannot replace provenance trials if there is no correlation between morphological traits and isoenzyme variants. However, they can supply important information about genetic variation generally and the evolutionary factors responsible for the genetic structure observed. Such information is very useful, because the existence of genetic variation is the main prerequisite for a successful tree improvement project (Papageorgiou, 1998).

A direct comparison between patterns of genetic variation of morphological traits and isoenzymes gene markers have shown both positive (Guries and Ledig, 1981; Lagercrantz and Ryman, 1990), and negative correlations (Rajora et al., 1991).

When studies of variation and heterozygosity show clearly that a population has a narrow genetic base and was influenced by genetic drift or inbreeding, then this population should not be chosen as a provenance, since the amount of variation would not be sufficient, even for morphological traits. Patterns of differentiation among populations can supply information about gene flow and migration isolation. The investigation of such patterns is closely related to the identification of diversity centres. According to this information, the amount and range of provenances used in a provenance trial can be determined without any loss of time or money.

The natural variability existing within species is the result of complex interaction among various factors, like mutations, response to diversity habitat, breeding system, degree of outbreeding, hybridization, size of population, isolation, etc. This variability has always been the main safe guard system of living organisms, a mechanism created by nature. It constitutes a buffer against changes in the environment and climate. Genetic variability also constitutes the raw material for tree breeding. The higher the variability inside a population, the better the chances to select families and individuals with desirable characteristics (Puri, 1998a).

Genetic variation in natural forest populations is not uniformly distributed. Population genetic structure is described by the partitioning of genetic variation within and between populations of a species. In most out-crossed species, e.g., conifers with large continuous distribution, most of the genetic variation resides within the population (Hamrick and Godt, 1990). Small population size, restricted gene flow, inbreeding, and life history features such as the highly specific site/habitat requirements that result in populations fragmentation tend to increase genetic difference between populations. Gibson and Hamrick (1991) opined that the cycles of local extinction and colonization that characterized some meta-population species tend to exhibit more genetic differentiation between populations.

Solanki *et al.* (1999): At the National Research Centre for Agroforestry, studies on neem since 1988 indicated that it has good adaptability in semi-arid tropics with an annual increment of 55 cm for plant height and 1.38 cm for collar diameter in red soil. Seed viability was increased by 10-15 days through depulping of seeds. Substantial variability was present for all characters under study. Results of provenance trials showed that seedlings from Riva, Katni, Bhopal, Guna, Shivpuri and Karera districts were excellent. Studies on reproductive biology over 3 years (1993-96) suggest that neem is predominantly a self-pollinated species.

The interpretation of adaptation in natural plant populations is difficult. The safest and simplest is the analysis of climatic adaptation, through observing differences in populations of different origins and relating their response to ecological conditions at their location of origin (Matyas, 1996).

To demonstrate the effects of climate on intraspecific genetic variation, it is sufficient to plot provenance data against an important climate or geographic factor of the location of origin. Depending on the nature of the observed trait, adaptive genetic variation may exhibit certain geographic patterns; although linked to climate, the various traits may show independent patterns depending on the effect the climate has on that specific trait. Correlations between trait variation and climate parameters may suggest the adaptive pressure exerted on the trait – in a sense demonstrating its adaptive significance. Comparing different types of traits, the variation of growth and phenology related traits seems to be in closest agreement with climatic factors. Morphological and allozyme variation generally do not correlate well with climate (Matyas, 1996).

From a forestry point of view, growth and survival traits are the most important. In temperate and boreal forests, the adaptive variation of growth –related traits (height increment, bud break and formation, hardiness, etc.) are primarily shaped by the thermoperiod (Sarvas, 1972, 1974; Prokazin & Bogachev, 1975; Morgenstern, 1978). Growth and phenology data of provenances correlate best with thermal parameters whereas heat sum, temperature average, maxima and minima yield roughly the same results. This is also in agreement with experiments in controlled environments (Giertych & Farrar, 1962; Yeatman, 1966).

Global climate change may have a serious impact on genetic resources in tropical forest trees. Genetic diversity plays a critical role in the survival of populations in rapidly changing environments. Furthermore, most tropical plant species are known to have unique ecological niches, and therefore changes in climate may directly affect the distribution of biomes, ecosystems, and constituent species. Climate change may also indirectly affect plant genetic resources through effects on phenology, breeding systems, and plant-pollinator densities may be reduced, leading to reduction in genetic diversity through genetic drift and inbreeding. Tropical forest plants may respond to climate change through phenotypic plasticity, adaptive evolution, migration to suitable site, or extinction (Bawa and Dayanandan, 1998).

Exploration of the natural range of a species for provenance research may reveal that some populations are seriously endangered through clearance of the forest. Where conservation *in situ* cannot be guaranteed it may be necessary to collect seed to establish gene conservation plantations elsewhere (Bouvarel, 1970).

The phylogenetic variation is the genetic variation due to the phylogenetic differences within species. As an example of this variation, is the work of Chylarecki and Giertych (1969). They have measured 17 characters of cones collected from 32 natural stands of *Picea abies* growing in Poland. By biometrical comparison among populations, they were able to classify 32 populations into four phylogenetically different groups.

Genetic variation developed in connection with natural selection is divided into two groups, one being latitudinal which copes with latitudinal circumstances, while the other ecological, developed by selection caused by ecological conditions. Theoretically speaking, the latitudinal variation could be involved in the ecological variation in the broad sense. However, genetic variation related to different latitudes is quite unique in the sense that the variation is essentially in growth rhythms of trees in response to photo-periodism, and is thus often of primary interest in forest tree breeding as is repeatedly found in the so-called provenance test (Libby *et al.*, 1969 ; Campbell and Sorensen, 1973). So, the latitudinal variation is separated from the ecological variation to meet the interest of forest tree breeders.

The distributional and the family variation have nothing to do with environmental stresses. The distributional variation among populations occurs as the result of random genetic changes during successive dispersal of the species i.e., the so called genetic random drift, while the family variation occurs with in the population as the result of formation of small groups of related trees as families due to limited distance of dispersal of seed and possibly of pollen (Sakai *et al.*, 1975).

For effectively conserving forests genetic resources, it is very important before all to choose natural stands in which a large amount of genetic variation is maintained. Natural stands of *Cryptomeria japonica* vary remarkable w.r.t. the amount of genetic variation they keep. Furthermore, genetic variation in natural stands involves different kinds of variability. Thus, it should be emphasized that the conservation should be preceded by investigation by biochemical, morphological physiological and particularly ecological techniques to find locations in which the natural forests involve a large amount of genetic variation of this kind (Sakai *et al.*, 1975).

For seed source trials and even more for gene conservation, it is desirable to capture as much as possible of the potentially valuable genetic variation within the population. Estimates of population variability, together with knowledge of the natural breeding system, are essential in assessing the need for and methods of genetic conservation (Burley, 1976). Newton *et al.* (1993);*Swietenia spp.*(Meliaceae) are amongst the most economically important tropical hardwoods and yet little information exists concerning their patterns of genetic variation. Characterization of this variation is essential for defining more accurately the conservation status of mahogany populations and for their economic utilization. The loss of genetic variation through deforestation may be critical for these species, which are highly susceptible to pest attacks when grown in plantations. This paper assesses the current state of knowledge concerning the extent of genetic variation in wild populations of mahoganies, and highlights its potential importance. It is suggested that any conservation strategy developed for mahoganies should include a genetic selection and improvement programme, as well as the protection of natural stands

in *situ*. Techniques by which particular genotypes may be captured for *ex situ* conservation are briefly described.

Fisher's view that traits closely associated with fitness should exhibit low levels of additive genetic variance has been widely interpreted to mean low heritabilities (Falconer, 1981; Mousseau and Roff, 1987). Fisher postulated that differences between heritabilities among traits categories are the result of differences in additive genetic variance, but recent theoretical considerations by Price & Schluter (1991) contest this idea. They proposed that the reason life history traits have low heritabilities is because such traits arecomposites of many morphological, physiological and behavioural traits. Because life history traits are composites of multiple under lying traits, they are subject to numerous sources of environmental variation which in turn inflates phenotypic variance, thus decreasing the heritability.

Primary goal of many genetic studies is the estimation of variance components associated with individual traits and of covariance terms between traits. Heritability, the proportion of variation in a trial that is contributed by average effects of genes, may be calculated from variance components. The heritability of a trait gives an indication of the ability of a population to respond to selection, and thus, the potential of that population to evolve (Lande, 1982; Mousseau & Roff, 1987).

Most of important traits in crops are quantitative and are controlled jointly by many loci. With maize (*Zea mays* L.) as a model species, it has been found through computer simulation that gene information is most useful in selection when few loci (e.g., 10) control the trait. With many loci (≥50), the least squares estimates of gene effects become imprecise. Gene information consequently improves selection efficiency among hybrids by only 10% or less, and actually becomes detrimental to selection as more loci become known. Increasing the population size and trait heritability to improve the estimates of gene effects also improves phenotypic selection, leaving little room for improvement of selection efficiency via gene information. The typical reductionist approach in genomics therefore has limited potential for enhancing selection for quantitative traits in hybrid crops (Bernardo, 2001).

Breeders have successfully improved crops despite not knowing the genes affecting quantitative traits. The numbers of genes controlling quantitative traits in different crops are yet unknown, although rough estimates include 69 loci for oil and 173 loci for protein content in the maize kernel (Dudley and Lambert, 1992). Experiments in many plant species have indicated that few quantitative trait loci have large effects, whereas many loci have smaller effects (Kearsey and Farquhar, 1998). Will knowing all the genes for a quantitative trait in crops further enhance breeding progress?

Suppose the identity and function of quantitative trait loci become known through extensive analysis of sequence homology, map posi$iton, gene expression, or genetic

path ways (Bowen and Luedtke, 1997; Somerville and Somerville, 1999). If inbreds differ at only a few loci with large effects, then information regarding gene function may be directly useful in selection, e.g., "cherry-pick" as many desirable genes as possible into one single-cross hybrid. It becomes increasingly difficult to accumulate all the desirable genes into one hybrid if the inbreds differ at an increasingly large number of loci. Consequently, the effects of the individual genes need to be quantified for the information to be useful in selection (Kennedy *et al.,* 1992).

Perhaps the practical value of knowing all the genes in hybrid crops would be in creating new genetic variation. If the identity and function of important genes for a quantitative trait become known, then new genetic variation can be created by over-expressing genes, targeted mutagenesis, or searching for novel genes in other germplasm sources (Tranksley and McCouch, 1997). But after new genetic variation has been assembled in a breeding population, selection based primarily on trait phenotypes would be the preferred approach for improving inbreds and hybrids.

Strong directional, and to some degree stabilizing, selection usually erodes only additive genetic variance while not affecting dominance variance. Consequently, traits closely associated with fitness should exhibit high levels of dominance variance. Traits that were known to have been subject to intense directional selection (morphological traits for domestic species) had significantly higher dominance estimates than did traits that were assumed not to have been subject to strong selection (morphological traits for wild outbred species)(Crnokrak and Roff, 1995).

Strong directional selection (and to a lesser degree, stabilizing selection; Lee & Parsons, 1968; Lacy, 1987) is predicted to erode additive genetic variance and, subsequently, decrease the heritability of a trait (Felsenstein, 1965). As a consequence, the response to selection will be reduced (but the reduction in response may result from other factors: see Falconer (1989) and Lande (1988). Traits for a population at equilibrium are predicted to have low additive genetic variance as it is assumed that selection has moulded them to an optimum (Hegmann & Dingle, 1982 for an alternative explanation). Because they are assumed to be subject to intense selection, traits most closely associated with fitness are predicated to have low heritabilities and subsequently relatively high dominance components (Wright, 1929). A number of studies (Mousseau & Roff, 1987) have shown that life history traits, which are assumed to be closely connected to fitness, have low heritabilities while morphological traits, which are assumed to be more distantly related to fitness have high heritabilities behavioural and physiological traits are usually intermediate to the above two types of traits). Because selection usually erodes only additive genetic variance (Lynch, 1994), although changes in gene frequency may also cause changes in nonadditive variance as well, one would predict that the opposite pattern should be found in terms of dominance variance: life

history traits should have relatively high levels of dominance variance while morphological traits should have low levels of dominance variance. In addition to eroding additive variance, selection is also expected to act directly on genetic dominance, resulting in a further relative increase of dominance variance to total genetic variance (Lynch, 1994). Therefore, before deciding on particular breeding design for estimating heritabilities, it is critical to take into consideration the type of traits being measured to obtain accurate estimates.

Newton (1996) argued whether tree improvement conflicts with the conservation of biodiversity? Tree breeders rarely consider ecological implications of their activities, but with recent global initiatives focusing on the conservation of biodiversity, the activities of tree breeders require critical examination. As *ex situ* approaches to genetic conservation will always be inadequate in the long term, greater attention needs to be paid to in situ conservation of the genetic resources on which tree breeders depend. The deployment of genetically improved material may have direct negative impacts on the status of native biodiversity, by the introduction of exotic species or non-native sources of germplasm. To avoid such conflicts between tree improvement and biodiversity conservation, new approaches need to be developed. It is suggested here that tree improvement should to be redefined as a rural development activity, with emphasis on the development of local genetic resources through the participation of local communities. In this way , in situ conservation and genetic improvement may be fully integrated, enabling forest genetic resources to be developed in a sustainable manner.

3. Material and Methods

The present investigation was carried out at Department of Genetics, CCS Haryana Agricultural University, Hisar, India during year 1995-2000. A total of 221 germplasm accessions from Northern, Western, Central and Southern India, representing a wide spectrum of geographical diversity, were studied in the following manner:

Tree architectural parameters were subjected to univariate analyses, simple correlation, multiple regression analyses, principal component and principal factor analyses.

Leaf morphological parameters were studied for variability and simple correlation.

Endocarp morphological parameters were studied for variability, genetic parameters (phenotypic coefficient of variation, genotypic coefficient of variation, heritability and genetic advance); simple, genotypic phenotypic and environmental correlation; path analysis; multiple regression analysis; D^2 analysis, hierarchical cluster analysis; principal component and principal factor analysis; temporal stability (predictability) analysis in different growing seasons.

Seed biochemical parameters (oil content, and profile of major fatty acids) were studied for variability, simple correlation, multiple regression analysis, hierarchical cluster analysis, principal component and factor analysis and stability (predictability) analysis in different growing seasons. Protein content and azadirachtin content of seeds was studied for variability, and simple correlation.

Evaluation of different provenance collections under *in vitro* (seed viability) and nursery conditions (comparative growth of seedlings of provenance collections).

RAPD (Random Amplified DNA Polymorphism) profiles of individual plants and pooled samples.

In vitro callusing and production of azadirachtin in tissue culture .

3.1. Material

3.1.1. Germplasm

Extensive survey of Neem trees was made during July-September, 1995, 1997 and 1998. All the agroclimatic zones of Haryana state and some parts of neighbouring states (Rajasthan, Himachal Pradesh, Delhi and Panjab) were covered. In addition to this, a total number of 59 seed samples were received from Neem Network participants from Tamil-Nadu, Karnataka, Uttar Pradesh, Maharashtra, Panjab and other sources. Detail of locations covered along with number of trees are given in **Appendix 1.**.

3.1.2. Nomenclature of the accession numbers adopted

Each tree was given a unique accession number. Nomenclature system adopted for accession numbers is explained in **Appendix 2.**.

3.1.3. Explants for tissue culture studies

A variety of explants were used from field grown plants, belonging to different provenances and age groups (**Appendix 3.**).

Mature fruits were collected from a tree of Hisar (Haryana) provenance. Within 48 hours of collection, fruits were depulped and endocarps were washed thoroughly with tap water and air dried at room temperature for 2-3 days. Thereafter, endocarps were kept in cotton cloth bags and stored at a temperature of 25 ± 2^{O}C.

Actively growing shoots were excised from parent tree with the help of a sharp edged scatier, and kept immersed in an antioxidant solution containing 0.5 g l^{-1} of ascorbic acid, 0.5 g l^{-1} of citric acid and 0.1 g l^{-1} PVP during transportation to laboratory and while excising different explants from the shoots. All the explants were excised from the shoots maintaining uniformity in size and shape of explants. Description of source plant and dimensions of explants are given in **Appendix 4.**.

3.1.4. Glassware

Glassware used during the course of study was of boro-silicate quality and procured from Borosil India Limited or Corning Glass Company. Conical flasks of specified volume of 100 and 150 ml volume were used for culturing of explants except for axillary buds where test tubes of 25mm x 150mm and 25mm x 200mm dimensions were also used ~~in addition to test tubes~~. Test tubes were also used for some of the rooting experiments.

3.1.5. Chemicals

All the inorganic reagents used were of analytical grade and were procured from BDH India, Glaxo India, SRL India & E. Merck India. Sucrose, type A agar agar, activated charcoal, myo-inositol and EDTA sodium salt were purchased from Hi-media, India. The plant growth regulators, reagents for DNA isolation, PCR vitamins, amino-acids and casein hydrolysate were obtained from Sigma Chemical Company, USA, Perkin Elmer, USA, and Promega, USA.

3.2. Methods

3.2.1. Germplasm collection and storage

Trees were selected keeping an isolation distance of ≥ 50m. Mature neem fruits were collected from trees. Method followed for processing of fruits and storage of endocarps is given in section **3.1.3.**.

3.2.2. Tree architecture

Fifty one sites of Haryana, Panjab, Rajasthan, Delhi, and Himachal Pradesh were surveyed for recording the tree architecture data during June-September 1998. Extent of phenotypic variation in natural population and estimation of association among morphological characters was done. Description of sampling sites considered for studies of tree architecture is given in **Appendix 5.**.

Following observations were recorded for the trees under survey:

Girth at breast height (m): The girth of the individual tree was recorded with the help of measuring tape. Measurements were taken at a height of 1.37m from ground level.

Girth was converted to diameter by following formula:

Diameter = Girth / π

where value of π was taken as 22 / 7

Basal girth (m): The girth of the individual tree was recorded from the basal portion of the stem, nearest to the ground, with the help of measuring tape.

Total height (m): Total height of standing tree is the perpendicular distance from the top of the leading shoot to the ground level. The total height of tree was recorded with the help of Suunto clinometer height meter.

Clear bole height (m): Clear bole height is the distance between ground level and crown point. The crown point is the position of the first crown forming living or dead branch. Clear bole height of all the sampled tree was taken with the help of a steel tape directly.

Unforked height (m): The stem height up to the point where main stem remains leading was recorded with the help of scale pole.

Crown spread (m): It was calculated by measuring the linear distance between two extremely leading shoots, passing along the stem in horizontal line. Similarly the length was measured between two extremities (leading shoots) in the other direction (at right angle to previous measurement) along the stem in a line. The mean was computed to get the crown spread (diameter).

Stem straightness: Trees were visually graded from 1 (least straight) to 5 (most straight) in the following manner:

Stem straightness index:

1 = deviation of $\geq 15^{o}$ from vertical plane ;

2 = deviation of $\geq 10 < 15^{o}$ from vertical plane;

3 = deviation of $\geq 5 < 10^{o}$ from vertical plane;

4 = deviation of $< 5^{o}$ from vertical plane;

5 = straight.

Crown shape: For this purpose the trees were visually graded from 1 ~~(least straight)~~ to 5 ~~(most straight)~~ based on crown shape:

Crown shape index: 1 = mushroom shape; 2 = round;
3 = oblong; 4 = conical; 5 = cylindrical

Tree health: Tree health was marked as good (G) and average (A).

Branch angle: In the parameter of branch angle, the angle of main branches was ignored. Angle of majority (90%) branches was considered.

Branch angle index: + = Small; ++ = medium; +++ = high

Apical dominance: Trees were visually graded on the basis of apical dominance as follows: - = nil; +/- = very low / negligible; + = low; ++ = moderate; +++ = high

Intensity of leafiness and colour of leaves: Trees were visually graded on the basis of intensity and colour of leaves as follows:

Leafiness index: - = nil; +/- = very low / negligible; + = low;
++ = moderate; +++ = high;

Colour of leaves: G = green; Y = yellow; DG = dark green; LG = light green;
YG = yellowish green; G+Y = green & yellow;
DG+LG = Dark green & light green;
LG+YG = Light green & yellowish green;

Fruiting intensity: Trees were visually graded on the basis of Fruiting intensity as follows: - = nil; +/- = very low / negligible; + = low; ++ = moderate; +++ = high

Grading as plus tree: Trees were visually graded on the basis presence or absence of traits useful for agroforestry system as follows:

P = poor; G = good; A = average; C = candidate plus tree;
Selected as poor/average/candidate plus tree for agroforestry purpose,

Remarks about suitability of tree for seed production/agroforestry purpose based on visual observations.

Ratios of different parameters: Ratios were calculated between all the quantitative parameters of the tree architecture as follows:

Total height : dbh

Clear bole height : total height

Unforked height : total height

Crown spread : dbh

Crown spread : total height

3.2.3. Endocarp morphology

A total of 47 trees were selected from 12 provenances for studying the endocarp morphology. Description of sampling sites considered for studying endocarp morphological parameters is given in **Appendix 6.**. Endocarp morphological parameters selected are listed below:

20 endocarp weight*

Endocarp length (mm)

Endocarp breadth (mm)

Endocarp weight (g)

Seed weight (g)

Endocarp coat weight (g)

Number of seeds per endocarp and their arrangement

Seed weight : Endocarp weight

Endocarp length : endocarp breadth

For all the parameters 5 unique random samples were considered. Endocarp length and breadth was recorded with the help of a vernier calipers.

*Three unique random samples were taken.

3.2.4. Seed oil content

Oil content was determined for 28 seed samples representing 6 provenance collections (Haryana, Rajasthan, Maharashtra and Uttar Pradesh). Oil content of oven dried (60^0C for 24hr) whole seeds (including endocarp) was measured by NMR (Nuclear Magnetic Resonance) technique (Medsen, 1976).

3.2.5. Fatty acid profile of seeds

Fatty acid analysis was performed for 28 seed samples representing 6 provenance collections (Haryana, Rajasthan, Maharashtra and Uttar Pradesh). Oil from the kernels was extracted in petroleum ether using standard Soxhlet apparatus. Methyl esters of each oil sample were prepared by the method of Luddy et al. (1968) and separated in Hewlett Packard (Model NO. 5730) A, 7030 Boeblingen, Germany) gas chromatograph, equipped with flame ionization detector, and stainless steel column (305 x 3.175 mm) packed with 20 % diethylene glycol succinate (DEGS) absorbed on 60-80 mesh chromosorb W. The column temperature at 190° C, and nitrogen (carrier gas) flow rate of 35 ml / min. was maintained. The peaks were identified by comparison of their retention times with those of standard fatty acid methyl esters, obtained from Sigma Chemical Co., USA. The area under individual peak was calculated by the formula: half the base x height, and converted directly into relative percentage.

3.2.6. Azadirachtin content of seeds:

Azadirachtin content (%) was estimated for seven seed samples representing five provenance collections of Haryana.

Principle: Azadirachtin in the sample was dissolved in Acetonitrile :Water and analyzed at 217nm by HPLC.

Apparatus: HPLC system model LC-10AD with UV/VIS detector model SPD-10A and C-R7A Plus Chromatopack (Plotter-cum-integrator) and was operated with the following parameters:

a) Column: RP-18e, E.Merck, 12.5cm X 4 mm; SS; 5μ particle size

b) Mobile phase = Acetonitrile : Water (35:65)

c) Flow rate = 1.0ml / min.

d) Detector wavelength = 217nm

e) Retention time = Around 6.0 min.

f) Micro syringe = 25μl capacity (Hamilton, USA)

Reagents:

a) Reference standard: 96% purity azadirachtin from M/S Trifolio-M, GMBH, Germany

b) Acetonitrile: HPLC Grade, E.Merck

c) Water: Triple distilled using all quartz distillation unit

Preparation of standard solution: 4.56mg of standard Azadirachtin was weighed on the analytical balance (Mettler AE 240, 0.01mg sensitivity) into 100ml volumetric flask and dissolved it in Acetonitrile and made upto the mark with Acetonitrile. 100μl of the standard solution was diluted to 1.0ml with water using 200-1000μl transferpette E.Merck.

Preparation of sample solution:

Cleaned Neem seeds (about 50 gm) were crushed using waring blender to remove outer nut shell and the shell was removed manually. Decorticated kernels (around 20gm) was crushed to fine powder using waring blender. 10gm of Neem kernel powder was weighed accurately and transferred into a 100ml stoppered measuring cylinder (class A) and made upto the mark using DM water. Shaken well for 2min. and continue shaking similarly every hour upto 8hr. Filter the extract after 8hr using Whatman No. 2 filter paper. Transfer 100μl of this filtrate into a 5ml STD volumetric flask and made upto the mark with HPLC water. Shake well and filter this solution using 0.45μ nylon filter.

Estimation: 20 µl of standard azadirachtin (containing 0.00465 mg ml^{-1}) and sample solution (containing 2.0mg ml^{-1}) were injected to get area reproducibility (within 2% variation) for each two consecutive injections.

Calculations:

% Azadirachtin in the sample = A1 / A2 x M2 / M1

where

A1 = Peak area of Azadirachtin in sample solution

A2 = Peak area of Azadirachtin in reference standard

M1 = Concentration of sample in the injected volume

M2 = Concentration of reference standard in the injection volume

3.2.7. Seed protein estimation

Seed protein estimation (as per the method given by Mckenzie and Wallace, 1954) was done for 10 whole seed samples representing six provenance collections of Haryana, Rajasthan, and Delhi. The whole seeds (alongwith endocarp) were used for the analysis.

3.2.8. Leaf morphology

Six leaf morphological characteristics of 13 trees of Hisar provenance were studied. Ten replications were studied for each characteristics. The characteristics under study were,

1. Leaf length
2. No. of leaflets per leaf
3. Length of leaflets
4. Number of serrations on side away from rachis
5. Number of serrations on side towards rachis
6. Presence of terminal leaf

Bottom most leaflet was considered for length and number of serrations. Vernier callipers was used to measure the lengths.

3.2.9. In vitro and Field evaluation of germplasm

Seed viability was tested in the seed collected from 21 trees representing 6 provenance collections (Haryana, Delhi and Rajasthan) after 6 months of storage at 25±2°C. Five seeds were put per petri plate lined with moist blotting paper. Germination parameters (germination percentage and radicle length) were scored after 10 days of incubation at 28±2°C.

Seeds from 27 trees representing 17 provenance collections (from Haryana, Rajasthan and Delhi) from all over India were taken for evaluation in the nursery. Seeds were sown in beds from August 31, to September 4, 1998. Two characters were studied viz. seedling

height and collar diameter. Six replications per treatment were taken. Data were recorded on November 29, 1998.

3.2.10. DNA fingerprinting

DNA isolation:

Genomic DNA from Neem leaves was isolated by CTAB extraction method of Murray and Thompson (1980) with minor modifications. Leaf tissue samples were taken from trees / nursery grown seedlings. Five grams of leaf tissue was ground in liquid nitrogen using a sterilized, pre-chilled mortar and pestle. Care was taken that leaf powder did not become moist, as under wet conditions DNAase would become active. Ground leaf tissue was mixed with 15 ml CTAB (**Appendix 9.**) extraction buffer in sterilized 50 ml centrifuge tube and the mixture was incubated at 65°C for 3 hrs in water bath. The tubes were removed from water bath, after cooling for 5 minutes, 10 ml of chloroform : octanol solution (24:1) was added. Sample was mixed thoroughly by gently inverting the tubes several times. It was centrifuged for 10 minutes at 8000 rpm. The aqueous phase was then transferred to a fresh 50 ml tube and again washed with chloroform : octanol solution. The DNA was precipitated with equal volume of ice cold isopropanol. DNA was hooked out and placed in wash I for washing for 20 min. followed by washing with wash II for 2-3 minutes. Washing solution was removed and DNA was dried overnight in refrigerator and dissolved in TE buffer.

DNA quantitation:

The nitrogenous bases constituting DNA absorb UV light. Spectrophotometeric measurement of UV absorbance by nucleic acid in solution is a simple and fast method of determining DNA concentration. DNA has a maximum absorbance at about 260 nm. Based on extinction coefficient an optical density (OD) of 1.0A at 260 nm, corresponds to approximately 50 μg/ml of double standard DNA. Concentration of DNA was determined by the following formula (Maniatis et al., 1989):

$$\text{Concentration of DNA } (\mu g/ml) = OD_{260} \times 50 \times \text{dilution factor}$$

Quality of DNA:

Quality of DNA was checked by submerged horizontal agarose (0.8%) gel electrophoresis.

DNA samples:

Fifteen DNA samples from individual plants representing 8 provenance collections (Haryana, Himachal Pradesh, and Tamil Nadu) and five pooled DNA samples representing five provenance collections (Haryana, Delhi, and Karnataka) were considered for RAPD analysis. Detail of DNA samples is given in **Appendix 7.** and **Appendix 8.**.

Primers: Random primers supplied by Operon Technologies Inc., USA were used for RAPD analysis. Detail of primers used are given in **Appendix 11.**.

RAPD analysis:

Polymerase chain reaction (PCR) was carried out in 20 μl of reaction mixture containing 50 ng of genomic DNA, 1 unit of Taq polymerase, 10 mM tris HCl (pH 9.0), 50mM KCl, 0.1% triton × 100, 2 mM $MgCl_2$, 100 μM of each 4 dNTPs and 0.2 μM of Primer. The reaction mixture in 0.2 ml Micro Amp tubes were placed in an automated thermal cycle (Gene amp2400, Perkin-Elmer). The temperature cycles used in PCR are given in **Appendix 10.**.

Gel electrophoresis:

DNA fragments generated by amplification were separated by agarose gel submerged horizontal electrophoresis. 1.5 per cent agarose solution in 1x TAE buffer **(Appendix 7.)** was used for preparation of gel. The mould was washed with distilled water and dried. The mould was wiped with ethanol swab air dried and ends were sealed with tape. After melting the agarose, ethidium bromide was added in the gel at a concentration of 5 μg/100ml. Gel solution was then poured onto the mould to get a 0.5 cm thick gel. Well forming comb was inserted in the gel and it was allowed to set for about 30 minutes. Comb was removed gently and mould sealing tapes were removed from both the ends. Gel tray was placed in the electrophoresis chamber filled with 1x TAE buffer. DNA sample was prepared by adding loading dye solution @ 1 ml/10 μl. Samples were loaded in the wells by a micropipette. Cover was placed on chamber and was connected to the power supply of M/s Atto, Japan. The electrophoresis was carried out at 60V constant voltage for 3-4 hours. Gel was run until the dye front was with in about four centimetres of the other end of gel.

PCR amplification products were viewed by fluorescence under UV light using UV transilluminator. Molecular weight of different fragments was determined by using Hind III-Eco RI double digest λ DNA as standard marker. The gel was photographed using coloured or monochrome 200 ASA film / thermal imaging system and yellow filter.

Evaluation of polymorphism and data analysis:

Data generated from detection of polymorphic amplified fragments by RAPD assays were analysed by employing the following equation of Nei and Li (1979) :

Similarity (F) = 2 Mx / My + Mz

Dissimilarity = (1 - F)

Where,

Mx = number of shared fragments between genotypes y and z

My = number of scored fragments of genotype y

Mz = number of scored fragments of genotype z

An agglomerative method of clustering the accessions was employed utilizing the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Jaccard's coefficient.

Genotype Discriminating Efficiency*:

For a given RAPD assay and n number of genotypes

Genotype Discriminating efficiency = (A x 100) / B

where,

B (all possible pairs of genotypes which are to be distinguished) = [n(n - 1)] / 2

A (Number of pairs of genotypes actually distinguished) = {[n(n - 1)] / 2} - P

where n is the total number of genotypes, P is the number of pairs of genotypes which could not be distinguished.

*This formula was devised by us to assess the efficiency of fingerprinting. This formula has not been reported elsewhere.

3.2.11. Tissue culture studies

Sterilization of equipments: Glassware was thoroughly washed with tap water (to remove residual detergent) after overnight soaking in dilute teepol solution. Thereafter, it was rinsed with single glass distilled water and dried in an oven at 180°C for 30 minutes. The glassware viz. pipettes, beakers, pasteur pipettes, etc., scalpels and forceps were dry heat sterilized at a temperature of 180°C at least for 2 h in a hot bed oven whereas, the plastic ware viz. beakers, measuring cylinders and assembly for filter sterilization were sterilized by autoclaving at 1.2 kg cm^{-2} pressure for 15 min.

Preparation of stock solutions and their storage: Separate stock solutions (1x to 30x) were prepared for hormones, vitamins, amino-acids, chelating agent, major salts and minor salts. These stocks were stored in glass containers at a temperature of 4°C ± 1°C in a refrigerator. Stocks of chelating agent and IAA were kept in amber coloured bottles for protection from light. All the stocks were consumed within a month of their preparation.

Sterilization of stock solutions: All the stock solutions of plant growth regulators were filter sterilized through Whatman filter (0.3 μm pore size), and stored at a temperature of 4°C ± 1°C and used with in 30 days of preparation.

Culture media:

In all the experiments, Murashige and Skoog (1962) medium in full or half strength was used (**Appendix 12.**). Basal MS medium without plant growth regulators was used for seed germination. A variety of supplements (AC, PVP, etc.) and hormones were added to the basal MS medium and the resulting combinations were coded accordingly (**Appendix 13.**). In general, the full and half strength of basal MS medium were used. The various media formulated differ with respect to plant growth regulators, agar

concentration, AC, PVP (soluble and insoluble type). All the constituents of the medium were mixed and final volume made prior to pH testing and pH of the medium was adjusted to 5.85 ± 0.03 with the help of 1N HCl and/or 1N NaOH. Following the melting of medium at a pressure of 1.2 kg cm^{-2} a standard volume of the medium was suspended in the culture vessels. The volume of medium dispensed in the conical flasks was 35 ml ± 5 ml, while in test tubes the volume dispensed was 20 ml ± 3 ml. After plugging the culture vessels with cotton plugs, these were autoclaved at a pressure of 1.2 kg cm^{-2} for 15 min and stored at 25°C ± 1°C and used within 10 days of the preparation.

Disinfection of explants:

Seeds were surface sterilized with 0.1% aqueous solution (w/v) of $HgCl_2$ for 4 minutes. Afterwards seeds were split into 2-4 pieces and placed on medium. Interval of subculture was kept at 30-35 days. Twenty seeds were kept for each treatment.

Vegetative explants were washed thoroughly under tap water for 10-15 min., with a few drops of teepol added, followed by a quick dip in 90% ethanol for 5-10 s. Thereafter, explants were treated with 0.1% (w/v) solution of $HgCl_2$ for 10-12 min. The explants were washed thoroughly in sterile single glass distilled water by 4-5 full volume rinses to remove the traces of surface sterilants used.

Establishment of the aseptic primary culture of vegetative explants:

After surface sterilization steps are completed, the explants were transferred to the 0.5 g l^{-1} of ascorbic acid, 0.5 g l^{-1} of citric acid and 0.1 g l^{-1} PVP solution, and inoculated immediately in order to avoid the undesirable browning/phenolic oxidation of the plant material. Leaves and stem segments were inoculated horizontally on to the medium while the axillary buds and shoot tips were implanted vertically. Depending on the size and type of explants, 3-5 explants were inoculated in one conical flask while 1-2 axillary buds in the test tubes.

Subculturing:

Although a two step propagation system (shoot regeneration and root induction) was adopted, but in some cases the regenerated shoots/calli were subcultured on a fresh medium (similar to or different than the first medium) at an interval of 30-40 days to promote shoot/callus proliferation.

Rooting:

The shoots produced *in vitro* were used for root induction by inoculation of shoots on to the media formulations containing growth regulators with full and half strength MS medium. In all the experiments intended for root induction, the regenerated shoots were excised from the explant and any accompanying callus was removed from the shoots before keeping on the rooting medium.

Incubation conditions:

All the seed, explant, callus and suspension cultures were incubated at a constant

temperature of 25 ± 1°C with light (fluorescent white light; intensity 4000 lux) / dark cycles of 16h/8h.

For the experiments aimed at rooting of regenerated shoots, the culture tubes were kept in diffused light and lower end of the shoots was maintained in the dark by inserting the bottom of culture tubes in a sand box.

Observations and record of data:

From time to time the plant cultures were observed in fluorescent white light for the following attributes :

Time taken by the explants/inoculum for initiation of response; callusing intensity, callus colour, softness and friability of callus, callus necrosis; number of explants or calli exhibiting roots/shoots/shoot buds/callus.

3.2.12. Statistical analysis

Univariate statistical analyses:

The mean value of each character was computed by dividing the totals by corresponding number of observations.

$$\bar{X} = \Sigma Xi \ / \ n$$

where,

Xi = value of the variable

n = total number of observations

Range: The lowest and the highest values for each character were recorded.

Standard deviation (σ): Standard deviation was calculated by squaring the deviation of each observation from the mean, adding the squares, dividing by the number of observations and extracting the square root according to the formula:

$$\sigma = \sqrt{\frac{\Sigma(X_i - \bar{X})^2}{N}}$$

where,

X_i = value of the given variable
$\bar{X}$ = arithmetic mean
N = total number of observations

't'-test: In order to test the significance of difference between means 't'-test was performed as suggested by Bailey (1997).

Analysis of variance: Analysis of variance based on randomised block design for various experiments was applied (Panse and Sukhatme, 1967).

Standard error (SE): Standard error for difference of two means were calculated with the help of error mean square from the analysis of variance table like:

$$SE(d) = \sqrt{\frac{2\,EMS}{r}}$$

where,

EMS = Error mean square

r = number of replications

Critical difference (CD): Critical difference for all the characters was calculated to compare the treatment means. Critical difference were calculated with the help of standard error for the difference of two means and tabulated value of t at 5% level of significance for error degree of freedom like:

CD = SE(d) x t at error degrees of freedom (P=0.05)

Univariate genetic parameters:

Genotypic and phenotypic coefficients of variation were estimated by the formula suggested by Burton (1952) for each character as follows:

$$PCV = (\sigma_p / \bar{X}) \times 100$$

$$GCV = (\sigma_g / \bar{X}) \times 100$$

where,

$\bar{X}$ = mean of that particular character

σ_p = phenotypic standard deviation

σ_g = genotypic standard deviation

Heritability:

Heritability in broad sense was calculated according to the formula suggested by Singh and Chaudhary (1979) for each character.

$$h^2 = (\sigma^2_g / \sigma^2_p) \times 100$$

where,

σ^2_g = genotypic variance

σ^2_p = phenotypic variance

Genetic Advance: Estimates of appropriate variance components were substituted for the parameters to predict expected genetic gain as suggested by Lush (1949).

The expected genetic advance was calculated at 5 per cent selection intensity for each character as:

$$\text{Genetic Advance (\% of mean)} = \{(K \sigma_p h^2) / \bar{X}\} \times 100$$

where,

K = Selection differential (2.06)

σ_p = Phenotypic standard deviation

h^2 = Heritability in broad sense

$\bar{X}$ = Mean for the particular character over all the genotypes

Stability Analysis: Temporal stability was assessed for endocarp morphological characteristics and seed oil content using the model given by Eberhart and Russel (1966).

Bivariate statistical analyses:

Genotypic and phenotypic correlation coefficients were calculated for each character as suggested by Singh and Chaudhary (1979).

Multivariate statistical analyses:

Multiple regression: Multiple regression analysis was performed for morphological traits and biochemical traits as suggested by Bailey (1994).

Path coefficient analysis: The genotypic correlation coefficients obtained were subjected to this analysis. Path coefficients were obtained according to the method described by Dewey and Lu (1959).

D^2 analysis: It is a measure for group distance based on multiple characters and was given by Mahalanobis (1928, 1930, 1930). With $x_1, x_2, x_3, \ldots\ldots x_p$ as the multiple measurements available on each individual and $d_1, d_2, d_3, \ldots\ldots d_p$ as $x_1 - x_1, x_2 - x_2 \ldots\ldots$ $x_p - x_p$ respectively, being the difference in the means of two populations, Mahalanobis D^2 statistic is defined as follows:

$$D^2_x = \Sigma^p_i \Sigma^p_j (\lambda^{ij}) d_i d_j$$

where, x is the number of traits in consideration, p is the number of genotypes, λ^{ij} is the matrix reciprocal to the common dispersion matrix and the d_i and d_j are the difference between the mean values of the two genotypes for n_i and n_j characters, respectively. In a still simpler form, it is:

$$D^2 = \Sigma^x_i d^2 = \Sigma^x_i (y_i^j - y_i^k)^2$$

Here, y is the uncorrelated variable which varies from I = 1 to x, i.e. number of characters. The superscripts j and k to y represent a pair of any two genotypes, e.g., 1 - 2, 1 - 3, 1 - 4; …., 2 - 3, 3 - 4, 4 - 5, ….; 3 - 4, 3 - 5, 3 - 6; …., (n - 1)n (where n is the number of populations).

In brief the calculation of D^2 involved the following steps:

Transformation of the original measurements to uncorrelated variables.

Calculation of the mean values of transformed characters.

Calculation of D^2 values: D^2 values between pairs of corresponding mean values of the transformed characters. Thus a total n(n-1) / 2 possible combinations among 106 values of D^2 were computed for the experiment and arranged in the form of matrix.

The determination of group constellations: The D^2 values presented in matrix form were arranged in increasing order of magnitude. Grouping of the genotypes into different clusters was done using Tocher's method (Rao, 1952). At first, two most closely associated strains were chosen and then a third strain was located which had smaller average D^2 value from the first two. Similarly the fourth was chosen and added to first three and change in average D^2 value within a cluster due to inclusion of additional genotype was assessed and so on. So long the change of D^2 value was not high, the genotypes were grouped in one cluster. If the change was appreciable then the newly added genotype was considered as out of the cluster. The genotypes of the first cluster were then omitted and the rest were treated similarly for the formation of other clusters. The idea behind this exercise was that any two genotypes belonging to the same cluster should at least on the average show a smaller D^2 value than those belonging to these groups.

Intra and Inter-cluster D^2 values were calculated as the sum of $n(n - 1)/2$ D^2 values among the genotypes within a cluster divided by $n(n-1)/2$. The single genotype had no intra-cluster D^2 values. For calculating inter-cluster D^2 value, all possible D^2 values between the genotypes of two clusters were added and then divided by $n1 \times n2$ (the number of genotypes in two clusters).

Intra and Inter-cluster distances: The square root of average D^2 value represents the distances within and between two clusters.

Intra cluster mean values: The cluster mean for a particular character is the summation of mean values of genotypes included in a cluster divided by number of genotypes in the same cluster. The values were calculated separately for each cluster and each character.

Hierarchical cluster analysis: Cluster analysis is a method of displaying the similarities and dissimilarities between pairs of genotypes of a set. A commonly used method for forming clusters is hierarchical cluster analysis, using one of the two methods: agglomerative or divisive. In agglomerative hierarchical cluster analysis (used in the present study), clusters are formed by grouping cases into bigger and bigger clusters until all cases are member of a single cluster. This procedure attempts to identify relatively homogeneous groups of cases (or variables) in separate cluster and combines clusters until only one is left.

There are several alternatives available to carry out agglomerative hierarchical cluster analysis. They are between groups linkage, within-group linkage, nearest neighbour, furthest neighbour, centroid clustering, median clustering, Ward's method, etc. For the present investigation between-group linkage or UPGMA (Unweighted pair group method using arithmetic averaged) was used as it is suggested to be best and most commonly used method (Romesburg, 1990).

UPGMA defines the distance between two clusters as the average of the distances between all pairs of cases in which one member of the pair is from each of the clusters. It considers only distances between pairs of cases between different clusters. Distance or similarity measures are generated by the Proximity procedure. Several options are available to measure similarity and dissimilarity (proximity) e.g., Euclidean, Squared Euclidean, Chebychev, Minkowski, etc. are the dissimilarity measures and Pearson correlation and Cosine are the similarity measures.

In the present study, Squared euclidean distance was used for endocarp and seed morphological and biochemical data; and RAPD data and Jaccard similarity coefficient was used for RAPD data (Jaccard, 1908).

Once the distance matrix was calculated, the actual formation of clustering was carried out. The first two cases combined were those that have the smallest distance between them. The distance between the two clusters was calculated as the average of the distances between all the pairs of cases in which one member of pair is from each of the clusters, e.g., If cases 1 and 2 form cluster A and cases 3, 4 and 5 form cluster B, the distance between cluster A and B is taken as to be the average of the distances between the following pairs of cases: (1,3) (1,4) (1,5)(2,3)(2,4)(2,5). This differs from the linkage methods in that it uses information about all pairs of distances, not just the nearest or the farthest. For this, it is usually preferred to single and complete linkage methods. This process continued until all cases were merged into single cluster.

At the first step, all the cases were considered separate clusters i.e., there were as many clusters as there were cases. At the second step, two of the cases having least distance were combined into a single cluster. At the third step, either a third case is added to the cluster already containing two cases, or two additional cases were merged into a new cluster. At every step, either individual case was added to clusters or already existing clusters were combined. Once a cluster was formed, it could not be split; it could only be combined with other cluster. Thus, this method did not allow cases to separate from clusters to which they have been allocated.

Based on the process of cluster formation dendrogram was produced using rescaled distances so that the ratio of distances between steps is preserved and problem of large distances was overcome. The dendrogram showed the clusters being combined and the values of coefficient (distance) at each step. In other words, dendrogram is the transformation of proximity matrix into a tree. The tree makes it easy to see the similarities and dissimilarities between all pairs of objects.

The number of clusters we want the classification to have, determines where to cut the tree. This is quite a subjective matter. However, Romesburg (1990) suggested a strategy to cut the tree at some point within a wide range of the resemblance coefficient for which the number of clusters remain constant, because a wide range indicates that the clusters

are well separated in the attribute space. This means that the decision of where to cut the tree is least sensitive to error when the width of range is the largest. This method of Romesburg was used to determine the number of clusters to be retained.

Principal Component Analysis: It is a multivariate technique to reduce the data with large number of correlated variables into a substantially smaller set of new variables, through linear combination of the variables that accounts most of the variation present in the original variables. Principal components are generally estimated either from correlation matrix or variance-covariance matrix. When the variables are measured in different units, scale effects can influence the composition of derived components. In such situations, it becomes desirable to standardize the variables and the correlation matrix comes to the rescue. In the present investigation correlation matrix was used to extract the principal components.

Suppose the vector of observations $X = (X_1, X_2, \ldots\ldots X_p)$ had correlation matrix R. the eigen values and associated eigen vectors of correlation matrix were determined by solving the determinantal equation:

$$|R - \lambda I| \; v = 0$$

Being $(\lambda_1, v_1), (\lambda_2, v_2), \ldots\ldots (\lambda_p, v_p)$ the eigen values and eigen vector pairs of R with $\lambda_1 \geq \lambda_2 \geq \ldots\ldots \lambda_p$, then the first principal component Y1, was calculated as:

$$Y_1 = v_1'X = v_{11}X_1 + v_{12}X_2 + \ldots\ldots + v_{1p}X_p$$

such that the variance of Y_1 was maximised subject to the constraint that the sum of squared weights was equal to one (i.e. $\sum^{p}_{j=1} v_{1j}^{2} = 1$). The second principal component was calculated as:

$$Y_2 = v_2'X = v_{21}X_1 + v_{22}X_2 + \ldots\ldots + v_{2p}X_p.$$

and this was uncorrelated with first principal component and

$$\sum^{p}_{j=1} v_{2j}^{2} = 1.$$

The principal components were uncorrelated with each other and their variances were equal to the eigen values $\lambda_1, \lambda_2, \ldots\ldots \lambda_p$ i.e.,

$$V(Y_j) = \lambda_j \quad (j = 1 \ldots\ldots p)$$

and the total variance in p variables was calculated by

$$\sum^{p}_{j=1} v(Y_j) = tr\,(R) = p = \lambda_1 + \lambda_2 + \ldots\ldots \lambda_p = \sum^{p}_{j=1} \lambda_j$$

Therefore, the proportion of the total variance explained by j^{th} principal component was estimated as:

$$\lambda_j / p \quad (j = 1 \ldots\ldots p)$$

In view of above, the standardized principal components were given by:

$$Y^*_1 = Y_1/\sqrt{\lambda_1} \quad Y^*_2 = Y_2/\sqrt{\lambda_2}, \ldots\ldots Y^*_p = Y_p/\sqrt{\lambda_p}$$

For deciding number of principal components to be retained, Kaiser's (1958) suggestion of dropping those principal components of correlation matrix with eigen roots less than one, was followed.

Principal Factor Analysis:

For further investigation Principal factor analysis was carried out as it has many added advantages over principal component analysis. It is closely related to principal component analysis, but differs in that it assumes a definite model, where each observed variable is expressed linearly in terms of common factor and unique factor. The common factor account for the correlation among the variables, while each observed variable is expressed linearly in terms of common factor and unique factor. The common factors account for the correlation among the variables, while each unique factor accounts for the remaining variance (including error) of that variable. Moreover, in principal component analysis total variation contained in a set of variables is considered, whereas in factor analysis interest centres only on that part of variance, which is shared by the common factors.

The common factor analytical model can be written in the following form:

$$Xj = I_{j1} f_1 + I_{j2} f_2 + \ldots\ldots + I_{jm} f_m + e_j \qquad \ldots\ldots \textbf{(i)}$$

Where each of the p observed variables has been described in terms of a common factor and a unique factor.

For a set of p variables, linear model (i) can be written in a slightly expanded form as:

$$
\begin{array}{llllll}
X1 = I_{11} f_1 + I_{12} f_2 & + \ldots\ldots + & I_{1m} f_m & + e_1 & \\
X1 = I_{11} f_1 + I_{12} f_2 & + \ldots\ldots + & I_{1m} f_m & + e_1 & \\
\vdots \quad \vdots \quad \vdots & & \vdots & \vdots & \\
\vdots \quad \vdots \quad \vdots & & \vdots & \vdots & \\
X1 = I_{11} f_1 + I_{12} f_2 & + \ldots\ldots + & I_{1m} f_m & + e_1 & \ldots\ldots \textbf{(ii)}
\end{array}
$$

Such a set of equations is called factor pattern. Factor analysis yields not only pattern but also correlation between variables and the factors. A table of correlation is called factor structure. A structure and pattern both are necessary in order to furnish a complete solution.

The factor model in matrix notation can be written as:

$$X = LF + e \qquad \ldots\ldots \textbf{(iii)}$$

Where, X = (p x l) vector of observed variable

L = (p x m) matrix of unknown constants called factor loadings.

$$\begin{matrix} l_{11} & l_{12} & \ldots\ldots\ldots\ldots & l_{1m} \\ l_{21} & l_{22} & \ldots\ldots\ldots\ldots & l_{2m} \\ l_{31} & l_{32} & \ldots\ldots\ldots\ldots & l_{3m} \\ : & : & & : \\ : & : & & : \\ l_{p1} & l_{p2} & \ldots\ldots\ldots\ldots & l_{pm} \end{matrix}$$

F = (m x I) vector of unobservable variables called common factors,

$F' = (f_1, f_2, \ldots\ldots\ldots f_m)$

e = (p x I) vector of unobservable variables called specific factors,

$e' = (e_1, e_2, \ldots\ldots\ldots e_p)$

It is obvious from each equation in (ii) that the variance of a variable X consists of two parts, viz.,

$$V(X) = h_j^2 + \phi_j \ (j = 1 \ldots\ldots\ldots p)$$

$$= l_{j1}^2 + l_{j2}^2 + \ldots\ldots\ldots\ldots + l_{jm}^2 + \phi_j$$

Where, h_j^2 is that portion of the variance of jth variable contributed by **m** common factors, also called communality and the specific variance (ϕ_j) contributed by specific factors. The jth communality is the sum of squares of the loading or common factor coefficients of jth variable.

Principal component and maximum likelihood methods of factor extraction are most commonly used besides other.

The advantage of using principal component method is that no distributional assumption is required whereas, maximum likelihood assumes that the general form of population distribution is known and follows multivariate normal distribution. Therefore, principal component method was used in the present study.

As the initial factor loading were not clearly interpretable, the factor axes were rotated using Varimax rotation (Kaiser, 1958) which is the most popular method of rotation. By this the variance of the squared loading across a factor was maximised which corresponded to spreading out of the squares of loading on each factor as much as possible. It made possible to obtain groups of large and negligible coefficients in different columns of the rotated factor loading.

Principal factor scores were determined using Anderson-Rubin method which is a modification of Bartlett's method (scores have mean = 1 and the sum of squares of unique factors over the range of variables is minimized), which ensures orthogonality of estimated factors. The scores produced have mean = 1 and SD = 1 and are uncorrelated. Plotting of different genotypes was done using their individual factors scores taking different principal factors as axes.

Statistical analysis in most of the cases was carried out using SPSS statistical package (version 7.52) and for some in house programmes developed at Computer Centre, The College of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar.

3.2.18 Photography

Microphotography was done with the help of SLR Type, Olympus PM-6 camera mounted on the compound microscope / stereozoom dissecting microscope. The macrophotographs were taken, using SLR Type Cameras (fitted with macro-lenses).

4. Experimental Results

The present investigation was carried out at Department of Genetics, CCS Haryana Agricultural University, Hisar, India during year 1995-2000. A total of 221 germplasm accessions of Neem A. Juss. from Northern, Western, Central and Southern India, representing a wide spectrum of geographical diversity, were studied in the following manner:

Tree architectural parameters were subjected to univariate analyses, simple correlation, multiple regression analyses, principal component and principal factor analyses.

Leaf morphological parameters were studied for variability and simple correlation.

Endocarp morphological parameters were studied for variability, genetic parameters (phenotypic coefficient of variation, genotypic coefficient of variation, heritability and genetic advance); simple, genotypic, phenotypic and environmental correlation; path analysis; multiple regression analysis; D^2 analysis, hierarchical cluster analysis; principal component and principal factor analysis; temporal stability analysis in different growing seasons.

Seed biochemical parameters (oil content, and profile of major fatty acids) were studied for variability, simple correlation, multiple regression analysis, hierarchical cluster analysis, principal component and factor analysis and stability (predictability) analysis in different growing seasons. Protein content and azadirachtin content of seeds was studied for variability, and simple correlation,

Evaluation of different provenance collections under *in vitro* (seed viability) and nursery conditions (comparative growth of seedlings of provenance collections).

RAPD (Random Amplified DNA Polymorphism) profiles of individual plants and pooled samples.

In vitro callusing and production of azadirachtin in tissue culture .

The results of the present investigation are presented under the following categories heads:

4.1 Survey and germplasm collection

Extensive survey of Neem trees was made during July-September in the years 1995, 1997 and 1998. All the agroclimatic zones of Haryana state (Rajasthan, Panjab, Delhi, Himachal Pradesh) and some parts of neighbouring states were covered under the survey. In addition to this, a total of 51 seed samples were received from Neem Network participants from Tamil-Nadu, Karnataka, Uttar Pradesh, Panjab and other sources in the year 1998. Passport data of the trees covered under survey is given in **Table** 4.1.1.

4.2. Tree architecture

Of 136 trees selected/surveyed, a total of 58 candidate plus trees (CPTs) for agroforestry; 45 CPTs for seed production as well as agroforestry and 47 CPTs only for seed production were

Table 4.1.1. Passport data of Neem (*Azadirachta indica* A. Juss) germplasm recorded from Haryana, Rajasthan, Delhi, Himachal Pradesh & Panjab

Accession	Date of collection	Geographical location	GBH (m)	Basal girth (m)	Height (m)	Clear bole (m)	Unforked height (m)	Crown spread (m)	Tree health	Crown shape index
RaJoBa01	13 Jul	Jodhpur	1.19	1.49	11.25	2.66	2.66	13.09	G	1
RaJoBa02	13 Jul	Jodhpur	1.56	1.93	13.50	3.90	4.92	14.72	G	2
RaJoUn01	13 Jul	Jodhpur	0.47	0.61	8.55	1.30	1.30	6.09	G	1
RaJoUn02	13 Jul	Jodhpur	0.52	0.69	7.05	2.43	2.43	7.01	A	3
RaJoUn03	13 Jul	Jodhpur	0.52	0.77	14.10	2.34	2.34	5.97	G	3
RaJoBa03	13 Jul	Jodhpur	0.57,1.18	1.23	11.70	0.99	0.99	11.72	G	1
RaAlPc01	14 Jul	Alwar	0.77	0.98	4.69	2.43	2.43	8.82	G	2
RaAlVc01	14 Jul	Alwar	1.21	1.08	9.90	2.41	2.41	9.84	G	2
RaAlAs01	14 Jul	Alwar	2.44	2.06	18.00	2.55	2.55	10.55	G	2
HaHiNc01	18 Jul	Nangal Chhoti	1.33	1.96	10.50	3.48	3.48	9.20	G	2
RaChBh01	18 Jul	Bhorugram	0.26, 0.87, 1.20	1.48	10.65	0.64	0.64	12.07	G	2
RaChBh02	18 Jul	Bhorugram	0.16	0.22	6.00	2.47	2.47	2.45	G	4
HaHiBa01	19 Jul	Barwala	0.99	1.19	11.10	2.00	2.00	3.45	G	2
HaHiGa01	19 Jul	Gaibipur	0.98	1.09	9.00	3.20	5.00	6.40	A	4
HaHiGa02	19 Jul	Gaibipur	0.83	0.75	10.50	2.00	4.40	8.40	G	3
HaHiSu01	19 Jul	Surewala	0.73	0.92	9.75	2.00	2.00	7.84	G	2
HaFaSa01	19 Jul	Sanyana	0.84	1.13	9.45	0.39	5.85	NA	G	3
HaFaDh01	19 Jul	Dhani Gopal	0.81	0.95	9.75	2.50	2.50	8.25	G	3
HaFaJh01	19 Jul	Fatehabad	0.90	1.10	12.15	3.10	3.50	9.20	G	3
HaFaRd01	19 Jul	Fatehabad	1.00	1.00	10.50	1.60	1.60	9.49	G	3

Table 4.1.1. (continued)

Accession	Date of collection	Geographical location	GBH (m)	Basal girth (m)	Height (m)	Clear bole (m)	Unforked height (m)	Crown spread (m)	Tree health	Crown shape index
HaSiAf01	19 Jul	Sirsa	0.32	0.35	17.10	3.48	6.90	7.50	G	3
HaHiAg01	19 Jul	Agroha	1.76	1.96	12.00	4.50	13.50	6.50	G	NA
HaHiPi01	20 Jul	Peeranwali	0.47	0.45	13.05	1.62	1.62	12.30	G	3
HaHiUn01	21 Jul	Hisar	0.31,0.76, 0.94	0.80	12.00	0.70	0.70	11.88	G	3
HaHiUn02	21 Jul	Hisar	0.89	0.90	10.80	NA	NA	9.08	G	1
HaHiUn03	21 Jul	Hisar	0.80	0.88	9.00	2.20	2.20	9.78	G	2
HaHiUn04	21 Jul	Hisar	0.60	0.80	10.80	2.10	2.10	8.01	G	2
HaHiUn05	30 Jul	Hisar	0.85	1.00	10.15	2.36	2.36	9.47	G	2
HaHiUn06	30 Jul	Hisar	1.10	1.20	12.25	1.15	1.15	9.50	G	3
HaHiUn07	30 Jul	Hisar	0.99	1.15	11.80	3.00	4.54	11.14	G	2
HaHiUn08	30 Jul	Hisar	0.72	0.89	9.85	2.36	9.08	8.75	A	3
HaHiUn09	30 Jul	Hisar	0.54	0.63	3.88	1.87	2.71	6.94	A	2
HaHiUn10	21 Jul	Hisar	0.62	0.82	7.52	3.30	3.62	6.58	A	2
HaHiUn11	24 Jul	Hisar	2.03	2.90	14.35	0.60	0.60	11.65	G	1
HaHiUn12	24 Jul	Hisar	1.10	1.30	10.75	2.82	4.80	8.83	G	2
HaHiUn13	24 Jul	Hisar	1.02,0.90	1.72	11.65	0.27	0.27	10.68	G	3
HaHiUn14	24 Jul	Hisar	1.85	2.20	11.75	2.84	3.84	9.38	G	2
HaHiUn15	24 Jul	Hisar	1.97	2.69	11.20	2.34	6.00	12.94	A	3
HaHiUn16	28 Jul	Hisar	0.99	1.29	11.80	4.00	4.00	10.10	A	2
HaHiUn17	21 Jul	Hisar	0.60	0.80	10.80	2.10	2.10	10.25	G	2
HaHiNa01	21 Jul	Nalwa	1.17	1.45	10.20	2.69	2.69	10.75	G	3
HaHiNa02	21 Jul	Nalwa	2.31	3.16	13.65	2.00	2.00	6.76	A	2
HaBhDu01	21 Jul	Dulehari	1.60	2.10	11.25	2.46	2.46	13.20	G	1
HaMaMm01	21 Jul	Mahendragarh	2.76	2.51	12.00	1.83	1.83	13.29	G	1

Table 4.1.1. (continued)

Accession	Date of collection	Geographical location	GBH (m)	Basal girth (m)	Height (m)	Clear bole (m)	Unforked height (m)	Crown spread (m)	Tree health	Crown shape index
HaMaJo01	22 Jul	Jonawas	1.71	2.40	13.20	2.29	2.29	9.85	G	3
HaMaNs01	22 Jul	Nangal Sirohi	2.90	2.49	15.00	1.79	1.79	18.95	G	1
HaMaNs02	22 Jul	Nangal Sirohi	0.80, 0.92, 0.45	1.52	7.50	0.28	0.28	9.33	A	1
HaMaHu01	22 Jul	Hudina	1.69	1.69	11.40	1.83	2.65	NA	A	2
HaMaHu02	22 Jul	Hudina	0.46	0.43	5.40	2.27	2.27	4.75	A	2
HaMaHu03	22 Jul	Hudina	1.30	1.45	11.25	2.57	2.57	12.00	A	2
HaMaHu04	22 Jul	Hudina	0.29	0.38	4.50	2.00	2.44	3.36	A	3
HaMaAj01	22 Jul	Ajamnagar	0.58	0.84	6.75	2.47	3.77	6.20	A	2
HaMaAj02	22 Jul	Ajamnagar	0.75	0.61	7.50	2.24	3.84	7.41	G	2
HaMaRd01	22 Jul	Narnaul	3.54	3.58	18.00	2.30	2.30	16.15	A	3
HaMaSb01	22 Jul	Saraibahadur	1.20	1.24	10.65	0.59	0.59	NA	A	2
HaMaSb02	22 Jul	Saraibahadur	0.85, 0.93	1.41	9.70	0.58	0.58	9.36	A	3
HaMaSb03	22 Jul	Saraibahadur	N.A	NA	NA	NA	NA	NA	NA	NA
HaReKu01	22 Jul	Kutubpur	1.35	1.13	12.00	3.90	6.00	10.00	A	3
HaReSu01	22 Jul	Suthari	1.10	1.24	10.50	2.31	2.31	8.96	G	2
HaReRd01	23 Jul	Bawal	2.03	2.05	15.00	2.10	2.10	17.35	G	2
HaGuBa01	23 Jul	Bilaspur	1.40	1.72	13.50	2.88	2.88	10.00	A	3
HaGuMa01	23 Jul	Manesar	1.17	1.35	16.95	3.00	8.55	12.28	G	3
HaGuMa02	23 Jul	Manesar	1.50	1.71	15.00	2.44	3.54	12.96	G	3
HaGuKr01	23 Jul	Gurgaon	0.64	0.80	11.25	3.54	3.30	10.40	G	3
HaGuKr01	23 Jul	Gurgaon	1.49	1.60	12.00	2.66	4.50	NA	G	2
HaGuKr03	23 Jul	Gurgaon	0.55	0.62	9.90	3.60	3.70	NA	G	4

Table 4.1.1. (continued)

Accession	Date of collection	Geographical location	GBH (m)	Basal girth (m)	Height (m)	Clear bole (m)	Unforked height (m)	Crown spread (m)	Tree health	Crown shape index
HaGuSi01	23 Jul	Silani	1.38	1.53	15.00	2.88	2.88	12.00	G	3
HaGuSi02	23 Jul	Silani	0.77, 0.76	1.15	12.75	0.41	0.41	6.19	G	5
HaJaLa01	23 Jul	Lakaria	1.00	1.10	8.00	2.00	2.00	7.78	A	2
HaRoBm01	25 Jul	Bhaini Maharajpur	1.80	1.95	12.00	3.34	3.34	13.07	A	1
HaRoBm02	25 Jul	Bhaini Maharajpur	1.38,1.22	2.06	9.90	0.82	0.82	12.98	A	1
HaRoBh01	25 Jul	Bhaini Mato	1.29	1.64	10.80	2.43	2.43	8.30	A	2
HaRoMa01	25 Jul	Madina	1.74	2.57	17.40	3.60	5.40	11.91	A	4
HaRoMa02	25 Jul	Madina	2.19	2.81	15.00	4.08	4.08	11.35	A	3
HaRoRh01	25 Jul	Rohad	1.29	1.54	11.55	3.90	3.90	11.03	G	2
HaRoRh02	25 Jul	Rohad	2.47	2.41	13.65	2.77	2.77	12.82	A	1
HaRoSa02	25 Jul	Sankhol	1.45	1.64	15.00	5.25	NA	14.28	A	2
DeNaJm01	25 Jul	Najafgarh	N.A.	NA	NA	NA	NA	NA	NA	NA
DeNaJm02	25 Jul	Najafgarh	0.62	0.82	6.50	2.23	4.00	6.62	A	2
DeNaSb01	25 Jul	Najafgarh	2.10	3.14	14.10	2.69	2.69	14.85	A	1
DeNaAn01	25 Jul	Najafgarh	0.90	1.11	9.90	3.17	4.17	10.85	G	2
HaGuBh01	26 Jul	Bhondsi	2.20	2.65	15.60	2.91	2.91	11.80	A	3
HaGuDh01	26 Jul	Dhunaella	2.64	4.62	17.25	3.00	3.00	15.49	G	3
HaGuSh01	26 Jul	Salaheri	1.61	1.82	11.70	2.00	2.44	14.00	A	1
HaGuSh02	26 Jul	Salaheri	1.46	1.77	10.80	2.99	4.00	9.88	G	2
HaGuSa01	26 Jul	Salamba	1.30	1.60	12.60	2.48	3.50	9.80	A	5
HaFrPa01	26 Jul	Palwal	1.58	1.97	13.50	5.00	5.00	10.98	A	3
HaFrPa02	26 Jul	Palwal	2.50	2.45	18.00	3.97	NA	15.36	A	3
HaFrBl01	26 Jul	Badkha	1.35	1.50	17.20	2.38	2.38	8.49	A	3

Table 4.1.1. (continued)

Accession	Date of collection	Geographical location	GBH (m)	Basal girth (m)	Height (m)	Clear bole (m)	Unforked height (m)	Crown spread (m)	Tree health	Crown shape index
DeNrPb01	27 Jul	Pusa	1.95	2.35	19.80	3.30	3.30	19.75	A	2
DeNrPb02	27 Jul	Pusa	1.55	1.90	18.60	3.78	3.78	17.10	A	2
DeNrPd01	27 Jul	Pusa	1.84	2.42	18.00	2.69	2.69	16.21	G	1
DeNrPd02	27 Jul	Pusa	2.50	3.15	16.80	1.41	1.41	16.79	A	1
DeNrPd03	27 Jul	Pusa	2.80	2.25	15.60	1.32	1.32	16.80	A	1
HaSoKu01	27 Jul	Kundli	1.64	1.96	16.80	2.57	6.48	13.08	A	2
HaHiSh01	31 Jul	Sheikhpura	1.65	1.93	11.55	1.52	4.20	11.95	A	2
HaHiGb01	31 Jul	Gujjar Bara	1.17	1.35	9.90	2.00	4.26	12.55	A	2
HaHiGb02	31 Jul	Gujjar Bara	0.38	0.46	4.98	1.09	1.48	5.12	A	2
HaHiGb03	31 Jul	Gujjar Bara	0.69	0.86	7.65	2.00	2.00	7.52	A	2
HaHiNa01	31 Jul	Narnaund	0.66, BL	0.86	8.70	0.45	2.69	5.48	A	3
HaHiNa02	31 Jul	Narnaund	1.08	1.32	10.50	3.05	4.08	5.95	A	3
HaHiNa03	31 Jul	Narnaund	0.80	0.96	10.65	2.00	2.00	7.62	A	3
HaJiGu01	31 Jul	Gulkani	1.49	1.73	11.70	3.12	3.12	11.39	A	3
HaJiGu02	31 Jul	Gulkani	1.85	2.18	13.20	1.83	3.24	14.31	A	1
HaJiKa01	31 Jul	Kandela	0.85	1.10	7.50	3.00	3.00	6.75	A	3
HaJiKa02	31 Jul	Kandela	0.79	1.04	12.00	1.41	1.41	9.15	A	3
HaJiAl01	31 Jul	Alewa	3.12	3.70	15.00	2.20	2.20	21.20	G	1
HaJiAl02	31 Jul	Alewa	1.43	1.57	8.70	1.44	1.44	10.17	G	1
HaKrNi01	01 Aug	Nilokheri	1.70, 1.17	1.08	15.00	1.15	1.15	14.57	A	1
HaKuKs01	01 Aug	Kurukshetra	2.27	2.54	12.00	2.50	2.50	21.50	A	2
HaKuKs02	01 Aug	Kurukshetra	4.43	6.12	15.00	1.40	2.47	17.26	A	3
HaKaGb01	01 Aug	Kaithal	2.82	4.15	11.70	2.90	3.80	12.20	A	2
HaKaSc01	01 Aug	Kaithal	1.68	1.75	11.40	1.70	1.70	14.60	A	1
HaKaTb01	01 Aug	Kaithal	2.75	3.70	12.30	2.45	2.45	12.93	A	1

Table 4.1.1. (continued)

Accession	Date of collection	Geographical location	GBH (m)	Basal girth (m)	Height (m)	Clear bole (m)	Unforked height (m)	Crown spread (m)	Tree health	Crown shape index
HaKaTc01	01 Aug	Kaithal	0.83	0.98	8.40	2.34	2.34	5.78	A	3
HaKaSv01	01 Aug	Kaithal	1.14	1.53	11.25	3.60	4.50	NA	A	4
HaKaKe01	07 Aug	Keorke	1.20	1.30	12.10	2.60	5.56	7.40	A	3
HaAmDh01	08 Aug	Dhin	1.26	1.51	18.00	1.21	1.21	7.75	A	3
HaYaJa01	08 Aug	Jagadhari	1.46	1.56	14.40	1.39	1.39	13.65	A	1
HaYaJa02	08 Aug	Jagadhari	1.20	1.44	12.60	3.24	3.60	12.57	A	2
HaYaTa01	8 Aug	Tajewala	1.80	2.08	10.50	2.46	2.46	11.30	A	1
HpSrBa01	8 Aug	Batamandi	1.90	2.10	15.90	1.95	1.95	9.96	A	3
HpSrSa01	8 Aug	Satiwala	1.90	2.05	9.45	1.30	1.30	6.05	A	3
HpSrMn01	8 Aug	Moginand	0.80	0.88	9.00	2.00	2.70	8.33	A	3
HpSrCm01	8 Aug	Chhota Moginand	0.79	0.88	18.00	1.73	1.73	5.87	A	3
HaAmLa01	9 Aug	Laha	2.60	2.73	14.30	1.00	1.00	12.44	A	1
HaAmGk01	9 Aug	Garhi Kataha	1.23	1.44	8.40	1.33	1.33	11.11	A	1
HaAmNa01	9 Aug	Naggal	1.14	1.35	7.70	3.50	7.70	8.38	A	2
HpPwFp01	9 Aug	Parwanoo	0.86, 1.14, 1.46	1.46	9.00	0.83	0.83	7.73	A	2
HaKlCa01	9 Aug	Kalka	0.91	0.94	8.55	2.64	2.64	5.84	A	2
HaKlNs01	9 Aug	Kalka	1.35	1.97	10.20	3.00	3.00	8.09	A	3
HaKlSc01	9 Aug	Kalka	2.58	NA	12.60	1.46	1.46	12.44	A	2
PbPtSa01	10 Aug	Salampur	2.00	2.08	9.99	0.30	0.30	13.69	G	1
PbPtCa01	10 Aug	Patiala	2.33	NA	18.00	3.60	3.60	17.82	A	4
HaKlPi01	12 Aug	Pinjore	2.50	2.24	11.80	2.34	2.34	13.07	A	2
HaKlPi02	13 Aug	Pinjore	1.86	1.93	10.30	2.60	2.61	9.80	A	2
HaKlRc01	14 Aug	Kalka	1.65	2.06	13.50	2.00	2.00	9.34	A	2
HaKlKh01	14 Aug	Kalka	1.59	1.56	12.64	1.47	1.47	12.20	A	1

Table 4.1.1. (continued)

Accession Number	Stem straight-ness index	Branch angle	Apical domin-nance	Intensity & color of leaves	Fruiting intensity	Selected as poor / average / candidate plus tree for agroforestry	Remarks
RaJoBa01	4	++	+	+++ DG	++	P	SP+AF
RaJoBa02	5	+	++	+++DG	++	A	SP+AF, F, B
RaJoUn01	5	+	++	++DG	++	A	AF, B
RaJoUn02	NA	++	+	++DG	+	A	SP
RaJoUn03	NA	+	++	++LG+DG	+	C	AF, B
RaJoBa03	5	+	+	+++DG	+++	P	AF, B
RaAlPc01	NA	++	++	+++DG	+++	A	SP, B
RaAlVc01	NA	++	+	+++LG+DG	+	A	SP
RaAlAs01	NA	++	+	+++DG	++	A	SP
HaHiNc01	NA	+++	+	+++DG	+	P	
RaChBh01	NA	++	+	+++DG	+++	P	SP
RaChBh02	5	++	+++	+++DG	-	C	AF
HaHiBa01	NA	++	+	+++DG	+++	A	SP
HaHiGa01	NA	++	++	++LG+DG	-	C	AF
HaHiGa02	NA	+	++	+++DG	+++	C	SP+AF
HaHiSu01	4	+	+	+++DG	+	A	
HaFaSa01	4	+/++	++	+++DG	++	C	SP+AF
HaFaDh01	4	+	++	+++DG	++	A	SP+AF
HaFaJh01	5	+	++	+++DG	+/-	C	AF
HaFaRd01	NA	+	++	++DG	+++	A	SP+AF
HaSiAf01	NA	+	++	++DG	+	C	AF
HaHiAg01	NA	NA	NA	++DG	NA	C	SP+AF
HaHiPi01	4	+/++	++	++LG +YG	++	A	SP

Table 4.1.1. (continued)

Accession #	Stem straight-ness index	Branch angle	Apical domin-nance	Intensity & color of leaves	Fruiting intensity	Selected as poor / average / candidate plus tree for agroforestry	Remarks
HaHiUn01	5	+/++	+	++DG	+	A	
HaHiUn02	1	+/++	+	++DG+LG	++	P	SP
HaHiUn03	4	++/+++	++	++DG	++	A	AF
HaHiUn04	4	+/++	++	++DG	+	C	AF
HaHiUn05	5	+/++	++	++DG	-	C	SP+AF
HaHiUn06	5	+/++	++	++DG	-	A	
HaHiUn07	4	+/++	+++	+++DG	+/-	C	SP+AF
HaHiUn08	5	+/++	++	++DG+LG		C	SP+AF
HaHiUn09	1	++/+++	+	++DG+LG		P	
HaHiUn10	4	++/+++	++	++LG	-	A	SP+AF
HaHiUn11	4	+/++	+	++DG	++	P	SP
HaHiUn12	4	++/+++	++	++DG	+/-	C	SP+AF
HaHiUn13	4	++/+++	++	++DG	-	A	SP, B
HaHiUn14	3	++/+++	+	++DG+LG	+	A	SP
HaHiUn15	4	+/++	+++	++YG+LG	-	C	SP+AF
HaHiUn16	5	+/++	++	++LG	-	C	AF
HaHiUn17	4	+/++	+	++LG	++	P	
HaHiNa01	4	++	++	++DG	-	A	SP+AF
HaHiNa02	4	++	+	++LG	-	A	SP+AF
HaBhDu01	NA	++	+	+++DG	-	A	
HaMaMm01	NA	++	+	+++DG	+	P	
HaMaJo01	NA	++	-	++DG	-	C	AF
HaMaNs01	NA	++	+	+++DG	-	A	SP
HaMaNs02	NA	++	-	+++LG	-/+	P	
HaMaHu01	4	++	++	++LG	++	A	SP

Table 4.1.1. (continued)

Accession #	Stem straight-ness index	Branch angle	Apical domin-nance	Intensity & color of leaves	Fruiting intensity	Selected as poor / average / candidate plus tree for agroforestry	Remarks
HaMaHu02	NA	++	++	++DG	++	A	
HaMaHu03	NA	++	+	++LG	++	A	SP+AF
HaMaHu04	4	++	+	++LG	+	A	AF
HaMaAj01	NA	+++	+	++LG	+	A	SP+AF, F
HaMaAj02	NA	++/+++	++	+++DG	+	A	SP+AF
HaMaRd01	NA	++/+++	++	++DG	+	P	
HaMaSb01	NA	++	+	++LG	++	P	SP
HaMaSb02	NA	++	++	++DG	++	P	SP+AF, B
HaMaSb03	NA	NA	NA	NA	NA	NA	NA
HaReKu01	4	++	++	++DG	+	C	SP+AF
HaReSu01	NA	++	++	+++DG	++	A	SP+AF
HaReRd01	4	+	+	++DG	+	A	SP+AF
HaGuBa01	4	+/++	+	++DG	++	C	SP+AF
HaGuMa01	NA	++	++	++DG	+	C	SP+AF
HaGuMa02	NA	++	+	++DG	++	C	SP+AF
HaGuKr01	4	+	++	++DG	+	C	AF
HaGuKr01	NA	+	+	++LG	+	C	AF
HaGuKr03	NA	+	++	++LG	+	C	AF
HaGuSi01	NA	+	++	++DG	++	C	SP+AF
HaGuSi02	4	+	+++	++DG	+/-	C	AF
HaJaLa01	NA	++/+++	+	++DG	++	P	
HaRoBm01	NA	++/+++	+	++LG+DG	+	P	SP
HaRoBm02	NA	++	+	++LG+DG	+	A	SP, B
HaRoBh01	NA	+/++	+	+++DG	++	A	SP
HaRoMa01	5	++	+++	++LG	+/-	C	AF

Table 4.1.1. (continued)

Accession #	Stem straight-ness index	Branch angle	Apical domin-nance	Intensity & color of leaves	Fruiting intensity	Selected as poor / average / candidate plus tree for agroforestry	Remarks
HaRoMa02	NA	++	+	++DG+LG	+	A	SP
HaRoRh01	NA	++	+	++DG+LG	+++	A	SP
HaRoRh02	NA	++	+	++DG+LG	+++	A	SP
HaRoSa02	4	++/+++	++	++DG+LG	+/-	C	AF, B
DeNaJm01	NA	NA	NA	NA	NA	C	AF
DeNaJm02	3	+/++	++	++DG+LG	++	C	AF+TF
DeNaSb01	NA	+/++	+	++DG	++	A	SP
DeNaAn01	NA	++	+	+++DG	+	C	SP+AF
HaGuBh01	4	++/+++	+	++DG+LG	++	C	SP+AF
HaGuDh01	NA	++/+++	+	++DG+LG	+	C	SP+AF
HaGuSh01	4	NA	+	++DG	+	A	SP+AF
HaGuSh02	NA	NA	+	+++DG	+	A	SP+AF
HaGuSa01	4	NA	++	++DG+LG	++	C	SP+AF
HaFrPa01	4	+/++	++	++DG+LG	+	C	SP+AF
HaFrPa02	NA	+/++	++	++DG+LG	+	C	SP+AF
HaFrBl01	NA	+/++	+++	++DG+LG	+/-	C	AF
DeNrPb01	5	+/++	++	++DG	++	C	SP+AF
DeNrPb02	NA	+/++	++	++DG+LG	+	C	SP+AF
DeNrPd01	NA	++/+++	+	++DG	++	A	SP
DeNrPd02	NA	++/+++	+	++DG+LG	+/-	A	SP
DeNrPd03	NA	++/+++	+	++DG+LG	++	P	SP
HaSoKu01	4	+/++	++	++DG+LG	+++	C	SP+AF
HaHiSh01	4	+/++	+	++DG+LG	-	C	SP+AF
HaHiGb01	NA	++/+++	+	+++DG+LG	-	C	SP+AF
HaHiGb02	NA	+/++	+/-	+++DG+LG	+/-	P	SP
HaHiGb03	NA	+/++	+	++DG+LG	+	A	SP

Table 4.1.1. (continued)

Accession #	Stem straight-ness index	Branch angle	Apical domin-nance	Intensity & color of leaves	Fruiting intensity	Selected as poor / average / candidate plus tree for agroforestry	Remarks
HaHiNa01	NA	++/+++	+	++DG+LG	+/-	P	SP+AF
HaHiNa02	NA	+/++	++	++DG+LG	+/-	C	SP
HaHiNa03	NA	+/++	++	++LG	+/-	C	SP+AF
HaJiGu01	NA	+/++	++	++LG	+	C	SP+AF
HaJiGu02	3	+/++	++	++LY	+	A	SP
HaJiKa01	4	++/+++	++	++LG	+/-	C	AF
HaJiKa02	4	++/+++	++	++DG	+	C	AF, B
HaJiAl01	3	++/+++	+	++DG	++	A	SP
HaJiAl02	4	++/+++	+	++DG	++	A	SP
HaKrNi01	5	+/++	+	++DG	++	A	SP, B
HaKuKs01	5	+/++	+	++DG+LG	++	A	SP, B
HaKuKs02	4	+/++	++	++DG+LG	+	C	SP+AF
HaKaGb01	4	++/+++	+	++LG	+	P	SP
HaKaSc01	4	++/+++	+	++DG	+/-	P	SP
HaKaTb01	4	++/+++	+	++DG+LG	++	P	SP
HaKaTc01	4	++/+++	++	++DG+LG	-	A	SP
HaKaSv01	5	++/+++	+++	++LG	+/-	C	SP+AF
HaKaKe01	5	++/+++	++	++DG	-	C	AF
HaAmDh01	5	++	++	++LG	-	C	SP+AF
HaYaJa01	3	++/+++	+	++LG	+/-	A	SP
HaYaJa02	5	++/+++	++	++LG	-	C	SP
HaYaTa01	3	++	+	++LG	-	A	SP
HpSrBa01	4	++	+	++LG	++	A	SP
HpSrSa01	5	++/+++	+	++DG	++	A	SP
HpSrMn01	4	+/++	++	++LG	-	C	SP
HpSrCm01	5	++	++	++LG	+	C	SP+AF

Table 4.1.1. (continued)

Accession #	Stem straight-ness index	Branch angle	Apical domin-nance	Intensity & color of leaves	Fruiting intensity	Selected as poor / average / candidate plus tree for agroforestry	Remarks
HaAmLa01	5	++/+++	+	++DG+LG	+	A	SP
HaAmGk01	1	++/+++	+	++DG+LG	+++	C	SP+AF
HaAmNa01	4	++/+++	+++	++DG+LG	++	C	SP+AF
HpPwFp01	4	+/++	+	++DG+LG	+/-	A	SP, B
HaKlCa01	3	++/+++	++	++LG	++	P	SP
HaKlNs01	3	++/+++	+	++DG+LG	+++	C	SP+AF
HaKlSc01	5	++/+++	+	++DG	+	A	SP
PbPtSa01	4	+/++	+	++DG	+/-	P	SP
PbPtCa01	4	+/++	++	++DG+LG	+	C	SP+AF
HaKlPi01	4	+/++	++	++DG+LG	+	A	SP
HaKlPi02	5	+/++	+	++DG+LG	++	C	SP+AF, B
HaKlRc01	5	++/++	++	++DG+LG	++	A	SP+AF, B
HaKlKh01	4	+/++	+	++DG	+++	A	SP, B

- = nil; +/- = very low / negligible; + = low; ++ = moderate; +++ = high; P = poor; G = good; A = average; C = candidate plus tree; G = green; Y = yellow;
DG = dark green; LG = light green; YG = yellowish green; G+Y = green & yellow; DG+LG = Dark green & light green; G+YG = Light green & yellowish green; B = fruits turning brown on tree;
SP = suitable for seed production; AF = suitable for agroforestry; F = flowering; NA = not available;
TF = two flowering seasons; BL = branch lopped;
Crown shape index: 1 = mushroom shape; 2 = round; 3 = oblong; 4 = conical; 5 = cylindrical
Stem straightness index: 1 = deviation of $\geq 15^0$ from vertical plane (crooked); 2 = deviation of $\geq 10 < 15^0$ from vertical plane; 3 = deviation of $\geq 5 < 10^0$ from vertical plane; 4 = deviation of $\nless 5^0$ from vertical plane; 5 = straight
In the parameter of branch angle, the angle of main branches is ignored. Angle of majority (90%) branches is considered.
In the Accession # of trees : first two letters indicate the state; next two letters indicate the district; next two letters indicate village/location.
e.g. states: Ha = Haryana; Ra = Rajasthan; De = Delhi; Hp = Himachal Pradesh; Pb = Panjab; districts : Hi = Hisar; Jo = Jodhpur; Al = Alwar; Ch = Churu; Fa = Fatehabad; Si = Sirsa; Ma = Mahendrgarh; Bh = Bhiwani; Re = Rewari; Gu = Gurgaon; Jh = Jhajjar; Ro = Rohtak; Fr = Faridabad; Pu = Pusa; So = Sonepat; Ji = Jind; Kr = Karnal; Ka = Kaithal; Ku = Kurukshetra; Am = Ambala; Ya = Yamunanagar; Kl = Kalka; Pt = Patiala; Sr = Sirmour; Pw = Parwanoo; Nr = New Rajendra Nagar; Na = Najafgarh.

marked. It was observed that in the year 1998, ~~fruit setting~~ in arid & semi-arid areas of Haryana and Rajasthan surveyed, fruit setting was poorer compared to the year 1995 and 1997. Incidentally in these areas, damage of immature fruits by birds was also more pronounced than in other agroclimatic regions. In some areas, particularly in Bhiwani and Mahendragarh, there was no fruit setting at all in the year 1998.

Six trees with conical crown shape were found scoring four with respect to crown shape index. Two trees were found to show cylindrical crown shape scoring five with respect to crown shape index. Twenty three trees were found to have completely straight stem scoring five with respect to stem straight. index, while 46 trees ~~were found to have completely straight stem~~ scored four with respect to stem straight. index. Sixteen trees were found to have unusual character of fruits turning brown on the tree itself. Sixteen trees were observed to have low branch angle.

4.2.1. Variation with respect to tree architecture parameters

Phenotypic variation with respect to dbh in some provenance collections of Neem is given in **Table** 4.2.1.1. Diameter at breast height (dbh) exhibited a wide range in different provenances (0.05 m- 1.41 m). Mean dbh in different provenance collections varied from 0.27 m to 0.69 m. Highest mean diameter (0.69 m) was observed in Kaithal + Kurukshetra provenance. Lowest mean diameter was observed in Jodhpur (0.27 m) provenance. Alwar + Rewari provenance exhibited highest level cv (65.69). Lowest level of cv (31.26) was observed in Rohtak + Jhajjar provenance.

Phenotypic variation with respect to basal girth in some provenance collections of Neem is given in **Table** 4.2.1.2. Basal girth exhibited a wide range in different provenances (0.22 - 6.12). Mean basal girth in different provenance collections varied from 1.03 m to 2.70 m. Highest mean basal girth (2.70 m) was observed in Kaithal + Kurukshetra provenance. Lowest mean basal girth was observed in Hisar + Sirsa (1.03 m) provenance. Kaithal + Kurukshetra provenance exhibited highest level cv (67.47). Lowest cv (25.66) was observed in Kalka + Pinjore provenance.

Phenotypic variation with respect to height : dbh in some provenance collections of Neem is given in **Table** 4.2.1.3. Height : dbh exhibited a wide range in different provenances (7.50 - 171.00).Mean height : dbh in different provenance collections varied from 21.57 to 50.13. Highest mean height : dbh (50.13) was observed in Jodhpur provenance. Lowest mean height : dbh was observed in Kaithal + Kurukshetra (21.57) provenance. Hisar + Sirsa provenance exhibited highest level cv (91.42) followed by Bhiwani+Bhorugram (90.05). Lowest cv (18.50) was observed in Rohtak + Jhajjar provenance.

Phenotypic variation with respect to height in some provenance collections of Neem is given in **Table** 4.2.1.4. Height exhibited a wide range in different provenances (3.88 m - 19.80 m).

Mean height in different provenance collections varied from 10.28 m to 15.12 m. Highest mean height (15.12 m) was observed in Delhi provenance. Lowest mean height was observed in Jind + Narnaund (15.12 m) provenance. Alwar + Rewari provenance exhibited highest level cv (40.28) followed by Sirmour (34.73). Lowest level cv (15.24) was observed in Gurgaon + Faridabad provenance.

Phenotypic variation with respect to clear bole height : height in some provenance collections of Neem is given in **Table** 4.2.1.5. Clear bole height : height exhibited a wide range in different provenances (0.02 - 0.52). Mean Clear bole height : height in different provenance collections varied from 0.15 to 0.24. Highest mean clear bole height : height (0.24) was observed in Hisar + Sirsa provenance. Lowest mean clear bole height : height was observed in Sirmour provenance (0.15). Ambala + Yamunanagar + Patiala provenance exhibited highest level cv (81.00) followed by Alwar + Rewari (66.63). Lowest cv (36.28) was observed in Hisar + Sirsa provenance.

Phenotypic variation with respect to clear bole in some provenance collections of Neem is given in **Table** 4.2.1.6. Clear bole exhibited a wide range in different provenances (0.27 m - 5.00 m). Mean clear bole in different provenance collections varied from 1.75 m to 2.87 m. Highest mean clear bole (2.87 m) was observed in Rohtak + Jhajjar provenance. Lowest mean clear bole was observed in Sirmour (1.75 m) provenance. Ambala + Yamunanagar + Patiala provenance exhibited highest level cv (62.95) followed by Jodhpur (45.87). Lowest cv (18.28) was observed in Sirmour provenance.

Phenotypic variation with respect to unforked height : height (Co-efficient of Apical Dominance) in some provenance collections of Neem is given in **Table** 4.2.1.7. Unforked height : height exhibited a wide range in different provenances (0.02 - 1.00). Mean value of unforked height : height in different provenance collections varied from 0.165 to 0.346. Highest mean unforked height : height (0.346) was observed in Hisar + Sirsa provenance. Lowest mean unforked height : height was observed in Sirmour (0.165 m) provenance. Ambala + Yamunanagar + Patiala provenance exhibited highest level cv (76.64) followed by Hisar (71.98). Lowest cv (32.58) was observed in Rohtak + Jhajjar provenance.

Phenotypic variation with respect to unforked height in some provenance collections of Neem is given in **Table** 4.2.1.8. Unforked height exhibited a wide range in different provenances (0.27 m - 9.08 m). Mean unforked height in different provenance collections varied from 1.92 m to 3.95 m. Highest mean unforked height (3.95 m) was observed in Hisar + Sirsa provenance. Lowest mean unforked height was observed in Sirmour (1.92 m) provenance. Ambala + Yamunanagar + Patiala provenance exhibited highest level cv (99.95) followed by Hisar (66.37). Lowest cv (30.52) was observed in Sirmour provenance.

Phenotypic variation with respect to crown spread : dbh in some provenance collections of Neem is given in **Table** 4.2.1.9. Crown spread : dbh exhibited a wide range in different

Table 4.2.1.1. Variation w.r.t. dbh in some provenance collections of *Azadirachta indica*

Site	Range	mean	sd	cv	se
1	0.20 - 0.89	0.5633	0.2214	39.295	0.0738
2	0.24 - 0.84	0.4858	0.1534	31.577	0.4043
3	0.32 - 0.79	0.5200	0.1626	31.263	0.0575
4	0.09 - 1.13	0.4647	3.3053	65.691	0.074
5	0.15 - 65	0.3144	0.1549	49.27	0.0365
6	0.12 - 0.99	0.405	0.2309	57.014	0.0667
7	0.26 - 1.41	0.6925	0.3701	53.448	0.1309
8	0.36 - 0.83	0.5037	0.1652	32.79	0.0584
9	0.25 - 0.60	0.4250	0.2021	47.546	0.101
10	0.29 - 0.80	0.5043	0.1667	33.06	0.063
11	0.15 - 0.50	0.2750	0.141	51.259	0.0575
12	0.05 - 0.68	0.3800	0.2165	56.978	0.0884
13	0.10 - 0.56	0.2944	0.1206	40.97	0.4042
14	0.25 - 0.80	0.4755	0.1747	36.748	0.0527

Table 4.2.1.2. Variation w.r.t. basal girth in some provenance collections of *Azadirachta indica*

Site	Range	mean	sd	cv	se
1	0.82 - 3.15	2.1222	0.7941	37.4198	0.2647
2	1.15 - 4.62	1.9492	0.91852	47.1238	0.26515
3	1.10 - 2.81	2.01	0.5739	28.5527	0.2029
4	0.38 - 3.58	1.5388	0.86661	56.3168	0.21018
5	0.45 - 2.90	1.2456	0.69468	55.7733	0.16373
6	0.46 - 3.70	1.5167	0.83943	55.347	0.24232
7	0.98 - 6.12	2.7025	1.82325	67.4654	0.64461
8	1.35 - 2.73	1.7737	0.481	27.1882	0.17006
9	0.88 - 2.10	1.4775	0.69023	46.7164	0.34511
10	0.94 - 2.24	1.7371	0.44574	25.6598	0.16847
11	0.61 - 1.93	1.12	0.52394	46.7809	0.2139
12	0.22 - 3.16	1.7283	0.96495	55.8315	0.39394
13	0.35 - 1.96	1.0344	0.42705	41.2837	0.14235
14	0.88 - 2.24	1.6427	0.52849	32.1715	0.15934

Table 4.2.1.3. Variation w.r.t. height : dbh in some provenance collections of *Azadirachta indica*

Site	Range	mean	sd	cv	se
1	17.53 - 37.96	28.7700	7.0647	24.5557	2.3549
2	20.54 - 53.13	31.7717	10.07427	31.70835	2.90819
3	17.22 - 31.64	24.375	4.51042	18.50429	1.59467
4	7.50 - 37.50	26.2565	9.92391	37.79606	2.4069
5	17.17 - 87.00	39.225	16.79578	42.81908	3.9588
6	15.15 - 48.00	29.6167	10.21337	34.48523	2.94834
7	10.64 - 32.61	21.5700	9.39022	43.5337	3.31994
8	15.61 - 45.00	25.4562	10.15209	39.88056	3.5893
9	15.75 - 72.00	37.5625	24.40318	64.96689	12.20159
10	14.75 - 29.48	22.8129	5.088	22.30323	1.92308
11	27.00 - 82.94	46.635	20.74136	44.47596	8.46762
12	20.07 - 42.60	42.8833	38.61458	90.04567	15.76433
13	21.43 - 171.00	50.1256	45.82446	91.41936	15.27482
14	14.75 - 72.00	28.1764	15.79762	56.06685	4.76315

Table 4.2.1.4. Variation w.r.t. height in some of provenance collections of *Azadirachta indica*

Site	Range	mean	sd	cv	se
1	6.50 - 19.80	15.1222	4.3413	28.708	1.4471
2	10.80 - 17.25	14.3208	2.1822	15.238	0.6299
3	8.00 - 17.40	12.2875	2.9827	24.274	1.0545
4	4.50 - 18.00	10.6406	4.2855	40.275	1.0394
5	3.88 - 14.35	10.6917	2.2867	21.388	0.539
6	4.98 - 15.00	10.2775	2.7461	26.72	0.7927
7	8.40 - 15.00	12.2375	2.1064	17.213	0.7447
8	7.70 - 18.00	11.9862	3.48770	29.098	1.2331
9	9.00 - 18.00	13.0875	4.5454	34.731	2.2727
10	8.55 - 13.50	10.8557	1.8514	17.055	0.6998
11	7.05 - 14.10	11.025	2.7573	25.01	1.1257
12	6.00 - 11.25	10.375	2.48	23.904	1.0125
13	9.00 - 12.15	11.3167	2.4048	21.25	0.8016
14	8.55 - 14.10	11.6673	3.0859	26.449	0.9304

Table 4.2.1.5. Variation w.r.t. clear bole height : height in some provenance collections of *Azadirachta indica*

Site	Range	mean	sd	cv	se
1	0.08 - 0.34	0.1867	0.0917	49.0990	0.0306
2	0.03 - 0.37	0.175	0.07982	45.61677	0.02304
3	0.06 - 0.34	0.2137	0.09767	45.6975	0.03453
4	0.03 - 0.52	0.2188	0.14581	66.63434	0.03536
5	0.02 - 0.48	0.2044	0.13039	63.77971	0.03073
6	0.12 - 0.40	0.2117	0.08189	38.68844	0.02364
7	0.08 - 0.28	0.1862	0.07308	39.23899	0.02583
8	0.03 - 0.45	0.1712	0.13871	80.99981	0.04944
9	0.10 - 0.22	0.145	0.05259	36.27525	0.0263
10	0.09 - 0.31	0.2014	0.08532	42.36166	0.03225
11	0.08 - 0.34	0.2117	0.09621	45.45428	0.03927
12	0.06 - .041	0.2383	0.12512	52.50067	0.05108
13	0.15 - 0.38	0.2433	0.08015	32.94086	0.02671
14	0.09 - 0.31	0.1809	0.07751	42.84932	0.02337

Table 4.2.1.6. Variation w.r.t. clear bole in some provenance collections of *Azadirachta indica*

Site	Range	mean	sd	cv	se
1	3.78 - 1.32	2.5733	0.8223	31.9546	0.2741
2	0.41 - 5.00	2.6975	1.02759	38.09427	0.29664
3	0.82 - 3.60	2.8675	1.09816	38.29691	0.38826
4	0.28 - 3.90	2.1365	0.78871	36.91681	0.19129
5	0.27 - 4.00	2.1111	0.96843	45.8733	0.22826
6	1.09 - 3.05	1.9717	0.59516	30.18561	0.1718
7	1.15 - 2.60	2.1300	0.62967	29.56202	0.22262
8	0.30 - 3.50	1.8037	1.13554	62.9545	0.40147
9	1.30 - 2.00	1.745	0.319	18.28131	0.1595
10	0.83 - 3.00	2.1257	0.75546	35.53938	0.28553
11	0.99 - 3.90	2.27	1.04103	45.8607	0.425
12	0.64 - 3.48	2.29	0.94276	41.16864	0.38488
13	1.60 - 4.50	2.7089	0.93392	34.47621	0.3113
14	0.83 - 3.00	1.9873	0.6402	32.21523	0.19302

Table 4.2.1.7. **Variation for unforked height: height in some provenance collections of *Azadirachta indica***

Site	Range	mean	sd	cv	se
1	0.08 - 0.62	0.2556	0.1821	71.275	0.0607
2	0.03 - 0.50	0.2417	0.12386	51.254	0.03575
3	0.08 - 0.34	0.245	0.07982	32.58	0.02822
4	0.04 - 0.56	0.2759	0.18611	67.461	0.04513
5	0.02 - 0.92	0.3256	0.23432	71.977	0.05523
6	0.12 - 0.43	0.2742	0.14422	38.015	0.03008
7	0.08 - 0.46	0.2325	0.11865	51.034	0.04195
8	0.03 - 1.00	0.2437	0.31802	76.64	0.11243
9	0.10 - 0.30	0.1650	0.09146	55.436	0.04573
10	0.09 - 0.31	0.2014	0.08532	42.362	0.03225
11	0.08 - 0.36	0.2233	0.11075	49.592	0.40521
12	0.06 - 0.41	0.2383	0.12512	52.501	0.05108
13	0.15 - 0.64	0.3456	0.17161	49.664	0.0572
14	0.09 - 0.31	0.1882	0.02494	45.142	0.02561

Table 4.2.1.8. **Variation for unforked height in some provenance collections of *Azadirachta indica***

Site	Range	mean	sd	cv	se
1	1.32 - 6.48	3.3156	1.57417	47.4785	0.52072
2	0.41 - 8.55	3.4575	1.93708	56.0257	0.55918
3	0.82 - 5.40	3.0925	1.40985	45.5893	0.49845
4	0.28 - 6.00	2.4565	1.2637	51.4439	0.30649
5	0.27 - 9.08	3.1828	2.11229	66.3663	0.49787
6	1.41 - 4.26	2.7025	1.49195	40.5054	0.31522
7	1.15 - 5.56	2.7462	1.36591	49.7373	0.48292
8	0.30 - 3.60	2.3737	2.37484	99.95	0.83963
9	1.30 - 2.70	1.9200	0.58589	30.5151	0.29294
10	0.83 - 3.00	2.1271	0.75652	35.5651	0.28593
11	0.99 - 4.92	2.4400	1.38643	56.8211	0.5660
12	0.64 - 3.48	2.2900	0.94276	41.1686	0.38488
13	1.60 - 7.62	3.9467	2.20535	55.879	0.73511
14	0.83 - 3.00	2.0518	0.67623	32.9579	0.20389

Table 4.2.1.9. **Variation for crown spread : dbh in some provenance collections o** *Azadirachta indica*

Site	Range	mean	sd	cv	se
1	1.88 - 3.74	27.9900	6.63148	23.69235	2.21049
2	16.86 - 33.19	23.7750	4.59623	19.33222	1.32681
3	16.21 - 31.66	22.5162	5.22947	23.22532	1.84889
4	13.53 - 40.57	26.7882	8.36176	31.21431	2.02802
5	15.90 - 82.00	36.6656	15.60821	42.56914	3.67889
6	17.50 - 42.67	27.9083	7.53057	26.98325	2.17389
7	12.24 - 31.67	21.4075	7.65163	35.74277	2.70526
8	14.99 - 33.08	23.7625	6.11358	25.72787	2.16147
9	10.08 - 33.32	20.87	9.94101	47.63305	4.97051
10	16.33 - 23.92	19.15	2.7406	14.31127	1.03585
11	29.44 - 41.86	37.11	4.93827	13.30711	2.01604
12	9.94 - 49.00	30.675	15.35132	50.04505	6.26715
13	11.61 - 75.00	34.3111	17.52211	51.06833	5.8407
14	10.08 - 33.32	19.7755	5.90819	29.8764	1.78138

Table 4.2.1.10. **Variation for crown spread in some provenance collections of** *Azadirachta indica*

Site	Range	mean	sd	cv	se
1	6.62 - 19.75	14.6722	3.95394	26.94852	1.31798
2	6.19 - 15.49	11.1558	2.50671	22.46997	0.72362
3	7.78 - 13.07	11.155	2.06796	18.53847	0.73113
4	3.36 - 18.95	10.3629	4.19088	40.44102	1.01643
5	6.58 - 12.94	9.8478	1.73831	17.65184	0.40972
6	5.12 - 21.20	10.3057	4.45656	43.23959	1.28649
7	5.78 - 21.50	13.28	5.05581	38.07086	1.78749
8	7.75 - 13.69	11.3612	2.24506	19.76068	0.79374
9	5.87 - 9.96	7.5525	1.95694	25.91119	0.97847
10	5.84 - 13.07	9.4386	2.53773	26.88688	0.95917
11	5.97 - 14.72	9.7667	3.8711	39.6359	1.58037
12	2.45 - 13.20	9.0717	3.95111	43.55449	1.61303
13	6.40 - 13.45	8.5589	2.11717	24.73658	0.70572
14	5.84 - 14.72	8.7527	2.43278	27.79459	0.73351

Table 4.2.1.11. Variation for crown spread : height in some provenance collections of *Azadirachta indica*

Site	Range	mean	sd	cv	se
1	0.78 - 1.10	0.9833	0.101	10.271	0.0337
2	0.49 - 1.20	0.7883	0.1879	23.835	0.0542
3	0.68 - 1.31	0.9337	0.2032	21.76	0.0718
4	0.59 - 1.88	1.0076	0.287	28.487	0.0696
5	0.78 - 1.79	0.9556	0.2327	24.354	0.0549
6	0.57 - 1.41	0.9908	0.2354	23.753	0.0679
7	0.61 - 1.79	1.0725	0.3657	34.098	0.1293
8	0.43 - 1.37	1.0137	0.2919	28.798	0.1032
9	0.33 - 0.93	0.6325	0.2450	38.735	0.1225
10	0.68 - 1.11	0.7429	0.3221	43.354	0.1217
11	0.42 - 1.16	0.8950	0.2786	31.131	0.1137
12	0.41 - 1.17	0.8567	0.3279	38.274	0.1339
13	0.44 - 1.21	0.7789	0.2189	28.11	0.073
14	0.33 - 1.11	0.7027	0.2887	41.081	0.087

provenances (1.88 - 82.00). Mean crown spread : dbh in different provenance collections varied from 19.15 to 37.11. Highest mean crown spread : dbh (37.11) was observed in Jodhpur provenance. Lowest mean crown spread : dbh was observed in Kalka + Pinjore (21.57 m) provenance. Hisar + Sirsa provenance exhibited highest level cv (51.07) followed by Bhiwani+Bhorugram (50.05). Lowest cv (13.31) was observed in Jodhpur provenance.

Phenotypic variation with respect to Crown spread in some provenance collections of Neem is given in **Table** 4.2.1.10. Crown spread exhibited a wide range in different provenances (2.45 m - 21.50 m). Mean Crown spread in different provenance collections varied from 7.56 m to 14.67 m. Highest mean Crown spread (14.67 m) was observed in Delhi provenance. Lowest mean Crown spread was observed in Hisar + Sirsa (8.56 m) provenance. Bhiwani + Bhorugram provenance exhibited highest level cv (43.55) followed by Jind + Narnaund (43.24). Lowest cv (17.65) was observed in Hisar provenance.

Phenotypic variation with respect to crown spread : height in some provenance collections of Neem is given in **Table** 4.2.1.11. Crown spread : height exhibited a wide range in different provenances (0.33 - 1.88). Mean crown spread : height in different provenance collections varied from 0.63 to 1.07. Highest mean crown spread : height (1.07) was observed in Kaithal + Kurukshetra provenance. Lowest mean crown spread : height was observed in Sirmour provenance (0.63). Kalka + Pinjore provenance exhibited highest level cv (43.35) followed by Sirmour + Kalka + Pinjore(41.08). Lowest cv (10.27) was observed in Delhi provenance.

4.2.2. Association of tree architecture characteristics

Association of different morphological characteristics of trees in all the 14 sites under study (**Table** 4.2.2.1.) was studied. Diameter at breast height (dbh) was positively and significantly associated with basal girth, height, crown spread and crown spread : height; and negatively and significantly associated with and height : dbh, clear bole height : height, unforked height: height, unforked height and Crown spread : dbh. Basal girth was positively and significantly associated with dbh, height, crown spread; and negatively and significantly associated with and height : dbh, clear bole height : height, unforked height: height, unforked height, and crown spread : dbh.

Height : dbh was positively and significantly associated with height and crown spread : dbh; and negatively and significantly associated with dbh, basal girth, crown spread and crown spread : height. Height was positively and significantly associated with dbh, basal girth, height : dbh, and crown spread; and negatively and significantly associated with clear bole height : height, unforked height: height, crown spread : dbh and crown spread : height.

Clear bole height : height was positively and significantly associated with clear bole, unforked height: height, unforked height, crown spread; and negatively and significantly

associated with dbh, basal girth, height, and crown spread. Clear bole was positively and significantly associated with clear bole : height, unforked height: height and unforked height.

Unforked height : height was positively and significantly associated with clear bole height : height, clear bole, and unforked height; and negatively and significantly associated with dbh, basal girth, height, and crown spread. Unforked height was positively and significantly associated with clear bole height : height, clear bole and unforked height : height; and negatively and significantly associated with crown spread.

Crown spread : dbh was positively and significantly associated with height : dbh, and negatively and significantly associated with dbh, basal girth, height. Crown spread was positively and significantly associated with dbh, basal girth and height; and negatively and significantly associated with and height : dbh, unforked height: height. Crown spread : height was positively and significantly associated with dbh, and crown spread.; and negatively and significantly associated with and height : dbh, and height.

Association of different morphological characteristics of trees in site 1 (Delhi) (**Table 4.2.2.2.**) was studied. Diameter at breast height (dbh) was positively and significantly associated with basal girth, and crown spread; and negatively and significantly associated with and Height : dbh, Clear bole height : Height, Unforked height: Height, and Crown spread : dbh. Basal girth was positively and significantly associated with dbh, crown spread; and negatively and significantly associated with clear bole height : height, unforked height: height, and crown spread : dbh.

Height : dbh was positively and significantly associated with clear bole, unforked height and crown spread : dbh; and negatively and significantly associated with dbh. Height was positively and significantly associated with crown spread; and negatively and significantly associated with clear bole height : height, and unforked height: height.

Clear bole height : height was positively and significantly associated with, unforked height: height, crown spread : dbh; and negatively and significantly associated with dbh, basal girth, height, and crown spread. Clear bole was positively and significantly associated with height : dbh, and crown spread : dbh.

Unforked height : height was positively and significantly associated with clear bole height : height, and unforked height; and negatively and significantly associated with dbh, basal girth, height, and crown spread. Unforked height was positively and significantly associated with height : dbh, and unforked height : height; and negatively and significantly associated with crown spread.

Crown spread : dbh was positively and significantly associated with clear bole height : height and clear bole, and negatively and significantly associated with dbh, and basal girth. Crown spread was positively and significantly associated with dbh, basal girth and height; and

Table 4.2.2.1. Association of tree architectural characteristics of trees of *Azadirachta indica* in all the 14 sites under study

	dbh	Basal girth	Height : dbh	Height	Clear bole height : Height	Clear bole	Unforked height: Height	Unforked height	Crown spread : dbh	Crown spread	Crown spread : height
Diameter at breast height (dbh)	1.0000	0.9287**	-0.5792**	0.5666**	-0.3226**	0.0479	-0.3124**	-0.0550	-0.6740**	0.7007**	0.1753*
Basal girth	-	1.0000	-0.5416**	0.5272**	-0.3046**	0.0469	-0.3013**	-0.0497	-0.6204**	0.6388**	0.1597
Height : dbh	-	-	-	0.0208	0.0398	0.0149	0.0683	0.1106	0.7277**	-0.3857**	-0.4200**
Height	-	-	-	1.0000	-0.5377**	0.1421	-0.4284**	0.0794	-0.2510**	0.5907**	-0.2823**
Clear bole height : Height	-	-	-	-	1.0000	0.5932**	0.7099**	0.4119**	0.0664	-0.4045**	0.0858
Clear bole	-	-	-	-	-	1.0000	0.5451**	0.7187**	-0.1463	-0.0059	-0.1707
Unforked height: Height	-	-	-	-	-	-	1.0000	0.8134**	0.0888	-0.3634**	0.0467
Unforked height	-	-	-	-	-	-	-	1.0000	-0.0150	-0.0699	-0.1465
Crown spread : dbh	-	-	-	-	-	-	-	-	1.0000	-0.1491	0.1300
Crown spread	-	-	-	-	-	-	-	-	-	1.0000	0.5118**
Crown spread : height	-	-	-	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.2.2.2. Association of tree architectural characteristics of trees of *Azadirachta indica* in site 1

	dbh	Basal girth	Height : dbh	Height	Clear bole height : height	Clear bole	Unforked height: height	Unforked height	Crown spread : dbh	Crown spread	Crown spread : height
Diameter at breast height (dbh)	1.0000	0.8444**	-0.7801*	0.6528	-0.9439**	-0.5183	-0.9073**	-0.6603	-0.8400**	0.7720*	0.0537
Basal girth	-	1.0000	-0.6452	0.6576	-0.8007**	-0.2797	-0.8391**	-0.5412	-0.7318*	0.7292*	-0.0662
Height : dbh	-	-	1.0000	-0.0529	0.6112	0.8119**	0.5437	0.7143*	0.8972**	-0.2544	-0.4138
Height	-	-	-	1.0000	-0.7698*	0.1786	-0.7879*	-0.1614	-0.2934	0.9301**	-0.4683
Clear bole height : Height	-	-	-	-	1.0000	0.4725	0.8586**	0.4733	0.7999**	-0.7750*	0.2525
Clear bole	-	-	-	-	-	1.0000	0.2067	0.5200	0.7757*	0.0837	-0.2244
Unforked height: Height	-	-	-	-	-	-	1.0000	0.6900*	0.5775	-0.9139**	-0.0555
Unforked height	-	-	-	-	-	-	-	1.0000	0.4933	-0.4443	-0.5996
Crown spread : dbh	-	-	-	-	-	-	-	-	1.0000	-0.3447	0.0276
Crown spread	-	-	-	-	-	-	-	-	-	1.0000	-0.1133
Crown spread : height	-	-	-	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.2.2.3. Association of tree architectural characteristics of trees of *Azadirachta indica* in site 2

	dbh	Basal girth	Height : dbh	Height	Clear bole height : height	Clear bole	Unforked height: height	Unforked height	Crown spread : dbh	Crown spread	Crown spread : height
Diameter at breast height (dbh)	1.0000	0.9316**	-0.7866**	0.3728	0.2936	0.4088	-0.0494	-0.0101	-0.6113*	0.7344**	0.4413
Basal girth		1.0000	-0.6226*	0.3810	0.1565	0.2746	-0.1341	-0.0680	-0.5719	0.6448*	0.3209
Height : dbh	-	-	1.0000	0.1768	-0.4101	-0.5219	-0.1410	0.0214	0.5336	-0.6344*	-0.7413**
Height	-	-	-	1.0000	0.1175	0.1506	-0.0361	0.2707	-0.0810	0.3135	-0.3755
Clear bole height : Height	-	-	-	-	1.0000	0.8657**	0.4459	0.4291	-0.1004	0.3535	0.2509
Clear bole	-	-	-	-	-	1.0000	0.6385*	0.5836*	-0.2210	0.3884	0.2633
Unforked height: Height	-	-	-	-	-	-	1.0000	0.9399**	0.3800	0.2724	0.2935
Unforked height	-	-	-	-	-	-	-	1.0000	0.4467	0.3334	0.1385
Crown spread : dbh	-	-	-	-	-	-	-	-	1.0000	0.0620	0.1158
Crown spread	-	-	-	-	-	-	-	-	-	1.0000	0.7496**
Crown spread : height	-	-	-	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.2.2.4. Association of tree architectural characteristics of trees of *Azadirachta indica* in site 3

	dbh	Basal girth	Height : dbh	Height	Clear bole height : height	Clear bole	Unforked height: height	Unforked height	Crown spread : dbh	Crown spread	Crown spread : height
Diameter at breast height (dbh)	1.0000	0.8187*	-0.6126	0.6953	-0.0279	0.4568	0.0484	0.3894	-0.7451*	0.5971	-0.2673
Basal girth	-	1.0000	-0.2230	0.8575**	-0.3965	0.3800	-0.0487	0.4740	-0.4980	0.6509	-0.3053
Height : dbh	-	-	1.0000	0.1327	-0.2726	0.1112	0.3871	0.3804	0.4064	-0.3366	-0.3458
Height	-	-	-	1.0000	-0.3063	0.6657	0.3921	0.8311*	-0.5458	0.4762	-0.6193
Clear bole height : Height	-	-	-	-	1.0000	0.4713	0.5194	0.0800	-0.2095	-0.3039	-0.0728
Clear bole	-	-	-	-	-	1.0000	0.8752**	0.9006**	-0.5529	0.1159	-0.6638
Unforked height: Height	-	-	-	-	-	-	1.0000	0.8302*	-0.3305	-0.2086	-0.6479
Unforked height	-	-	-	-	-	-	-	1.0000	-0.4609	0.1569	-0.7448*
Crown spread : dbh	-	-	-	-	-	-	-	-	1.0000	0.0664	0.7113*
Crown spread	-	-	-	-	-	-	-	-	-	1.0000	0.3787
Crown spread : height	-	-	-	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

negatively and significantly associated with clear bole height: height and unforked height: height.

Crown spread : height was not significantly associated with any of the tree architecture characteristics. Association of different morphological characteristics of trees in site 2 (Gurgaon) (**Table** 4.2.2.3.) was studied. Diameter at breast height (dbh) was positively and significantly associated with basal girth, and crown spread; and negatively and significantly associated with height : dbh and crown spread : dbh.

Basal girth was positively and significantly associated with dbh, and crown spread; and negatively and significantly associated with and height : dbh. Height : dbh was negatively and significantly associated with dbh, basal girth, crown spread and crown spread : height.

Height was not significantly associated with any of the tree architecture characteristics. Clear bole height : height was positively and significantly associated with clear bole. Clear bole was positively and significantly associated with clear bole : height, unforked height: height and unforked height.

Unforked height : height was positively and significantly associated with clear bole and unforked height. Unforked height was positively and significantly associated with clear bole and Unforked height : height.

Crown spread : dbh was negatively and significantly associated with dbh. Crown spread was positively and significantly associated with dbh, basal girth and crown spread : height; and negatively and significantly associated with height : dbh. Crown spread : height was positively and significantly associated with crown spread : dbh.; and negatively and significantly associated with and height : dbh.

Association of different morphological characteristics of trees in site 3 (Rohtak + Jhajjar) (**Table** 4.2.2.4.) was studied. Diameter at breast height (dbh) was positively and significantly associated with basal girth; and negatively and significantly associated with crown spread : dbh. Basal girth was positively and significantly associated with dbh, and height. Height : dbh was not significantly associated with any of the characters under study. Height was positively and significantly associated with basal girth, and unforked height. Clear bole height : height was not significantly associated with any of the tree architecture characteristics. Clear bole was positively and significantly associated with unforked height: height and unforked height. Unforked height : height was positively and significantly associated with clear bole, and unforked height. Unforked height was positively and significantly associated with height, clear bole and unforked height : height; and negatively and significantly associated with crown spread : height.

Crown spread : dbh was positively and significantly associated with crown spread : height, and negatively and significantly associated with dbh. Crown spread was not significantly associated with any of the tree architecture characteristics.

Crown spread : height was positively and significantly associated with crown spread : dbh; and negatively and significantly associated with unforked height. Association of different morphological characteristics of trees in site 4 (Alwar + Rewari) (**Table** 4.2.2.5.) was studied. Diameter at breast height (dbh) was positively and significantly associated with basal girth, height and crown spread; and negatively and significantly associated with and height : dbh, clear bole height : height, unforked height: height, and Crown spread : dbh.

Basal girth was positively and significantly associated with dbh, height, and crown spread; and negatively and significantly associated with height : dbh, height, clear bole height : height, unforked height: height, and crown spread : dbh.

Height : dbh was positively and significantly associated with clear bole height : height, unforked height : height; and negatively and significantly associated with dbh, basal girth, height, and crown spread.

Height was positively and significantly associated with dbh, basal girth, and crown spread; and negatively and significantly associated with height : dbh, clear bole height : height, unforked height: height, crown spread : dbh.

Clear bole height : height was positively and significantly associated with height : dbh, and unforked height: height; and negatively and significantly associated with dbh, basal girth, height, and crown spread. Clear bole was positively and significantly associated with unforked height: height and unforked height.

Unforked height : height was positively and significantly associated with height : dbh, clear bole height : height, clear bole, and unforked height; and negatively and significantly associated with dbh, basal girth, height, and crown spread. Unforked height was positively and significantly associated with clear bole and unforked height : height.

Crown spread : dbh was negatively and significantly associated with dbh, basal girth, height and crown spread. Crown spread was positively and significantly associated with dbh, basal girth and height; and negatively and significantly associated with and height : dbh, clear bole height : height, unforked height: height, crown spread : dbh. Crown spread : height was not significantly associated with any of the tree architecture characteristics.

Association of different morphological characteristics of trees in site 5 (Hisar) (**Table** 4.2.2.6.) was studied. Diameter at breast height (dbh) was positively and significantly associated with basal girth; and negatively and significantly associated with Height : dbh, and Crown spread : dbh.

Table 4.2.2.5. Association of tree architectural characteristics of trees of *Azadirachta indica* in site 4

	dbh	Basal girth	Height : dbh	Height	Clear bole height : height	Clear bole	Unforked height: height	Unforked height	Crown spread : dbh	Crown spread	Crown spread : height
Diameter at breast height (dbh)	1.0000	0.9320**	-0.6500**	0.8938**	-0.5229*	0.0999	-0.5803*	-0.0944	-0.8681**	0.8506**	-0.0587
Basal girth	-	1.0000	-0.7017**	0.8515**	-0.5957*	-0.1051	-0.7131**	-0.2803	-0.7539**	0.8211**	-0.0220
Height : dbh	-	-	1.0000	-0.5100*	0.4965*	0.1863	0.6034*	0.2983	0.4343	-0.7080**	-0.4627
Height	-	-	-	1.0000	-0.6831**	0.1674	-0.6428**	-0.0101	-0.8541**	0.7895**	-0.3058
Clear bole height : Height	-	-	-	-	1.0000	0.2198	0.7354**	0.1464	0.4356	-0.5828*	0.2630
Clear bole	-	-	-	-	-	1.0000	0.5556*	0.8902**	-0.3802	-0.0371	-0.2172
Unforked height: Height	-	-	-	-	-	-	1.0000	0.7004**	0.4099	-0.6395**	0.0544
Unforked height	-	-	-	-	-	-	-	1.0000	-0.1561	-0.1881	-0.2439
Crown spread : dbh	-	-	-	-	-	-	-	-	1.0000	-0.5882*	0.3521
Crown spread	-	-	-	-	-	-	-	-	-	1.0000	0.2472
Crown spread : height	-	-	-	-	-	-	-	-	-	-.	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.2.2.6. Association of tree architectural characteristics of trees of *Azadirachta indica* in site 5

	dbh	Basal girth	Height : dbh	Height	Clear bole height : height	Clear bole	Unforked height: height	Unforked height	Crown spread : dbh	Crown spread	Crown spread : height
Diameter at breast height (dbh)	1.0000	0.9697**	-0.6770**	0.4605	-0.2296	-0.0521	-0.1013	0.0763	-0.7413**	0.4162	-0.2021
Basal girth	-	1.0000	-0.6493**	0.4612	-0.2651	-0.1616	-0.1546	0.0007	-0.7103**	0.4375	-0.1826
Height : dbh	-	-	1.0000	0.2379	-0.2551	-0.1693	-0.3113	-0.2241	0.9235**	0.1941	-0.2264
Height	-	-	-	1.0000	-0.7286**	-0.2820	-0.6163**	-0.2139	-0.0109	0.7388**	-0.7332**
Clear bole height : Height	-	-	-	-	1.0000	0.6978**	0.6260**	0.3799	-0.1061	-0.6095**	0.4764*
Clear bole	-	-	-	-	-	1.0000	0.5094*	0.5843*	-0.2116	-0.3643	-0.0615
Unforked height: Height	-	-	-	-	-	-	1.0000	0.8750**	-0.1711	-0.4235	0.4287
Unforked height	-	-	-	-	-	-	-	1.0000	-0.2213	-0.1362	0.0334
Crown spread : dbh	-	-	-	-	-	-	-	-	1.0000	0.1855	0.1486
Crown spread	-	-	-	-	-	-	-	-	-	1.0000	-0.1609
Crown spread : height	-	-	-	-	-	-	-	-	-	-.	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.2.2.7. Association of tree architectural characteristics of trees of *Azadirachta indica* in site 6

	dbh	Basal girth	Height : dbh	Height	Clear bole height : height	Clear bole	Unforked height: height	Unforked height	Crown spread : dbh	Crown spread	Crown spread : height
Diameter at breast height (dbh)	1.0000	0.9972**	-0.7946**	0.7904**	-0.3937	0.0993	-0.2650	0.1997	-0.6253*	0.9414**	0.6249*
Basal girth	-	1.0000	-0.7650**	0.8071**	-0.3735	0.1348	-0.2670	0.1989	-0.6178*	0.9396**	0.5965*
Height : dbh	-	-	1.0000	-0.3981	0.0423	-0.2190	-0.1527	-0.4164	0.7303**	-0.6804*	-0.6561*
Height	-	-	-	1.0000	-0.5232	0.1197	-0.3711	0.2551	-0.5255	0.7938**	0.1904
Clear bole height : Height	-	-	-	-	1.0000	0.7209**	0.5636	0.1855	-0.1079	-0.5169	-0.3331
Clear bole	-	-	-	-	-	1.0000	0.4555	0.4847	-0.5618	-0.0621	-0.3149
Unforked height: Height	-	-	-	-	-	-	1.0000	0.7861**	-0.0978	-0.3114	-0.0984
Unforked height	-	-	-	-	-	-	-	1.0000	-0.4673	0.1472	-0.0221
Crown spread : dbh	-	-	-	-	-	-	-	-	1.0000	-0.3772	-0.0047
Crown spread	-	-	-	-	-	-	-	-	-	1.0000	0.7406**
Crown spread : height	-	-	-	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.2.2.8. Association of tree architectural characteristics of trees of *Azadirachta indica* in site 7

	dbh	Basal girth	Height : dbh	Height	Clear bole height : height	Clear bole	Unforked height: height	Unforked height	Crown spread : dbh	Crown spread	Crown spread : height
Diameter at breast height (dbh)	1.0000	0.9844**	-0.8874**	0.5629	-0.4026	-0.1433	-0.2711	-0.0577	-0.6240	-0.5618	0.3847
Basal girth	-	1.0000	-0.8953**	0.4420	-0.2602	-0.0177	-0.1484	0.0382	-0.7371*	-0.4635	0.2903
Height : dbh	-	-	1.0000	-0.2671	0.1187	-0.1588	0.2010	0.0492	0.5547	0.5977	-0.5789
Height	-	-	-	1.0000	-0.8666**	-0.6157	-0.4628	-0.1813	-0.0285	-0.5587	0.2103
Clear bole height : Height	-	-	-	-	1.0000	0.9080**	0.7442*	0.5386	-0.2123	-0.5439	-0.2433
Clear bole	-	-	-	-	-	1.0000	0.7713*	0.6774	-0.3504	-0.3131	-0.0681
Unforked height: Height	-	-	-	-	-	-	1.0000	0.9502**	-0.3884	-0.6087	-0.4897
Unforked height	-	-	-	-	-	-	-	1.0000	-0.4706	-0.4364	-0.4076
Crown spread : dbh	-	-	-	-	-	-	-	-	1.0000	0.2600	0.3352
Crown spread	-	-	-	-	-	-	-	-	-	1.0000	0.9256**
Crown spread : height	-	-	-	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Basal girth was positively and significantly associated with dbh; and negatively and significantly associated with and height : dbh, and crown spread : dbh. Height : dbh was positively and significantly associated with crown spread : dbh; and negatively and significantly associated with dbh, and basal girth. Height was positively and significantly associated with crown spread; and negatively and significantly associated with clear bole height : height, unforked height: height, and crown spread : height.

Clear bole height : height was positively and significantly associated with clear bole, unforked height: height, crown spread : height; and negatively and significantly associated with height, and crown spread. Clear bole was positively and significantly associated with clear bole : height, unforked height: height and unforked height.

Unforked height : height was positively and significantly associated with clear bole height : height, clear bole, and unforked height; and negatively and significantly associated with height. Unforked height was positively and significantly associated with clear bole and unforked height : height.

Crown spread : dbh was positively and significantly associated with height : dbh, and negatively and significantly associated with dbh, basal girth. Crown spread was positively and significantly associated with height; and negatively and significantly associated with clear bole height : height. Crown spread : height was positively and significantly associated with clear bole height : height.; and negatively and significantly associated with height.

Association of different morphological characteristics of trees in site 6 (Jind + Narnaund) (**Table** 4.2.2.7.) was studied. Diameter at breast height (dbh) was positively and significantly associated with basal girth, height, crown spread and crown spread : height; and negatively and significantly associated with Height : dbh, and Crown spread : dbh. Basal girth was positively and significantly associated with dbh, height, crown spread; and negatively and significantly associated with and height : dbh, and crown spread : dbh.

Height : dbh was positively and significantly associated with crown spread : dbh; and negatively and significantly associated with dbh, basal girth, crown spread and crown spread : height. Height was positively and significantly associated with dbh, basal girth, and crown spread. Clear bole height : height was positively and significantly associated with clear bole. Clear bole was positively and significantly associated with clear bole : height.

Unforked height : height was positively and significantly associated with unforked height. Unforked height was positively and significantly associated with unforked height : height. Crown spread : dbh was positively and significantly associated with height : dbh, and negatively and significantly associated with dbh, and basal girth. Crown spread was positively and significantly associated with dbh, basal girth, height and crown spread : height; and negatively and significantly associated with and height : dbh.

Crown spread : height was positively and significantly associated with dbh, basal girth, and crown spread; and negatively and significantly associated with height : dbh. Association of different morphological characteristics of trees in site 7 (Kaithal + Kurukshetra) (**Table** 4.2.2.8.) was studied. Diameter at breast height (dbh) was positively and significantly associated with basal girth; and negatively and significantly associated with and Height : dbh.

Basal girth was positively and significantly associated with dbh; and negatively and significantly associated with and height : dbh, and crown spread : dbh. Height : dbh was negatively and significantly associated with dbh, and basal girth. Height was negatively and significantly associated with clear bole height : height.

Clear bole height : height was positively and significantly associated with, clear bole, unforked height: height; and negatively and significantly associated with height. Clear bole was positively and significantly associated with clear bole height : height, and unforked height: height.

Unforked height : height was positively and significantly associated with clear bole height : height, clear bole, and unforked height. Unforked height was positively and significantly associated with unforked height : height. Crown spread : dbh was negatively and significantly associated with basal girth. Crown spread was positively and significantly associated with crown spread : height. Crown spread : height was positively and significantly associated with crown spread.

Association of different morphological characteristics of trees in site 8 (Ambala + Yamunanagar + Patiala) (**Table** 4.2.2.9.) was studied. Diameter at breast height (dbh) was positively and significantly associated with basal girth.

Basal girth was positively and significantly associated with dbh; and negatively and significantly associated with crown spread : dbh. Height : dbh was positively and significantly associated with height; and negatively and significantly associated with crown spread : height.

Height was positively and significantly associated with height : dbh, crown spread : height. Clear bole height : height was positively and significantly associated with clear bole, unforked height: height, and unforked height. Clear bole was positively and significantly associated with clear bole : height, unforked height: height and unforked height.

Unforked height : height was positively and significantly associated with clear bole height : height, clear bole, and unforked height. Unforked height was positively and significantly associated with clear bole height : height , clear bole and unforked height : height.

Crown spread : dbh was negatively and significantly associated with basal girth. Crown spread was not significantly associated with any of the tree architecture parameters. Crown spread : height was positively and significantly associated with height : dbh, and height.

Table 4.2.2.9. Association of tree architectural characteristics of trees of *Azadirachta indica* in site 8

	dbh	Basal girth	Height : dbh	Height	Clear bole height : height	Clear bole	Unforked height: Height	Unforked height	Crown spread : dbh	Crown spread	Crown spread : height
Diameter at breast height (dbh)	1.0000	0.9905**	-0.5706	0.1495	-0.5271	-0.5420	-0.4550	-0.4953	-0.6892	0.4805	0.0749
Basal girth	-	1.0000	-0.5553	0.1577	-0.4799	-0.4768	-0.4363	-0.4956	-0.7244*	0.4145	-0.0301
Height : dbh	-	-	1.0000	0.7219*	-0.1005	0.1091	-0.1326	-0.0326	0.2932	-0.4147	-0.7803*
Height	-	-	-	1.0000	-0.5671	-0.3280	-0.5486	-0.4635	-0.2075	-0.0699	0.8755**
Clear bole height : Height	-	-	-	-	1.0000	0.9320**	0.9413**	0.9672**	0.2786	-0.4404	-0.1706
Clear bole	-	-	-	-	-	1.0000	0.7926*	0.8829**	0.3810	-0.3194	0.0169
Unforked height: Height	-	-	-	-	-	-	1.0000	0.9829**	0.1226	-0.5129	0.1383
Unforked height	-	-	-	-	-	-	-	1.0000	0.1952	-0.4957	0.0569
Crown spread : dbh	-	-	-	-	-	-	-	-	1.0000	0.2718	0.2897
Crown spread	-	-	-	-	-	-	-	-	-	1.0000	0.5039
Crown spread : height	-	-	-	-	-	-	-	-	-	-.	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.2.2.10. Association of tree architectural characteristics of trees of *Azadirachta indica* in site 9

	dbh	Basal girth	Height : dbh	Height	Clear bole height : height	Clear bole	Unforked height: height	Unforked height	Crown spread : dbh	Crown spread	Crown spread : height
Diameter at breast height (dbh)	1.0000	0.9996**	-0.7778	-0.1048	-0.3293	-0.4344	-0.4418	-0.5814	-0.8747	0.2670	0.0118
Basal girth	-.	1.0000	-0.7721	0.0876	-0.3337	-0.4096	-0.4443	-0.5678	-0.8663	0.2910	0.0113
Height : dbh	-	-	1.0000	0.6725	-0.3327	0.2793	-0.2100	0.1266	0.4851	-0.3700	-0.6143
Height	-	-	-	1.0000	-0.8083	0.2481	-0.7270	-0.2231	-0.0799	0.0297	-0.8191
Clear bole height : Height	-	-	-	-	1.0000	0.3357	0.9907**	0.7507	0.6228	0.2634	0.9299
Clear bole	-	-	-	-	-	1.0000	0.4261	0.8628	0.7423	0.7399	0.3265
Unforked height: Height	-	-	-	-	-	-	1.0000	0.8198	0.7233	0.2673	0.8887
Unforked height	-	-	-	-	-	-	-	1.0000	0.9029	0.5611	0.6614
Crown spread : dbh	-	-	-	-	-	-	-	-	1.0000	0.1923	0.3892
Crown spread	-	-	-	-	-	-	-	-	-	1.0000	0.5026
Crown spread : height	-	-	-	-	-	-	-	-	-	-.	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.2.2.11. Association of tree architectural characteristics of trees of *Azadirachta indica* in site 10

	dbh	Basal girth	Height : dbh	Height	Clear bole height : height	Clear bole	Unforked height: height	Unforked height	Crown spread : dbh	Crown spread	Crown spread : height
Diameter at breast height (dbh)	1.0000	0.8190*	-0.8927**	0.5919	-0.1458	0.1332	-0.1458	0.1342	-0.5594	0.8755**	0.1279
Basal girth	-	1.0000	-0.7462	0.6271	-0.1252	0.2116	-0.1252	0.2122	-0.6339	0.6487	0.0371
Height : dbh	-	-	1.0000	-0.2248	0.0295	-0.1630	0.0295	-0.1652	0.6311	-0.6962	0.0787
Height	-	-	-	1.0000	-0.4339	-0.1094	-0.4339	-0.1099	-0.0627	0.7182	0.2421
Clear bole height : Height	-	-	-	-	1.0000	0.9314**	1.0000**	0.9314**	-0.3986	-0.4058	-0.3835
Clear bole	-	-	-	-	-	1.0000	0.9314**	1.0000**	-0.5569	-0.1447	-0.3503
Unforked height: Height	-	-	-	-	-	-	1.0000	0.9314**	-0.3986	-0.4058	-0.3835
Unforked height	-	-	-	-	-	-	-	1.0000	-0.5584	-0.1442	-0.3542
Crown spread : dbh	-	-	-	-	-	-	-	-	1.0000	-0.0953	0.3911
Crown spread	-	-	-	-	-	-	-	-	-	1.0000	0.3458
Crown spread : height	-	-	-	-	-	-		-	-	-.	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.2.2.12. Association of tree architectural characteristics of trees of *Azadirachta indica* in site 11

	dbh	Basal girth	Height : dbh	Height	Clear bole height : height	Clear bole	Unforked height: height	Unforked height	Crown spread : dbh	Crown spread	Crown spread : height
Diameter at breast height (dbh)	1.0000	0.9946**	-0.7384	0.5083	0.2736	0.6793	0.4394	0.7449	-0.7607	0.9606**	0.6887
Basal girth	-	1.0000	-0.7087	0.5617	0.1996	0.6326	0.3688	0.7025	-0.7384	0.9709**	0.6676
Height : dbh	-	-	1.0000	0.1777	-0.4207	-0.3777	-0.4851	-0.4229	0.2271	-0.8096	-0.9864**
Height	-	-	-	1.0000	-0.2822	0.3650	-0.1316	0.4061	-0.6986	0.4255	-0.1997
Clear bole height : Height	-	-	-	-	1.0000	0.7682	0.9716**	0.6966	-0.3811	0.1375	0.3906
Clear bole	-	-	-	-	-	1.0000	0.8652*	0.9813**	-0.8755*	0.4828	0.3090
Unforked height: Height	-	-	-	-	-	-	1.0000	0.8312*	-0.5274	0.2812	0.4278
Unforked height	-	-	-	-	-	-	-	1.0000	-0.8859*	0.5508	0.3350
Crown spread : dbh	-	-	-	-	-	-	-	-	1.0000	-0.5690	-0.1487
Crown spread	-	-	-	-	-	-	-	-	-	1.0000	0.7924
Crown spread : height	-	-	-	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Association of different morphological characteristics of trees was studied in site 9 (Sirmour) (**Table** 4.2.2.10.) was studied. Diameter at breast height (dbh) was positively and significantly associated with basal girth. Basal girth, Height : dbh, Height, Clear bole, Unforked height : height, Unforked height, Crown spread : dbh, Crown spread, and Crown spread : height were not significantly associated with any of the tree architecture characteristics. Clear bole height : height was positively and significantly associated with unforked height: height.

Association of different morphological characteristics of trees in site 10 (Kalka + Pinjore) is depicted in **Table** 4.2.2.11. Diameter at breast height (dbh) was positively and significantly associated with basal girth and crown spread and negatively and significantly associated with Height : dbh. Basal girth was positively and significantly associated with dbh. Height : dbh was positively and significantly associated with dbh. Height was not significantly associated with any of the tree architecture characteristics. Clear bole height : height was positively and significantly associated with clear bole, unforked height: height, and unforked height.

Clear bole was positively and significantly associated with clear bole : height, unforked height: height and unforked height. Unforked height : height was positively and significantly associated with clear bole height : height, clear bole, and unforked height. Unforked height was positively and significantly associated with clear bole height : height , clear bole and unforked height : height. Crown spread : dbh was not significantly associated with any of the tree architecture characteristics. Crown spread was positively and significantly associated with dbh. Crown spread : height was not significantly associated with any of the tree architecture characteristics.

Association of different morphological characteristics of trees was studied in site 11 (Jodhpur) (**Table** 4.2.2.12.) was studied. Diameter at breast height (dbh) was found to be positively and significantly associated with basal girth Crown spread.

Basal girth was positively and significantly associated with dbh, crown spread. Height : dbh was negatively and significantly associated with crown spread : height. Clear bole height : height was positively and significantly associated with unforked height: height. Clear bole was positively and significantly associated with unforked height: height and unforked height; and negatively and significantly associated with crown spread : dbh.

Unforked height : height was positively and significantly associated with clear bole height : height, clear bole, and unforked height. Unforked height was positively and significantly associated with clear bole and unforked height : height; and negatively and significantly associated with crown spread : dbh.

Crown spread : dbh was negatively and significantly associated with clear bole, unforked height. Crown spread was positively and significantly associated with dbh, and basal girth. Crown spread : height negatively and significantly associated with and height : dbh.

Association of different morphological characteristics of trees in site 12 (Bhiwani+Bhorugram) was studied (**Table** 4.2.2.13.) was studied. Diameter at breast height (dbh) was positively and significantly associated with basal girth and height; and negatively and significantly associated with Crown spread : dbh and Height : dbh. Basal girth was positively and significantly associated with dbh, and height; and negatively and significantly associated with height : dbh, and crown spread : dbh. Height : dbh was negatively and significantly associated with dbh, basal girth, height.

Height was negatively and significantly associated with dbh and basal girth. Crown spread : dbh was negatively and significantly associated with dbh and basal girth. Crown spread was positively and significantly associated with crown spread : height

Association of different morphological characteristics of trees in site 13 (Hisar+Sirsa) is given in **Table** 4.2.2.14 was studied. Diameter at breast height (dbh) was positively and significantly associated with basal girth; and negatively and significantly associated with height : dbh and crown spread : dbh.

Basal girth was positively and significantly associated with dbh and negatively and significantly associated with and height : dbh, and crown spread : dbh. Height : dbh was positively and significantly associated with height and crown spread : dbh; and negatively and significantly associated with dbh, and basal girth. Height was positively and significantly associated with height : dbh, and crown spread : dbh. Clear bole height : height was positively and significantly associated with, and clear bole, and unforked height: height. Clear bole was positively and significantly associated with clear bole : height, unforked height: height and unforked height; and negatively and positively associated with crown spread : height.

Unforked height : height was positively and significantly associated with clear bole height : height, clear bole, and unforked height; and negatively and significantly associated with crown spread : height, and crown spread. Unforked height was positively and significantly associated with clear bole and unforked height : height; and negatively and significantly associated with crown spread : height. Crown spread : dbh was positively and significantly associated with height : dbh, and height; and negatively and significantly associated with dbh and basal girth. Crown spread was positively and significantly associated with crown spread : height; and negatively and significantly associated with unforked height: height. Crown spread : height was positively and significantly associated with crown spread; and negatively and significantly associated with clear bole, unforked height : height, and unforked height.

Table 4.2.2.13. Association of tree architectural characteristics of trees of *Azadirachta indica* in site 12

	dbh	Basal girth	Height : dbh	Height	Clear bole height : height	Clear bole	Unforked height: height	Unforked height	Crown spread : dbh	Crown spread	Crown spread : height
Diameter at breast height (dbh)	1.0000	0.9763**	-0.8498*	0.9352**	-0.4348	0.1311	-0.4348	0.1311	-0.9335**	0.3803	0.1256
Basal girth	-	1.0000	-0.8402*	0.9713**	-0.5511	-0.0109	-0.5511	-0.0109	-0.8784*	0.3688	0.1032
Height : dbh	-	-	1.0000	-0.8979*	0.5677	-0.0548	0.5677	-0.0548	0.7297	-0.7508	-0.5732
Height	-	-	-	1.0000	-0.7075	-0.1680	-0.7075	-0.1680	-0.7788	-0.5216	0.2717
Clear bole height : Height	-	-	-	-	1.0000	0.7890	1.0000	0.7890	0.1131	-0.6047	-0.4550
Clear bole	-	-	-	-	-	1.0000	0.7890	1.0000	-0.4373	-0.2117	-0.1744
Unforked height: Height	-	-	-	-	-	-	1.0000	0.7890	0.1131	-0.6047	-0.4550
Unforked height	-	-	-	-	-	-	-	1.0000	-0.4373	-0.2117	-0.1744
Crown spread : dbh	-	-	-	-	-	-	-	-	1.0000	-0.1460	0.0795
Crown spread	-	-	-	-	-	-	-	-	-	1.0000	0.9607**
Crown spread : height	-	-	-	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.2.2.14. Association of tree architectural characteristics of trees of *Azadirachta indica* in site 13

	dbh	Basal girth	Height : dbh	Height	Clear bole height : height	Clear bole	Unforked height: height	Unforked height	Crown spread : dbh	Crown spread	Crown spread : height
Diameter at breast height (dbh)	1.0000	0.9769**	-0.7006*	-0.3560	0.5929	0.4021	0.4291	0.2206	-0.8214**	-0.0813	0.0523
Basal girth	-	1.0000	-0.6904*	-0.3483	0.6378	0.4423	0.3856	0.1860	-0.7954*	-0.0520	0.0725
Height : dbh	-	-	1.0000	0.8834**	-0.2926	0.2144	0.0224	0.4114	0.9176**	-0.1408	-0.5297
Height	-	-	-	1.0000	-0.1647	0.4423	0.1376	0.5671	0.7637*	-0.0510	-0.5540
Clear bole height : Height	-	-	-	-	1.0000	0.8041**	0.8163**	0.5999	-0.5773	-0.6232	-0.4670
Clear bole	-	-	-	-	-	1.0000	0.8176**	0.8826**	-0.1162	-0.5770	-0.7428*
Unforked height: Height	-	-	-	-	-	-	1.0000	0.8903**	-0.3126	-0.7026*	-0.6818*
Unforked height	-	-	-	-	-	-	-	1.0000	0.0734	-0.6206	-0.8285**
Crown spread : dbh	-	-	-	-	-	-	-	-	1.0000	0.2230	-0.1578
Crown spread	-	-	-	-	-	-	-	-	-	1.0000	0.8543**
Crown spread : height	-	--	-	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Association of different morphological characteristics of trees in site 14 (Sirmour + Kalka + Pinjore) (**Table** 4.2.2.15.) was studied. dbh was positively and significantly associated with basal girth and crown spread and negatively and significantly associated with height : dbh and crown spread : dbh. Basal girth was positively and significantly associated with dbh; and negatively and significantly associated with height : dbh and crown spread : dbh. Height : dbh was positively and significantly associated with height; and negatively and significantly associated with dbh and basal girth. Height was positively and significantly associated with height : dbh. Clear bole height : height was positively and significantly associated with, and clear bole, unforked height: height, and unforked height. Clear bole was positively and significantly associated with clear bole : height, unforked height: height and unforked height.

Unforked height : height was positively and significantly associated with clear bole height : height, clear bole, and unforked height. Unforked height was positively and significantly associated with clear bole height : height, clear bole and unforked height : height. Crown spread : dbh was negatively and significantly associated with dbh and basal girth. Crown spread was positively and significantly associated with dbh. Crown spread : height was not associated with any of the tree architecture parameters.

Qualitative data was analysed with the help of Kendall's rank correlation coefficient (τ). Based on data of 126 trees, association of branch angle index with apical dominance index was found to be negative and significant (-0.174) at five per cent level of significance. The association of branch angle index with fruiting intensity was found to be negative and non-significant (-0.077) at five per cent level of significance. The association of apical dominance index with fruiting intensity index was found to be negative and significant (-0.196) at five per cent level of significance. Crown shape index was found to be negatively and non-significantly associated with branch angle index (-0.133) at five per cent level of significance. The association of crown shape index with apical dominance index was found to be positive and significant (0.487) at one per cent level of significance. Association of crown shape index with fruiting index was found to be negative and significant (-0.204) at one per cent level of significance.(**Table** 4.2.2.16.)

4.2.3. Regression analysis of the tree architecture parameters

Multiple regression analysis of Clear bole height : Height on all other tree architecture characteristics in *Neem* in four provenances individually (Site 2 = Gurgaon + Faridabad, Site 4 = Alwar + Rewari, Site 5 = Hisar, Site 6 = Jind + Narnaund) and 14 sites combined was done (**Table** 4.2.3.1.). In individual provenances coefficient of determination (R^2) was very high (0.88 to 0.98), pointing to very high level of goodness of fitness of the regression line. For all the 14 sites combined, coefficient of determination (R^2) was very high (0.84). Multiple regression analysis Clear bole on all other tree architecture characteristics in Neem was done (**Table** 4.2.3.2.). In individual provenances coefficient of determination (R^2)

able 4.2.2.15. Association of tree architectural characteristics of trees of *Azadirachta indica* in site 14

	dbh	Basal girth	Height : dbh	Height	Clear bole height : height	Clear bole	Unforked height: height	Unforked height	Crown spread : dbh	Crown spread	Crown spread : height
lbh	1.0000	0.9052**	-0.6893*	0.0662	-0.0853	0.0836	-0.1994	-0.0534	-0.6928*	0.6869*	0.1293
3asal girth	-	1.0000	-0.7057*	0.0494	-0.0674	0.1208	-0.1975	-0.0343	-0.7564**	0.5311	0.0725
Ieight : dbh	-	-	1.0000	0.6049*	-0.2714	-0.1140	-0.201	-0.0577	0.5040	-0.4625	-0.3155
Ieight	-	-	-	1.0000	-0.5483	-0.1013	-0.5817	-0.1861	-0.0163	0.1375	-0.2803
Clear bole height : Height	-	-	-	-	1.0000	0.8701**	0.9600**	0.8773**	0.0373	-0.0928	-0.0511
Clear bole	-	-	-	-	-	1.0000	0.7959**	0.9502**	-0.0403	0.0994	-0.1774
Jnforked height: Height	-	-	-	-	-	-	1.0000	0.8908**	0.2499	-0.1010	0.0275
Jnforked height	-	-	-	-	-	-	-	1.0000	0.1983	0.0768	-0.0896
Crown spread : dbh	-	-	-	-	-	-	-	-	1.0000	-0.0071	0.2598
Crown spread	-	-	-	-	-	-	-	-	-	1.0000	0.4198
Crown spread : height	-	-	-	-	-	-	-	-	-	-	1.0000

*Significant at 1% level *Significant at 5% level of significance

ıble 4.2.2.16. Association amongst qualitative parameters of trees of *A. indica* based on Kendall's rank correlation coefficient (τ)

	Apical dominance index	Branch angle index	Crown Shape index	Fruiting intensity index
pical dominance index	1.000	-.174*	.487**	-.196*
ranch angle index		1.000	-.133	-.077
rown Shape index			1.000	-.204**

ignificant at the .05 level
significant at the .01 level
:126

Table 4.2.2.15. Association of tree architectural characteristics of trees of *Azadirachta indica* in site 14

	dbh	Basal girth	Height : dbh	Height	Clear bole height : height	Clear bole	Unforked height: height	Unforked height	Crown spread : dbh	Crown spread	Crown spread : height
dbh	1.0000	0.9052**	-0.6893*	0.0662	-0.0853	0.0836	-0.1994	-0.0534	-0.6928*	0.6869*	0.1293
Basal girth	-	1.0000	-0.7057*	0.0494	-0.0674	0.1208	-0.1975	-0.0343	-0.7564**	0.5311	0.0725
Height : dbh	-	-	1.0000	0.6049*	-0.2714	-0.1140	-0.201	-0.0577	0.5040	-0.4625	-0.3155
Height	-	-	-	1.0000	-0.5483	-0.1013	-0.5817	-0.1861	-0.0163	0.1375	-0.2803
Clear bole height : Height	-	-	-	-	1.0000	0.8701**	0.9600**	0.8773**	0.0373	-0.0928	-0.0511
Clear bole	-	-	-	-	-	1.0000	0.7959**	0.9502**	-0.0403	0.0994	-0.1774
Unforked height: Height	-	-	-	-	-	-	1.0000	0.8908**	0.2499	-0.1010	0.0275
Unforked height	-	-	-	-	-	-	-	1.0000	0.1983	0.0768	-0.0896
Crown spread : dbh	-	-	-	-	-	-	-	-	1.0000	-0.0071	0.2598
Crown spread	-	-	-	-	-	-	-	-	-	1.0000	0.4198
Crown spread : height	-	-	-	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.2.2.16. Association amongst qualitative parameters of trees of *A. indica* based on Kendall's rank correlation coefficient (τ)

	Apical dominance index	Branch angle index	Crown Shape index	Fruiting intensity index
Apical dominance index	1.000	-.174*	.487**	-.196*
Branch angle index		1.000	-.133	-.077
Crown Shape index			1.000	-.204**

* significant at the .05 level
** significant at the .01 level
N=126

Table 4.2.3.1. Multiple regression analysis of clear bole height : height on all other tree architecture characteristics in *Azadirachta indica*

Site	Constant	Regression Co-efficients										R^2
		b_1	b_2	b_3	b_4	b_5	b_6	b_7	b_8	b_9	b_{10}	
Global*	0.044548	0.003309	0.004757	0.000821	-0.005458	0.085383	0.579455	-0.055533	-0.000596	-0.001255	0.031062	0.841934
2	1.414952	-1.104361	-0.004519	-0.003387	-0.046491	0.086043	-3.026163	0.192018	-0.024890	0.050480	-0.072664	0.920363
4	0.332186	-0.345741	0.001897	-0.003527	0.010994	0.118989	1.677686	-0.225061	-0.007283	0.014940	-0.103135	0.976976
5	-0.076022	-0.400654	0.137287	0.002199	0.009391	0.105103	0.323140	-0.032485	0.004245	-0.029544	0.139081	0.880350
6	0.780150	0.875534	-0.316877	-0.019032	-0.000971	0.104948	1.329214	-0.147108	0.017031	0.036816	-0.905677	0.959763

* all the sites taken together

Table 4.2.3.2. Multiple regression analysis of clear bole on all other tree architecture characteristics in *Azadirachta indica*

Site	Constant	Regression Co-efficients										R^2
		b_1	b_2	b_3	b_4	b_5	b_6	b_7	b_8	b_9	b_{10}	
Global*	0.403950	-0.397539	-0.009620	-0.003435	0.030760	7.066772	-3.896968	0.527204	-0.005993	0.040995	-0.406293	0.820973
2	-0.072042	-1.008665	-0.201771	0.071680	-0.044831	7.697746	19.630135	-1.029665	-0.174283	0.599032	-3.872234	0.957002
4	-1.064141	1.081069	-0.021291	0.020463	-0.021636	5.202021	-7.902436	1.266254	0.009859	-0.067362	0.783196	0.965597
5	1.883470	4.370220	-1.061736	0.010737	-0.075162	6.235731	-7.071475	0.749311	-0.015134	-0.082922	0.598862	0.871306
6	4.731942	-4.895628	0.306868	0.085296	-0.439803	4.464297	0.388995	0.126534	-0.144034	0.572932	-2.551286	0.967595

*all the sites taken together

Table 4.2.3.3. **Multiple regression analysis of Unforked height: Height on all other tree architecture characteristics in *Azadirachta indica***

Site	Constant	Regression Co-efficients										R^2
		b_1	b_2	b_3	b_4	b_5	b_6	b_7	b_8	b_9	b_{10}	
Global*	0.102939	0.085709	-0.016508	-0.004025	-0.007915	0.535435	-0.403508	0.089747	0.000356	-0.008938	0.082095	0.943884
2	0.536958	-0.440110	0.002591	-0.001309	-0.015689	-0.175518	0.012726	0.067025	-0.010317	0.019068	-0.029231	0.998081
4	-0.236788	0.264920	-0.004235	0.002979	-0.007962	0.486797	-0.052448	0.124824	0.004889	-0.011931	0.093441	0.995899
5	0.015210	-0.096915	0.058112	-0.004355	0.019784	0.044070	-0.016255	0.099728	0.006273	-0.062663	0.337704	0.994947
6	-0.382167	-0.860039	0.308006	0.004415	0.005800	0.148624	0.001022	0.095289	-0.001964	-0.039531	6.523197	0.997223

*all the sites taken together

Table 4.2.3.4. **Multiple regression analysis of Unforked height on all other tree architecture characteristics in *Azadirachta indica***

Site	Constant	Regression Co-efficients										R^2
		b_1	b_2	b_3	b_4	b_5	b_6	b_7	b_8	b_9	b_{10}	
Global*	-1.062876	-0.775091	0.151653	0.004749	0.078719	-5.488161	0.629517	9.598652	-0.002371	0.072049	-0.663942	0.935849
2	-8.801280	7.348099	0.002950	0.007756	0.276969	2.411717	-0.144536	14.514151	0.197904	-0.400490	0.879296	0.998301
4	1.666289	-1.784307	0.013630	-0.021389	0.053019	-3.928153	0.505525	7.508410	-0.032300	0.083793	-0.693857	0.994650
5	-0.093366	1.071729	-0.596664	0.045192	-0.210550	-0.440389	0.171218	9.913314	-0.064491	0.634277	-3.369319	0.993819
6	3.962834	8.884545	-3.163022	-0.053177	-0.044357	-1.782515	0.036043	10.326221	0.029033	0.391618	-5.451921	0.997258

* all the sites taken together

was very high (0.87 to 0.97), pointing to very high level of goodness of fitness of the regression line. For all the 14 sites combined, coefficient of determination (R^2) was very high (0.82).

Multiple regression analysis of Unforked height: Height on all other tree architecture characteristics in Neem was done (**Table** 4.2.3.3.). In individual provenances coefficient of determination (R^2) was very high (0.994 to 0.998), pointing to very low level of goodness of fitness of the regression line. For all the 14 sites combined, coefficient of determination (R^2) was very high (0.94).

Multiple regression analysis of Unforked height on all other tree architecture characteristics in Neem was done (**Table** 4.2.3.4.). In individual provenances coefficient of determination (R^2) was very high (0.993 to 0.998), pointing to very low level of goodness of fitness of the regression line. For all the 14 sites combined, coefficient of determination (R^2) was very high (0.94).

4.3. Endocarp morphology

4.3.1. Variation with respect to endocarp morphological characteristics

Endocarp morphological characteristics of some trees of Hisar provenance were studied (**Table** 4.3.1.1.). Endocarp length showed a range of 0.69 to 1.75 cm. Highest value for Endocarp length (1.75 cm) was observed tree NGN1 of Pune provenance and lowest value for Endocarp length (0.69 cm) was observed tree T23 of Jodhpur provenance. Lowest mean Endocarp length (0.778 cm) was found in tree T23 of Jodhpur provenance. Highest mean endocarp breadth (0.812 cm) was found in tree H100 of Kalka provenance. Lowest mean Endocarp length (0.489 cm) was found in tree HSR03 of Hisar provenance. Highest coefficient of variation (14.73) for this character was observed tree T30 of Alwar provenance, while tree NMN4 of Pune provenance exhibited a lowest value of 2.07.

Endocarp breadth showed a range of 0.42 to 0.93 cm. Highest value for Endocarp breadth (0.93 cm) was observed in tree H099 of Kalka provenance and lowest value for Endocarp breadth (0.42 cm) was observed in tree T33 of Alwar provenance. Highest mean Endocarp breadth (cm) was found in tree H100 of Kalka provenance. Lowest mean Endocarp breadth (0.489 cm) was found in tree HSR03 of Hisar provenance. Highest coefficient of variation (22.69) for this character was observed in tree T30 of Alwar provenance while tree NMN4 of Pune provenance exhibited a lowest value of 2.42.

Endocarp weight showed a range of 0.05 to 0.413 gm. Highest value for Endocarp length (0.413 gm) was observed in tree T44 of Raipur provenance and lowest value for Endocarp weight (0.05 gm) was observed in tree HSR03 of provenance Hisar. Highest mean Endocarp weight (0.3116 gm) was found in tree T44 of Raipur provenance. Lowest mean Endocarp weight (0.0844 gm) was found in tree T33 of Alwar provenance. Highest coefficient of

Table 4.3.1.1. Variability w.r.t. endocarp morphological characteristics in some provenance collections of *Azadirachta indica*

Genotype number	Tree Id. No	Provenance	Year of collection	Endocarp length 1			Endocarp breadth 2			Endocarp weight 3			Seed weight 4		
				Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.
2	T3	Bikaner	1997	1.22-1.32	1.2480 (0.0421)	3.37	0.590-0.770	0.663 (0.0719)	10.84	0.178-0.267	0.212 (0.0389)	18.35	0.107-0.168	0.1312 (0.0275)	20.96
3	T4	Bikaner	1997	1.07-1.25	1.1820 (0.0740)	6.26	0.650-0.700	0.669 (0.0188)	2.81	0.162-0.187	0.1756 (0.0108)	6.15	0.077-0.089	0.0848 (0.0049)	5.78
4	T5	Bikaner	1997	1.11-1.21	1.1500 (0.0406)	3.53	0.560-0.630	0.613 (0.0299)	4.87	0.143-0.223	0.1894 (0.0289)	15.26	0.078-0.117	0.1004 (0.0141)	14.04
7	T41	Raipur	1997	1.23-1.50	1.3340 (0.1019)	7.64	0.660-0.840	0.704 (0.0766)	10.88	0.212-0.353	0.2462 (0.0599)	24.33	0.118-0.206	0.1374 (0.0384)	27.95
8	T42	Raipur	1997	1.48-1.62	1.5320 (0.0554)	3.62	0.670-0.720	0.697 (0.0217)	3.11	0.238-0.315	0.2792 (0.0311)	11.14	0.124-0.157	0.1422 (0.0141)	9.92
9	T43	Raipur	1997	1.20-1.40	1.3100 (0.0846)	6.46	0.580-0.685	0.635 (0.0379)	5.97	0.093-0.109	0.1858 (0.0115)	6.19	0.078-0.094	0.1014 (0.0067)	6.61
10	T44	Raipur	1997	1.48-1.68	1.5800 (0.0748)	4.73	0.695-0.810	0.737 (0.0438)	5.94	0.269-0.413	0.3116 (0.0578)	18.55	0.134-0.233	0.1652 (0.0390)	23.61
11	T45	Raipur	1997	1.48-1.66	1.532 (0.1314)	8.58	0.520-0.710	0.613 (0.0835)	13.62	0.160-0.281	0.2302 (0.0523)	22.72	0.081-0.191	0.1288 (0.0421)	32.69
12	T62	Bhatinda	1997	1.13-1.30	1.222 (0.0743)	6.08	0.640-0.715	0.661 (0.0311)	4.70	0.145-0.248	0.2098 (0.0400)	19.07	0.024-0.133	0.1024 (0.0445)	43.47
13	T63	Bhatinda	1997	1.140-1.420	1.27 (0.1114)	8.98	0.665-0.810	0.711 (0.0569)	8.00	0.197-0.341	0.2352 (0.0597)	25.38	0.109-0.197	0.1316 (0.0370)	28.12
14	T64	Bhatinda	1997	1.100-1.390	1.292 (0.1258)	9.74	0.645-0.735	0.686 (0.0351)	5.25	0.178-0.270	0.2188 (0.0381)	17.41	0.029-0.158	0.1102 (0.0489)	44.37
24	T5D	Delhi	1995	1.000-1.340	1.188 (0.1252)	10.54	0.600-0.825	0.668 (0.0914)	13.68	0.148-0.335	0.2 (0.0778)	38.90	0.042-0.185	0.094 (0.0542)	57.66
25	T7D	Delhi	1995	0.970-1.150	1.08 (0.0707)	6.55	0.505-0.575	0.556 (0.0290)	5.22	0.113-0.153	0.1396 (0.0186)	13.32	0.058-0.077	0.0704 (0.0088)	12.50
15	T23	Jodhpur	1997	0.690-0.870	0.778 (0.0712)	9.15	0.505-0.610	0.55 (0.0433)	7.87	0.067-0.103	0.091 (0.0148)	16.26	0.035-0.058	0.0484 (0.0083)	17.15
16	T24	Jodhpur	1997	0.880-0.980	0.944 (0.0416)	4.41	0.480-0.515	0.494 (0.0171)	3.46	0.084-0.102	0.0914 (0.0093)	10.18	0.039-0.059	0.0474 (0.0088)	18.57

Table 4.3.1.1. (continued)

Genotype number	Tree Id. No	Prove-nance	Year of collection	Endocarp length 1			Endocarp breadth 2			Endocarp weight 3			Seed weight 4		
				Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.
17	T20	Jodhpur	1997	0.970-1.070	1.014 (0.0404)	3.98	0.580-0.710	0.621 (0.0524)	8.44	0.087-0.154	0.1268 (0.0249)	19.64	0.005-0.051	0.033 (0.0218)	66.06
18	T22	Jodhpur	1997	0.900-1.040	0.964 (0.0611)	6.34	0.485-0.580	0.515 (0.0382)	7.42	0.090-0.141	0.1122 (0.0206)	18.36	0.038-0.062	0.0524 (0.0101)	19.27
19	T21	Jodhpur	1998	0.990-1.07	1.022 (0.0356)	3.48	0.565-0.745	0.636 (0.0679)	10.68	0.129-0.163	0.1532 (0.0144)	9.40	0.062-0.091	0.0181 (0.0090)	49.72
29	HSR01	Hisar	1998	1.010-1.220	1.092 (0.0850)	7.78	0.515-0.615	0.568 (0.0432)	7.61	0.079-0.192	0.1344 (0.0415)	30.88	0.013-0.104	0.0638 (0.0340)	53.29
30	HSR02	Hisar	1998	1.130-1.380	1.244 (0.0918)	7.38	0.535-0.615	0.565 (0.0322)	5.70	0.128-0.191	0.1504 (0.0244)	16.22	0.018-0.083	0.06 (0.0248)	41.33
1	T17	Hisar	1997	1.10-1.21	1.1680 (0.0432)	3.70	0.525-0.615	0.5760 (0.0445)	7.73	0.115-0.178	0.1536 (0.0268)	17.45	0.048-0.087	0.0706 (0.0141)	19.97
31	HSR03	Hisar	1998	0.950-1.030	0.992 (0.0363)	3.66	0.465-0.535	0.489 (0.0272)	5.56	0.050-0.105	0.0884 (0.0228)	25.79	0.004-0.057	0.0424 (0.0219)	51.65
32	HSR04	Hisar	1998	1.120-1.440	1.3 (0.1386)	10.66	0.470-0.575	0.516 (0.0441)	8.55	0.095-0.178	0.1386 (0.0354)	25.54	0.023-0.071	0.051 (0.0218)	42.75
33	HSR05	Hisar	1998	0.93-1.160	1.048 (0.0998)	9.52	0.480-0.560	0.522 (0.0303)	5.80	0.075-0.140	0.1176 (0.0259)	22.02	0.011-0.063	0.0506 (0.0223)	44.07
34	SHS-0	Hisar	1998	1.170-1.320	1.232 (0.0614)	4.98	0.515-0.625	0.572 (0.0492)	8.60	0.139-0.194	0.1658 (0.0229)	13.81	0.066-0.095	0.0812 (0.0127)	15.64
5	T18	Hisar	1997	1.27-1.50	1.3640 (0.0862)	6.32	0.640-0.725	0.687 (0.0382)	5.56	0.226-0.312	0.2636 (0.0363)	13.77	0.136-0.171	0.1522 (0.0148)	9.72
6	T52	Kaithal	1997	1.15-1.30	1.2060 (0.0611)	5.07	0.560-0.620	0.595 (0.0298)	5.01	0.154-0.214	0.1756 (0.0235)	13.38	0.083-0.111	0.0932 (0.0113)	12.12
36	H082	Kaithal	1998	0.910-1.120	1.024 (0.0770)	7.52	0.500-0.580	0.532 (0.0363)	6.82	0.090-0.135	0.1188 (0.0172)	14.48	0.042-0.058	0.0512 (0.0066)	12.89
26	H083	Kaithal	1998	1.22-1.38	1.314 (0.0817)	6.22	0.54-0.62	0.573 (0.0337)	5.88	0.143-0.194	0.1684 (0.0193)	11.46	0.045-.091	0.0748 (0.0177)	23.66

Table 4.3.1.1. (continued)

Genotype number	Tree Id. No	Prove-nance	Year of collection	Endocarp length 1			Endocarp breadth 2			Endocarp weight 3			Seed weight 4		
				Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.
37	H084	Kaithal	1998	1.060-1.320	1.186 (0.0979)	8.25	0.535-0.670	0.589 (0.0676)	11.48	0.148-0.229	0.176 (0.0351)	19.94	0.072-0.115	0.0866 (0.0176)	20.32
38	H085	Kaithal	1998	1.260-1.380	1.32 (0.0552)	4.18	0.505-0.565	0.537 (0.0217)	4.04	0.140-0.178	0.159 (0.0151)	9.50	0.049-0.089	0.0748 (0.0156)	20.86
27	T30	Alwar	1998	1.05-1.46	1.202 (0.1771)	14.73	0.485-0.810	0.592 (0.1343)	22.69	0.086-0.251	0.147 (0.0697)	47.14	0.029-0.135	0.0712 (0.0439)	61.66
28	T31	Alwar	1998	0.800-0.960	0.88 (0.0718)	8.16	0.500-0.575	0.532 (0.0353)	6.64	0.089-0.123	0.1028 (0.0144)	14.01	0.029-0.040	0.038 (0.0061)	16.05
39	T33 (1998)	Alwar	1998	0.830-1.160	1.008 (0.1333)	13.22	0.420-0.560	0.491 (0.0667)	13.58	0.052-0.122	0.0844 (0.0328)	38.86	0.015-0.043	0.026 (0.0123)	47.31
40	TGN3	Pune	1995	1.190-1.470	1.31 (0.1027)	7.84	0.510-0.655	0.607 (0.0565)	9.31	0.136-0.195	0.1736 (0.0238)	13.71	0.079-0.112	0.0942 (0.0124)	13.16
41	NMN4	Pune	1995	1.220-1.2880	1.252 (0.0259)	2.07	0.620-0.660	0.644 (0.0156)	2.42	0.163-0.222	0.1948 (0.0220)	11.29	0.092-0.118	0.1066 (0.0110)	10.32
42	TER2	Pune	1995	1.220-1.510	1.374 (0.1101)	8.01	0.585-0.615	0.601 (0.0119)	1.98	0.146-0.219	0.1874 (0.0302)	16.12	0.071-0.123	0.1008 (0.0203)	20.14
43	NGN1	Pune	1995	1.550-1.750	1.628 (0.0817)	5.02	0.520-0.625	0.557 (0.0422)	7.58	0.171-0.217	0.1972 (0.0240)	12.17	0.077-0.108	0.0952 (0.0133)	13.97
35	H100	Kalka	1998	1.100-1.190	1.132 (0.0383)	3.38	0.780-0.845	0.812 (0.0289)	3.56	0.209-0.263	0.231 (0.0206)	8.92	0.091-0.111	0.1066 (0.0127)	11.91
20	H101	Kalka	1998	1.300-1.460	1.404 (0.0669)	4.76	0.630-0.725	0.682 (0.0375)	5.50	0.123-0.280	0.21 (0.0605)	28.81	0.017-0.132	0.0874 (0.0436)	49.89
21	H102	Kalka	1998	0.850-1.080	1 (0.0962)	9.62	0.635-0.820	0.723 (0.0711)	9.83	0.08-0.203	0.1294 (0.0514)	39.72	0.010-0.105	0.0544 (0.0430)	79.04
22	H107	Kalka	1998	1.300-1.500	1.364 (0.0921)	6.75	0.665-0.795	0.703 (0.0537)	7.64	0.232-0.362	0.2624 (0.0559)	21.30	0.095-0.165	0.1204 (0.0267)	22.18
23	H099	Kalka	1995	1.070-1.22	1.154 (0.0594)	5.15	0.715-0.930	0.791 (0.0845)	10.68	0.174-0.294	0.2218 (0.0447)	20.15	0.085-0.160	0.114 (0.0280)	24.56
				C.D. at 5%	0.1055			0.0629			0.0442			0.0313	

Table 4.3.1.1. (continued)

Genotype number	Tree Id. No	Prove-nance	Year of collection	Endocarp coat weight 5			Number of seeds endocarp^{-1} 6			Seed weight : : Endocarp weight 7			Endocarp length : Endocarp breadth 8		
				Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.
2	T3	Bikaner	1997	0.027-0.155	0.0808 (0.0469)	58.04	1-2	1.4 (0.5477)	39.12	0.419-0.862	0.6328 (0.1587)	25.10	1.714-2.068	1.8946 (0.1429)	7.54
3	T4	Bikaner	1997	0.082-0.100	0.0908 (0.0079)	8.70	1-1	1 (0.0000)	-	0.457-0.476	0.4834 (0.0209)	4.32	1.529-1.894	1.7696 (0.1425)	8.05
4	T5	Bikaner	1997	0.065-0.106	0.0890 (0.0151)	16.97	1-1	1 (0.0000)	-	0.520-0.545	0.5312 (0.0127)	2.39	1.762-2.000	1.8788 (0.0911)	4.85
7	T41	Raipur	1997	0.094-0.147	0.1088 (0.0216)	19.85	1-2	1.2 (0.4472)	37.27	0.536-.0584	0.5554 (0.0181)	3.26	1.786-2.030	1.901 (0.0978)	5.14
8	T42	Raipur	1997	0.114-0.159	0.137 (0.0179)	13.07	1-1	1 (0.0000)	-	0.491-0.523	0.51 (0.0156)	3.06	2.153-2.266	2.1982 (0.0512)	2.33
9	T43	Raipur	1997	0.078-0.094	0.0844 (0.0062)	7.35	1-1	1 (0.0000)	-	0.530-0.565	0.5458 (0.0155)	2.84	1.920-2.397	2.069 (0.1887)	9.12
10	T44	Raipur	1997	0.135-0.180	0.1464 (0.0191)	13.05	1-2	1.2 (0.4472)	37.27	0.498-0.564	0.5268 (0.0248)	4.71	2.056-2.302	2.1466 (0.0974)	4.54
11	T45	Raipur	1997	0.079-0.125	0.1014 (0.0215)	21.20	1-2	1.4 (0.5477)	39.12	0.506-0.680	0.552 (0.0726)	13.15	2.338-2.904	2.5176 (0.2244)	8.91
12	T62	Bhatinda	1997	0.097-0.121	0.1074 (0.0123)	11.45	1-1	1 (0.0000)	-	0.166-0.556	0.4654 (0.1677)	36.03	1.725-2.000	1.8654 (0.1211)	6.49
13	T63	Bhatinda	1997	0.088-0.144	0.1036 (0.0229)	22.10	1-2	1.2 (0.4472)	37.27	0.537-0.578	0.5572 (0.0173)	3.10	1.714-1.914	1.7866 (0.0832)	4.66
14	T64	Bhatinda	1997	0.070-0.149	0.1086 (0.0282)	25.97	1-1	1 (0.0000)	-	0.163-0.622	0.4896 (0.1872)	38.24	1.654-2.093	1.8838 (0.1658)	8.80
24	T5D	Delhi	1995	0.077-0.150	0.106 (0.0269)	25.38	1-2	1.2 (0.4472)	37.27	0.284-0.552	0.4492 (0.1006)	22.40	1.624-2.016	1.7878 (0.1548)	8.66
25	T7D	Delhi	1995	0.055-0.077	0.0692 (0.0098)	14.16	1-1	1 (0.0000)	-	0.497-0.513	0.5048 (0.0058)	1.15	1.689-2.099	1.9468 (0.1597)	8.20
15	T23	Jodhpur	1997	0.032-0.054	0.0426 (0.0086)	20.19	1-2	1.2 (0.4472)	-	0.476-0.575	0.5324 (0.0422)	7.93	1.266-1.689	1.4204 (0.1632)	11.49
16	T24	Jodhpur	1997	0.041-0.048	0.044 (0.0027)	6.14	1-1	1 (0.0000)	37.27	0.464-0.584	0.5134 (0.0498)	9.70	1.709-2.042	1.914 (0.1252)	6.54

Table 4.3.1.1. (continued)

Genotype number	Tree Id. No	Prove-nance	Year of collection	Endocarp coat weight 5			Number of seeds endocarp^{-1} 6			Seed weight : : Endocarp weight 7			Endocarp length : Endocarp breadth 8		
				Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	
17	T20	Jodhpur	1997	0.078-0.111	0.0938 (0.0140)	14.93	1-1	1 (0.0000)	-	0.057-0.395	0.2426 (0.1484)	61.17	1.465-1.740	1.6396 (0.1160)	7.07
18	T22	Jodhpur	1997	0.049-0.079	0.0598 (0.0117)	19.57	1-2	1.2 (0.4472)	-	0.422-0.496	0.4666 (0.0334)	7.16	1.793-1.961	1.8736 (0.0637)	3.40
19	T21	Jodhpur	1998	0.062-0.079	0.0714 (0.0064)	8.96	1-1	1 (0.0000)	-	0.512-0.558	0.5332 (0.0185)	3.47	1.409-1.770	1.6176 (0.1321)	8.17
29	HSR01	Hisar	1998	0.053-0.088	0.0706 (0.0127)	17.99	1-1	1 (0.0000)	-	0.165-0.573	0.4432 (0.1663)	37.52	1.669-2.112	1.9284 (0.1672)	8.67
30	HSR02	Hisar	1998	0.059-0.120	0.0904 (0.0240)	26.55	1-1	1 (0.0000)	-	0.130-0.539	0.397 (0.1556)	39.19	2.093-2.250	2.2008 (0.0678)	3.08
1	T17	Hisar	1997	0.06-0.101	0.083 (0.0169)	20.36	1-1	1 (0.0000)	-	0.416-0.540	0.4596 (0.0539)	11.73	1.95-2.21	2.0340 (0.1126)	5.54
31	HSR03	Hisar	1998	0.041-0.055	0.047 (0.0053)	11.28	1-1	1 (0.0000)	-	0.080-0.538	0.4404 (0.2022)	45.91	1.925-2.168	2.032 (0.1015)	5.00
32	HSR04	Hisar	1998	0.072-0.109	0.0876 (0.0150)	17.12	1-1	1 (0.0000)	-	0.242-0.447	0.3538 (0.0801)	22.64	2.333-2.784	2.5192 (0.1700)	6.75
33	HSR05	Hisar	1998	0.056-0.078	0.067 (0.0080)	11.94	1-1	1 (0.0000)	-	0.147-0.500	0.408 (0.1473)	36.10	1.714-2.229	2.015 (0.2441)	12.11
34	SHS-0	Hisar	1998	0.073-0.099	0.0846 (0.0107)	12.65	1-1	1 (0.0000)	-	0.468-0.510	0.4892 (0.0179)	3.66	1.888-2.563	2.17 (0.2573)	11.86
5	T18	Hisar	1997	0.087-0.141	0.1114 (0.0218)	19.57	1-1	1 (0.0000)	-	0.548-0.615	0.5800 (0.0257)	4.43	1.862-2.083	1.9866 (0.0907)	4.57
6	T52	Kaithal	1997	0.071-0.103	0.0824 (0.0124)	15.05	1-1	1 (0.0000)	-	0.519-0.545	0.5314 (0.0105)	1.98	1.855-2.196	2.0306 (0.1367)	6.73
36	H082	Kaithal	1998	0.048-0.078	0.0676 (0.0117)	17.31	1-1	1 (0.0000)	-	0.390-0.467	0.433 (0.0305)	7.04	1.741-2.240	1.932 (0.2046)	39.53
26	H083	Kaithal	1998	0.055-0.103	0.0936 (0.0076)	8.12	1-1	1 (0.0000)	-	0.315-0.482	0.4394 (0.0699)	15.91	2.103-2.509	2.2972 (0.1613)	7.02
37	H084	Kaithal	1998	0.071-0.114	0.0894 (0.0186)	20.81	1-1	1 (0.0000)	-	0.467-0.536	0.4924 (0.0280)	5.69	1.832-2.280	2.0236 (0.1644)	8.12

Table 4.3.1.1. (continued)

Genotype number	Tree Id. No	Provenance	Year of collection	Endocarp coat weight 5			Number of seeds endocarp^{-1} 6			Seed weight : : Endocarp weight 7			Endocarp length : Endocarp breadth 8		
				Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.	Range	Mean (S.E.)	C.V.
38	H085	Kaithal	1998	0.076-0.091	0.0842 (0.0062)	7.36	1-1	1 (0.0000)	-	0.350-0.500	0.4668 (0.0655)	14.03	2.336-2.561	2.4596 (0.0992)	4.03
27	T30	Alwar	1998	0.050-0.116	0.0758 (0.0264)	34.83	1-3	1.4 (0. 8944)	63.89	0.322-0.538	0.4574 (0.0879)	19.22	1.802-2.165	2.0558 (0.1461)	7.11
28	T31	Alwar	1998	0.051-0.080	0.0648 (0.0136)	20.99	1-1	1 (0.0000)	-	0.248-0.427	0.37 (0.0716)	19.35	1.600-1.699	1.653 (0.0364)	2.20
39	T33	Alwar	1998	0.036-0.080	0.0584 (0.0215)	36.82	1-1	1 (0.0000)	-	0.231-0.352	0.304 (0 0489)	16.09	1.908-2.310	2.0578 (0.1524)	7.41
40	TGN3	Pune	1995	0.057-0.097	0.0794 (0.0143)	18.01	1-1	1 (0.0000)	-	0.419-0.580	0.512 (0.0598)	11.68	1.954-2.410	2.168 (0.1958)	9.03
41	NMN4	Pune	1995	0.071-0.104	0.0882 (0.0131)	14.85	1-1	1 (0.0000)	-	0.516-0.589	0.5484 (0.0286)	5.22	1.891-2.065	1.9452 (0.0684)	3.52
42	TER2	Pune	1995	0.075-0.104	0.0866 (0.0127)	14.67	1-1	1 (0.0000)	-	0.486-0.582	0.5354 (0.0396)	7.40	2.085-2.455	2.284 (0.1441)	6.31
43	NGN1	Pune	1995	0.085-0.112	0.102 (0.0119)	11.67	1-1	1 (0.0000)	-	0.469-0.503	0.4822 (0.0222)	4.60	2.592-3.192	2.9334 (0.2257)	7.69
35	H100	Kalka	1998	0.0109-0.140	0.1244 (0.0146)	11.74	1-1	1 (0.0000)	-	0.409-0.504	0.4614 (0.0393)	8.52	1.310-1.488	1.3962 (0.0824)	5.90
20	H101	Kalka	1998	0.103-0.148	0.1226 (0.0198)	16.15	1-1	1 (0.0000)	-	0.138-0.471	0.3888 (0.1407)	36.19	1.959-2.195	2.0604 (0.0857)	4.16
21	H102	Kalka	1998	0.052-0.098	0.075 (0.0167)	22.27	1-1	1 (0.0000)	-	0.125-0.540	0.3676 (0.2198)	59.79	1.317-1.436	1.3838 (0.0527)	3.81
22	H107	Kalka	1998	0.122-0.197	0.142 (0.0314)	22.11	1-1	1 (0.0000)	-	0.406-0.496	0.4588 (0.0339)	7.39	1.786-2.256	1.9476 (0.1825)	9.37
23	H099	Kalka	1995	0.089-0.134	0.1078 (0.0168)	15.58	1-2	1.2 (0.4472)	37.27	0.489-0.544	0.5108 (0.0202)	3.95	1.312-1.678	1.4698 (0.1503)	10.23
				C.D. (5 %)	0.0218			0.3177			0.1139			0.1797	

variation (47.14) for this character was observed in tree T30 of Alwar provenance while tree T4 of Bikaner provenance exhibited a lowest value of 6.15.

Seed weight showed a range of 0.004 to 0.233 gm. Highest value for Seed weight (0.233 gm) was observed in tree T44 of Raipur provenance and lowest value for Seed weight (0.004 gm) was observed in tree HSR03 of Hisar provenance. Highest mean Seed weight (0.1652 gm) was found in tree T44 of Raipur provenance. Lowest mean Seed weight (0.0141 gm) was found in tree T21 of Jodhpur provenance. Highest coefficient of variation (79.04) for this character was observed in tree H102 of Kalka provenance. while tree T4 of Bikaner provenance exhibited a lowest value of 5.78.

Endocarp coat weight showed a range of 0.027 to 0.197 gm. Highest value for Endocarp coat weight (0.197 gm) was observed in tree H107 of Kalka provenance and lowest value for Endocarp coat weight (0.027 gm) was observed in tree T3 of Bikaner provenance. Highest mean Endocarp coat weight (1.464 gm) was found in tree T44 of Raipur provenance. Lowest mean Endocarp coat weight (0.0426 gm) was found in tree T23 of Jodhpur provenance. Highest coefficient of variation (58.04) for this character was observed in tree T3 of Bikaner provenance, while tree T24 of Jodhpur exhibited a lowest value of 6.14.

Number of seeds endocarp^{-1} showed a range of one to 3. Highest value for number of seeds endocarp^{-1} (3) was observed in tree T30 of Alwar provenance and lowest value for and number of seeds endocarp^{-1} (1) was observed in all the provenance collections. Highest mean number of seeds endocarp^{-1} (1.4) was found in three trees namely, T3 of Bikaner provenance, T44 of Raipur provenance and T30 of Alwar provenance. Lowest number of seeds endocarp^{-1} (1) was found in T33 trees of different provenance. Highest coefficient of variation (63.89) for this character was observed in tree T30 of Alwar provenance followed by tree T3 of Bikaner and tree T45 of Raipur both of the exhibiting a coefficient of variation of 39.12, while 34 tree of different provenance collections exhibited nil coefficient of variation.

Seed weight : Endocarp weight showed a range of 0.057 to 0.862. Highest value for Seed weight : Endocarp weight (0.862) was observed in tree T3 of Bikaner provenance and low value for Seed weight : Endocarp weight (0.057) observed in tree T20 of Jodhpur provenance. Highest mean Seed weight : Endocarp weight(0.6328) was found in tree T3 of Bikaner provenance. Lowest mean Seed weight : Endocarp weight (0.2426) was found in tree T20 of Jodhpur provenance. Highest coefficient of variation (61.17) for this character was observed in tree 20 of Jodhpur provenance. while tree T7D of Delhi exhibited a lowest value of 1.15.

Highest value for Endocarp length : Endocarp breadth (3.192) was observed in tree NGN1 of Pune provenance and lowest value for Endocarp length : Endocarp breadth (1.266) was observed in tree T23 of Jodhpur provenance. Highest mean Endocarp length : Endocarp breadth (2.9334) was found in tree NGN1 of Pune provenance. Lowest mean Endocarp length : Endocarp breadth (1.3838) was

Table 4.3.1.2. **Percentage of trilocular endocarps containing 0-3 seeds in different trees of some provenance collections of *Azadirachta indica***

Accession Number of tree	Provenance	Total number of endocarps	Total number of seeds	Total number of seeds as %age of endocarps	Proportion (%) of endocarps showing			
					0* seed	1 seed	2 seeds	3 seeds
ALD1	Allahabad	11.00	11.00	100.00	0	100	0	0
DRN1	Dehradun	24.00	28.00	116.67	0	83.33	16.67	0
DRN2	Dehradun	16.00	19.00	118.75	12.5	56.25	31.25	0
DRN3	Dehradun	21.00	22.00	104.76	0	95.24	4.76	0
DRN4	Dehradun	25.00	25.00	100.00	0	100	0	0
KTL1	Kaithal	17.00	12.00	70.59	29.41	70.59	0	0
KTL2	Kaithal	13.00	10.00	76.92	23.08	76.92	0	0
KTL3	Kaithal	13.00	12.00	92.31	15.38	76.92	7.69	0
KTL4	Kaithal	14.00	10.00	71.43	28.57	71.43	0	0
KTL5	Kaithal	15.00	10.00	66.67	33.33	66.67	0	0
KTL6	Kaithal	10.00	10.00	100.00	0	100	0	0
HSR1	Hisar	10.00	12.00	120.00	0	80	20	0
HSR2	Hisar	10.00	12.00	120.00	0	80	20	0
HSR3	Hisar	10.00	11.00	110.00	0	90	10	0
HSR4	Hisar	10.00	10.00	100.00	0	100	0	0
HSR5	Hisar	10.00	10.00	100.00	0	100	0	0
HSR6	Hisar	10.00	11.00	110.00	0	90	10	0
HSR7	Hisar	10.00	10.00	100.00	0	100	0	0
BNR1	Bikaner	10.00	11.00	110.00	0	90	10	0
BNR2	Bikaner	10.00	13.00	130.00	0	70	30	0
BNR3	Bikaner	11.00	14.00	127.27	0	72.73	27.27	0
BNR4	Bikaner	10.00	11.00	110.00	0	90	10	0
BNR5	Bikaner	11.00	16.00	145.45	9.09	36.36	54.54	0
NMN4	Pune	10.00	10.00	100.00	0	100	0	0
TGN3	Pune	10.00	10.00	100.00	0	100	0	0
TER2	Pune	10.00	12.00	120.00	10	70	10	10
NGN1	Pune	11.00	11.00	100.00	0	100	0	0
KLK1	Pune	10.00	10.00	100.00	20	60	20	0
KLK2	Kalka	10.00	10.00	100.00	0	100	0	0
KLK3	Kalka	10.00	10.00	100.00	20	60	20	0
KLK4	Kalka	10.00	9.00	90.00	20	70	10	0
KLK5	Kalka	11.00	11.00	100.00	0	100	0	0
Mean		12.28	12.59	103.46	6.92	83.01	9.76	0.31
Range				70.59-145.45	0-33.33	56.25-100.00	0-54.54	0-10
S.D.				17.10	10.73	16.78	12.84	1.77
C.V.				33.49	155.11	20.22	131.60	565.69

*contains one shrivelled seed per endocarp

χ^2 = 141.263**, N = 393, d.f. = 62, P<0.01 (when 0, 1, and 2 seed(s) per endocarps are considered)

Table 4.3.1.3. Provenance variation w.r.t. percentage of endocarps containing 0-3 seeds in different provenance collections of *Azadirachta indica*

Provenance	Number of trees (total N of endocarps sampled)	Total Number of endocarps	Total Number of seeds as %age of endocarps	Mean (%) number of endocarps showing			
				0 seeds	1 seeds	2 seeds	3 seeds
Dehradun	4 (86)	94	109.30 (9.10)	2.63 (±5.26)	77.43 (±23.32)	11.28 (±11.66)	0.00
Kaithal	4 (57)	44	77.81 (10.06)	32.09 (±11.50)	95.83 (±8.33)	2.08 (±8.33)	0.00
Hisar	4 (40)	45	112.50 (9.57)	0.00	78.79 (±15.84)	10.61 (±7.92)	0.00
Bikaner	4 (41)	49	119.32 (10.82)	0.00	68.66 (±15.26)	15.67 (±7.63)	0.00
Pune	4 (41)	43	105.00 (10.00)	2.08 (±4.17)	89.58 (±20.83)	2.08 (±4.17)	2.08 (±4.17)
Kalka	4 (40)	39	97.50 (5.00)	15.56 (±10.42)	74.45 (±18.99)	12.78 (±9.49)	0.00
Pooled Mean			103.57	8.73	80.79	9.08	0.35
C.D.			11.38	10.97	NS	NS	NS

Table 4.3.2.1. Association of endocarp morphological characteristics of trees of *Azadirachta indica* in all the 43 genotypes combined

	Endocarp length	Endocarp breadth	Endocarp weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.4162**	0.7387**	0.7284**	0.1836**	0.1792*	0.6278**
Endocarp breadth	-	1.0000	0.7782**	0.7647**	0.3357**	0.1865**	-0.4310**
Endocarp weight	-	-	1.0000	0.8734**	0.3271**	0.4459**	0.0528
Endocarp coat weight	-	-	-	1.0000	0.1960**	0.4694**	0.0646
number of seeds endocarp^{-1}	-	-	-	-	1.0000	-0.0017	-0.1055
Seed weight : : Endocarp weight	-	-	-	-	-	1.0000	-0.0061
Endocarp length : endocarp breadth	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

found in tree H102 of Kalka provenance. Highest coefficient of variation (39.53) for this character was observed in tree H082 of Kaithal provenance, while tree T31 of Alwar provenance exhibited a lowest value of 2.20.

Data regarding percentage of trilocular endocarps containing 0-3 endocarp in different trees of some provenance collections of *Neem* was analysed (**Table** 4.3.1.2.). Proportion (of endocarps showing 0 seed varied from 0 to 33.33 per cent. Proportion of endocarps showing one seed varied from 56.25 to 100 per cent. Only one genotype (TER2 from Pune provenance) showed three seeds in one endocarp.

Provenance variation with respect to percentage of endocarps containing 0-3 seeds in different provenance collections of neem varied considerably (**Table** 4.3.1.3). Proportion of endocarps showing 0 seed varied from 0 to 32.09 per cent highest being in Kaithal provenance. The differences in the mean values were significant in 60 per cent of the paired wise comparisons of means. Proportion of endocarps showing one seed varied from 77.43 to 95.83 per cent highest being in Kaithal provenance. Proportion of endocarps showing two seeds varied from 2.08 to 15.67 per cent. Proportion of endocarps showing three seeds varied from 0 to 2.08 per cent.

Between provenance variation was found for total number of seeds as percentage of endocarps, which ranged from 77.81 to 119.32 per cent. Highest value for this characteristic was shown by Bikaner provenance and lowest by Kaithal provenance. The differences in the mean values were significant in 60 per cent of the paired wise comparisons of means.

4.3.2. Association of endocarp morphological characteristics

Association of endocarp morphological characteristics was studied using simple correlation. **Table** 4.3.2.1. contains the result of correlation when all the 43 trees were taken together. Endocarp length was positively and significantly associated with endocarp breadth, endocarp weight, endocarp coat weight, number of seeds endocarp^{-1}, seed weight : endocarp weight, and endocarp length : endocarp breadth.

Endocarp breadth was positively and significantly associated with endocarp length, endocarp weight, endocarp coat weight, number of seeds endocarp^{-1}, and seed weight : endocarp weight; and negatively and significantly associated with endocarp length : endocarp breadth. Endocarp weight was positively and significantly associated with endocarp length, endocarp breadth, endocarp coat weight, number of seeds endocarp^{-1}, and seed weight : endocarp weight.

Endocarp coat weight was positively and significantly associated with endocarp length, endocarp breadth, endocarp weight, number of seeds endocarp^{-1} and seed weight : endocarp weight. Number of seed endocarp^{-1} was positively and significantly associated with endocarp length, endocarp breadth, endocarp weight, and endocarp coat weight. Seed weight : endocarp

Table 4.3.2.2 Association of endocarp morphological characteristics of *Azadirachta indica* in T17

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.7874	0.5908	0.2198	0.7522	0.0000	-0.5653	-0.4357
Endocarp breadth	-	1.0000	0.9605**	0.6842	0.9502*	0.0000	-0.2429	-0.8977*
Endocarp weight	-	-	1.0000	0.8339	0.8879*	0.0000	-0.0153	-0.9810**
Seed weight :	-	-	-	1.0000	0.4865	0.0000	0.5347	-0.8485
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.4699	-0.8457
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	-0.0610
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.3. Association of endocarp morphological characteristics of *Azadirachta indica* in T3

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.9447*	0.9339*	-0.1945	0.8877*	-0.1736	-0.7035	-0.8563
Endocarp breadth	-	1.0000	0.9663**	-0.0479	0.8288	-0.0064	-0.6260	-0.9780**
Endocarp weight	-	-	1.0000	0.0300	0.8111	0.1057	-0.5957	-0.9293*
Seed weight :	-	-	-	1.0000	-0.5603	0.9743**	0.7778	-0.0663
Endocarp coat weight	-	-	-	-	1.0000	-0.4825	-0.9487*	-0.7313
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.6886	-0.1124
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	0.5223
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

weight was positively and significantly associated with endocarp length, endocarp breadth, endocarp weight, and endocarp coat weight. Endocarp length : endocarp breadth was positively and significantly associated with endocarp length; and negatively and significantly associated with endocarp breadth.

Individual trees showed considerable variation with respect to. association of endocarp morphological characteristics (**Table** 4.3.2.2. to **Table** 4.3.2.44.). Endocarp length was significantly and positively associated with endocarp breadth in nine trees while it was negatively and non-significantly associated with endocarp breadth in seven trees. In the rest of trees these two traits were positively and non-significantly associated.

Endocarp length was significantly and positively associated with endocarp weight in eight trees while it was negatively and non-significantly associated with endocarp weight in three trees. In the rest of trees these two traits were positively and non-significantly associated. Endocarp length was significantly and positively associated with seed weight in six trees while it was negatively and non-significantly associated with seed weight in three trees. In the rest of trees these two traits were positively and non-significantly associated.

Endocarp length was significantly and positively associated with endocarp coat weight in 13 trees while it was negatively and non-significantly associated with seed weight in three trees. In the rest of trees these two traits were positively and non-significantly associated. Endocarp length was significantly and positively associated with and number of seeds endocarp^{-1} in two trees while it was negatively and non-significantly associated with and number of seeds endocarp^{-1} in one tree. These two traits were positively and non-significantly associated in seven trees. In the rest of trees these there was no association between the two traits.

Endocarp length was significantly and negatively associated with seed weight : endocarp weight in three trees while it was negatively and non-significantly associated with seed weight : endocarp weight in 23 trees. In the rest of trees these two traits were positively and non-significantly associated. Endocarp length was significantly and negatively associated with endocarp length : endocarp breadth in one tree while these traits were positively and significantly associated in four trees. In the six trees these two traits were negatively and non-significantly associated. In the rest of trees these two traits were positively and non-significantly associated.

Endocarp breadth was significantly and positively associated with endocarp weight in 20 trees while it was negatively and non-significantly associated with endocarp weight in two trees. In the rest of trees these two traits were positively and non-significantly associated. Endocarp breadth was significantly and positively associated

Table 4.3.2.4. Association of endocarp morphological characteristics of *Azadirachta indica* in T4

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds $endocarp^{-1}$	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	-0.6261	0.7253	0.3380	0.7778	0.0000	-0.5755	0.9674**
Endocarp breadth	-	1.0000	-0.1563	0.4018	-0.4626	0.0000	0.7840	-0.8032
Endocarp weight	-	-	1.0000	0.7332	0.9064*	0.0000	-0.4121	0.6053
Seed weight :	-	-	-	1.0000	0.3773	0.0000	0.3164	0.1239
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.7580	0.7474
Number of seeds $endocarp^{-1}$	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	-0.6996
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.5. Association of endocarp morphological characteristics of *Azadirachta indica* in T5

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds $endocarp^{-1}$	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.4010	0.7169	0.6644	0.7550	0.0000	-0.6918	0.2890
Endocarp breadth	-	1.0000	0.9153*	0.9223*	0.8949*	0.0000	-0.5306	-0.7611
Endocarp weight	-	-	1.0000	0.9916**	0.9927**	0.0000	-0.6429	-0.4475
Seed weight :	-	-	-	1.0000	0.9687**	0.0000	-0.5388	-0.4908
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.7302	-0.4002
Number of seeds $endocarp^{-1}$	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	0.0707
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.6. Association of endocarp morphological characteristics of *Azadirachta indica* in T18

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.6959	0.9243*	0.8681	0.9511*	0.0000	-0.9511*	0.5102
Endocarp breadth	-	1.0000	0.9171*	0.9555*	0.8798*	0.0000	-0.7785	-0.2618
Endocarp weight	-	-	1.0000	0.9892**	0.9951**	0.0000	-0.9408*	0.1434
Seed weight :	-	-	-	1.0000	0.9699**	0.0000	-0.8835*	0.0197
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.9683**	0.2255
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	-0.3532
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.7. Association of endocarp morphological characteristics of *Azadirachta indica* in T52

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.0962	0.7289	0.6437	0.7948	0.0000	-0.8905*	0.6629
Endocarp breadth	-	1.0000	0.7111	0.7619	0.6530	0.0000	-0.1008	-0.6809
Endocarp weight	-	-	1.0000	0.9907**	0.9922**	0.0000	-0.5849	-0.0031
Seed weight :	-	-	-	1.0000	0.9659**	0.0000	-0.4693	-0.1036
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.6810	0.0888
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	-0.5775
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.8. Association of endocarp morphological characteristics of *Azadirachta indica* in T41

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.8928*	0.9059*	0.9052*	0.9004*	0.9108*	0.7815	-0.3074
Endocarp breadth	-	1.0000	0.9891**	0.9876**	0.9843**	0.9925**	0.8450	-0.7031
Endocarp weight	-	-	1.0000	0.9985**	0.9952**	0.9971**	0.8577	-0.6573
Seed weight :	-	-	-	1.0000	0.9882**	0.9989**	0.8847*	-0.6553
Endocarp coat weight	-	-	-	-	1.0000	0.9864**	0.8032	-0.6550
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.8851*	-0.6574
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	-0.5490
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.9 Association of endocarp morphological characteristics of *Azadirachta indica* in T42

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.7658	0.8467	0.7922	0.8468	0.0000	-0.5059	0.5202
Endocarp breadth	-	1.0000	0.9687**	0.9583*	0.9275*	0.0000	-0.4540	-0.1503
Endocarp weight	-	-	1.0000	0.9639**	0.9777**	0.0000	-0.5383	0.0106
Seed weight :	-	-	-	1.0000	0.8865*	0.0000	-0.2950	-0.0572
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.7028	0.0635
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	-0.1647
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.10. Association of endocarp morphological characteristics of *Azadirachta indica* in T43

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.0623	0.4458	0.5035	0.2848	0.0000	0.2213	0.7076
Endocarp breadth	-	1.0000	0.5998	0.3743	0.7092	0.0000	-0.4342	-0.6613
Endocarp weight	-	-	1.0000	0.9010*	0.8846*	0.0000	-0.0828	-0.0891
Seed weight :	-	-	-	1.0000	0.5948	0.0000	0.3577	0.1136
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0. 5377	-0.2872
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	0.4727
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.11. Association of endocarp morphological characteristics of *Azadirachta indica* in T44

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.6785	0.8147	0.8021	0.8274	0. 7470	0.6953	0.1894
Endocarp breadth	-	1.0000	0.9102*	0.9232*	0.8693	0. 9313*	0.8819*	-0.5927
Endocarp weight	-	-	1.0000	0.9974**	0.9893**	0. 9804**	0.9048*	-0.3152
Seed weight :	-	-	-	1.0000	0.9763**	0. 9722**	0.9324*	-0.3474
Endocarp coat weight	-	-	-	-	1.0000	0. 9814	0.8343	-0.2445
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.8389	-0.4169
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	-0.4146
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.12. Association of endocarp morphological characteristics of *Azadirachta indica* in T45

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.8219	0.6780	0.3524	0.9595**	0.8892*	-0.1515	-0.2518
Endocarp breadth	-	1.0000	0.6826	0.3704	0.9354*	0.9513*	-0.1482	-0.7575
Endocarp weight	-	-	1.0000	0.9187*	0.6357	0.5199	0.5793	-0.4052
Seed weight :	-	-	-	1.0000	0.2790	0.1454	0.8536	-0.2536
Endocarp coat weight	-	-	-	-	1.0000	0.9797**	-0.2599	-0.4895
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	-0.3900	-0.5923
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	0.0500
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.13. Association of endocarp morphological characteristics of *Azadirachta indica* in T62

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.1233	-0.2243	-0.4687	0.9614**	0.0000	-0.6339	0.8947
Endocarp breadth	-	1.0000	0.3716	0.2364	0.3536	0.0000	0.1037	-0.3264
Endocarp weight	-	-	1.0000	0.9628**	-0.2255	0.0000	0.8821*	-0.3175
Seed weight :	-	-	-	1.0000	-0.4805	0.0000	0.9765**	-0.4941
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.6576	0.7505
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	-0.5982
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.14. Association of endocarp morphological characteristics of *Azadirachta indica* in T63

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.8555	0.8326	0.8213	0.8416	0.7530	0.5179	0.4514
Endocarp breadth	-	1.0000	0.9871**	0.9746**	0.9965**	0.9719**	0.5858	-0.0757
Endocarp weight	-	-	1.0000	0.9974**	0.9932**	0.9903**	0.6917	-0.0981
Seed weight :	-	-	-	1.0000	0.9822**	0.9871**	0.7419	-0.0978
Endocarp coat weight	-	-	-	-	1.0000	0.9844**	0.6030	-0.0975
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.6707	-0.2259
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	-0.0086
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.15 Association of endocarp morphological characteristics of *Azadirachta indica* in T64

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.4925	0.3421	-0.2288	0.8597	0.0000	-0.5532	0.8420
Endocarp breadth	-	1.0000	-0.1130	-0.5014	0.7173	0.0000	-0.6919	-0.0549
Endocarp weight	-	-	1.0000	0.8182	-0.0676	0.0000	0.5383	0.4520
Seed weight :	-	-	-	1.0000	-0.6289	0.0000	0.9242*	0.0365
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.8759	0.5480
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	-0.2159
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

with seed weight in 13 trees while it was negatively and non-significantly associated with seed weight in five trees. In the rest of trees these two traits were positively and non-significantly associated.

Endocarp breadth was significantly and positively associated with endocarp coat weight in 20 trees while it was negatively and non-significantly associated with seed weight in one tree. In the rest of trees these two traits were positively and non-significantly associated. Endocarp breadth was significantly and positively associated with and number of seeds endocarp^{-1} in eight trees while it was negatively and non-significantly associated with and number of seeds endocarp^{-1} in two trees. These two traits were positively and non-significantly associated in one tree. In the rest of trees there was no association between the two traits. Endocarp breadth was significantly and positively associated with seed weight : endocarp weight in three trees; significantly and negatively associated in one tree while it was negatively and non-significantly associated in 22 trees. In the rest of trees these two traits were positively and non-significantly associated.

Endocarp breadth was significantly and negatively associated with endocarp length : endocarp breadth in one tree while it was positively and significantly associated with seed weight : endocarp weight in three trees. In the six trees these two traits were negatively and non-significantly associated. In the rest of trees these two traits were positively and non-significantly associated. Endocarp weight was significantly and positively associated with seed weight in 23 trees while it was positively and non-significantly associated with seed weight in rest of the trees.

Endocarp weight was significantly and positively associated with endocarp coat weight in 26 trees while it was negatively and non-significantly associated with seed weight in two tree. In the rest of trees these two traits were positively and non-significantly associated. Endocarp weight was significantly and positively associated with and number of seeds endocarp^{-1} in five trees while it was positively and non-significantly associated with number of seeds endocarp^{-1} in five trees.

Endocarp weight was significantly and positively associated with seed weight : endocarp weight in five trees; significantly and negatively associated in one tree while it was negatively and non-significantly associated in 14 trees. In the rest of trees these two traits were positively and non-significantly associated. Endocarp weight was significantly and negatively associated with endocarp length : endocarp breadth in three trees In 30 trees these two traits were negatively and non-significantly associated. In the rest of trees these two traits were positively and non-significantly associated. Seed weight was significantly and positively associated with endocarp coat weight in 11 trees while it was negatively and non-significantly associated with seed

Table 4.3.2.16. Association of endocarp morphological characteristics of *Azadirachta indica* in T23

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.1581	0.1855	0.2927	0.0352	0.0157	0.3045	0.7259
Endocarp breadth	-	1.0000	0.8446	0.5652	0.9070*	0.3227	-0.4484	-0.5644
Endocarp weight	-	-	1.0000	0.8705	0.8781*	0.3786	-0.1147	-0.4323
Seed weight :	-	-	-	1.0000	0.5290	0.6447	0.3876	-0.1500
Endocarp coat weight	-	-	-	-	1.0000	0.0261	-0.5746	-0.5997
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.5505	-0.2172
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	0.5635
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.17. Association of endocarp morphological characteristics of *Azadirachta indica* in T24

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	-0.4327	0.1830	0.0807	0.8778*	0.0000	-0.3740	0.8808*
Endocarp breadth	-	1.0000	0.2793	0.2860	0.0265	0.0000	0.3397	-0.8079
Endocarp weight	-	-	1.0000	0.9553*	0.3154	0.0000	0.8133	-0.0427
Seed weight :	-	-	-	1.0000	0.0208	0.0000	0.9466*	-0.2192
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.2879	0.5591
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	-0.4372
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.18. Association of endocarp morphological characteristics of *Azadirachta indica* in T20

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.4410	0.4635	0.0994	0.6676	0.0000	-0.0017	0.0664
Endocarp breadth	-	1.0000	0.2216	-0.2988	0.8570	0.0000	-0.3371	-0.8654
Endocarp weight	-	-	1.0000	0.8273	0.4888	0.0000	0.7568	-0.0215
Seed weight :	-	-	-	1.0000	-0.0857	0.0000	0.9885**	0.3592
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.1928	-0.5959
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	0.3491
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.19. Association of endocarp morphological characteristics of *Azadirachta indica* in T22

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.8777*	0.9805**	0.9415*	0.9084*	0.6957	0.1407	-0.0004
Endocarp breadth	-	1.0000	0.9279*	0.7350	0.9944**	0.9502*	-0.2703	-0.4797
Endocarp weight	-	-	1.0000	0.9328*	0.9503*	0.7812	0.0826	-0.1394
Seed weight :	-	-	-	1.0000	0.7741	0.5293	0.4333	0.1938
Endocarp coat weight	-	-	-	-	1.0000	0.9147*	-0.2293	-0.4123
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	-0.4454	-0.7075
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	0.8294
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.20. Association of endocarp morphological characteristics of *Azadirachta indica* in T21

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.6658	0.5511	0.4233	0.6396	0.0000	-0.1299	-0.4173
Endocarp breadth	-	1.0000	0.7335	0.8247	0.4861	0.0000	0.6306	-0.9526*
Endocarp weight	-	-	1.0000	0.9520*	0.9038*	0.0000	0.3630	-0.7350
Seed weight :	-	-	-	1.0000	0.7292	0.0000	0.6305	-0.8833*
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.0705	-0.4077
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	-0.8114
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.21. Association of endocarp morphological characteristics of *Azadirachta indica* in H101

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.6730	0.0592	-0.1310	0.4701	0.0000	-0.4413	0.2677
Endocarp breadth	-	1.0000	0.1255	-0.0388	0.4699	0.0000	-0.3588	-0.5323
Endocarp weight	-	-	1.0000	0.9802**	0.8995*	0.0000	0.8406	-0.0687
Seed weight :	-	-	-	1.0000	0.7951	0.0000	0.9303*	-0.0729
Endocarp coat weight	-	-	-	-	1.0000	0.0000	0.5215	-0.0497
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	-0.0157
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.22. Association of endocarp morphological characteristics of *Azadirachta indica* in H102

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.9174*	0.1326	-0.1571	0.8109	0.0000	-0.5367	0.2147
Endocarp breadth	-	1.0000	0.3848	0.1086	0.9023*	0.0000	-0.2752	-0.1910
Endocarp weight	-	-	1.0000	0.9524*	0.6212	0.0000	0.7532	-0.5718
Seed weight :	-	-	-	1.0000	0.3528	0.0000	0.9158*	-0.6080
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.0422	-0.1923
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	-0.6101
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.23. Association of endocarp morphological characteristics of *Azadirachta indica* in H107

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight :	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Endocarp length	1.0000	0.1739	0.3048	0.0276	0.5186	0.0000	-0.8732	0.6290
Endocarp breadth	-	1.0000	0.9647**	0.9408*	0.9147*	0.0000	0.0828	-0.6562
Endocarp weight	-	-	1.0000	0.9536*	0.9666**	0.0000	0.0138	-0.5272
Seed weight :	-	-	-	1.0000	0.8446	0.0000	0.3141	-0.7205
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.2430	-0.3242
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : endocarp weight	-	-	-	-	-	-	1.0000	-0.7335
Endocarp length : endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.2.24. Association of endocarp morphological characteristics of *Azadirachta indica* in H099

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds Endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	0.3152	0.4106	0.4219	0.3903	0.6210	0.3615	0.2398
Endocarp breadth	-	1.0000	0.8880*	0.9019*	0.8618	0.9194*	0.9355*	-0.8451
Endocarp weight	-	-	1.0000	0.9992**	0.9979**	0.9025*	0.9918**	-0.6841
Seed weight	-	-	-	1.0000	0.9945**	0.9175*	0.9947**	-0.6909
Endocarp coat weight	-	-	-	-	1.0000	0.8742	0.9834**	-0.6705
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.9199*	-0.587
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	-0.7577
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.25. Association of endocarp morphological characteristics of *Azadirachta indica* in T5D

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	0.7685	0.7910	0.7083	0.8604	0.6788	0.3249	0.1104
Endocarp breadth	-	1.0000	0.9699**	0.9192*	0.9528*	0.9600**	0.5132	-0.5502
Endocarp weight	-	-	1.0000	0.9802**	0.9168*	0.9702**	0.6613	-0.4665
Seed weight	-	-	-	1.0000	0.8197	0.9383*	0.7953	-0.4947
Endocarp coat weight	-	-	-	-	1.0000	0.9151*	0.3098	-0.3522
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.5710	-0.5916
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	-0.3652
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.26. Association of endocarp morphological characteristics of *Azadirachta indica* in T7D

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	0.0304	0.6836	0.7192	0.6487	0.0000	-0.0545	0.7468
Endocarp breadth	-	1.0000	0.7106	0.6891	0.7274	0.0000	-0.7647	-0.6422
Endocarp weight	-	-	1.0000	0.9978**	0.9982**	0.0000	-0.7125	0.0502
Seed weight	-	-	-	1.0000	0.9921**	0.0000	-0.6650	0.0920
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.7528	0.0120
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	0.4698
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.27. Association of endocarp morphological characteristics of *Azadirachta indica* in H083

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	0.3308	0.0543	-0.1893	0.5760	0.0000	-0.3925	0.6267
Endocarp breadth	-	1.0000	0.9056*	0.6640	0.7453	0.0000	0.3613	-0.5279
Endocarp weight	-	-	1.0000	0.9181*	0.3938	0.0000	0.7223	-0.7048
Seed weight	-	-	-	1.0000	-0.0026	0.0000	0.9371*	-0.7294
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.3510	-0.0867
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	-0.6651
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.28. Association of endocarp morphological characteristics of *Azadirachta indica* in T30

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	0.9821**	0.9940**	0.9847**	0.9907**	0.8143	0.7704	-0.9059*
Endocarp breadth	-	1.0000	0.9870**	0.9741**	0.9887**	0.9075*	0.7280	-0.9687**
Endocarp weight	-	-	1.0000	0.9955**	0.9875**	0.8337	0.8058	-0.9284*
Seed weight	-	-	-	1.0000	0.9680**	0.8132	0.8572	-0.9333*
Endocarp coat weight	-	-	-	-	1.0000	0.8511	0.7043	-0.9349*
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.5123	-0.9713**
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	-0.6502
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.29. Association of endocarp morphological characteristics of *Azadirachta indica* in T31

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	0.9774**	0.8906*	-0.0854	0.9845**	0.0000	-0.8100	0.7622
Endocarp breadth	-	1.0000	0.9190*	0.0463	0.9551*	0.0000	-0.7103	0.6086
Endocarp weight	-	-	1.0000	0.3460	0.9057*	0.0000	-0.5045	0.5449
Seed weight	-	-	-	1.0000	-0.0843	0.0000	0.6354	-0.4255
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.8229	0.7709
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	-0.8448
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.30. Association of endocarp morphological characteristics of *Azadirachta indica in* HSR01

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	0.3381	0.7737	0.5993	0.9252*	0.0000	0.2924	0.5639
Endocarp breadth	-	1.0000	0.7804	0.8963*	0.1529	0.0000	0.8774	-0.5858
Endocarp weight	-	-	1.0000	0.9629**	0.6922	0.0000	0.7735	-0.0251
Seed weight	-	-	-	1.0000	0.4719	0.0000	0.9080*	-0.2736
Endocarp coat weight	-	-	-	-	1.0000	0.0000	0.0986	0.6499
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	-0.5127
Endocarp length : Endocarp breadth	-		-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.31. Association of endocarp morphological characteristics of *Azadirachta indica in* HSR02

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	0.9256*	0.1698	-0.7143	0.9107*	0.0000	-0.9248*	0.6798
Endocarp breadth	-	1.0000	0.1861	-0.6797	0.8915*	0.0000	-0.8867*	0.3517
Endocarp weight	-	-	1.0000	0.5243	0.4756	0.0000	0.1011	0.0852
Seed weight	-	-	-	1.0000	-0.4997	0.0000	0.9000*	-0.4338
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.8270	0.5348
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	-0.5639
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

weight in eight trees. In the rest of trees these two traits were positively and non-significantly associated.

Seed weight was significantly and positively associated with number of seeds endocarp^{-1} in six trees while it was positively and non-significantly associated with and number of seeds endocarp^{-1} in five trees. In rest of the trees there was no association between these two traits. Seed weight was significantly and positively associated with seed weight : endocarp weight in 16 trees; significantly and negatively associated in one tree while it was negatively and non-significantly associated in five trees. In the rest of trees these two traits were positively and non-significantly associated.

Seed weight was significantly and negatively associated with endocarp length : endocarp breadth in two trees In 30 trees these two traits were negatively and non-significantly associated. In the rest of trees these two traits were positively and non-significantly associated. Endocarp coat weight was significantly and positively associated with and number of seeds endocarp^{-1} in five trees while it was positively and non-significantly associated with and number of seeds endocarp^{-1} in four trees. In rest of the trees there was no association between these two traits.

Endocarp coat weight was significantly and positively associated with seed weight : endocarp weight in 16 trees; significantly and negatively associated in one tree while it was negatively and non-significantly associated in five trees. In the rest of trees these two traits were positively and non-significantly associated.

Endocarp coat weight was significantly and negatively associated with endocarp length : endocarp breadth in two trees. In 27 trees these two traits were negatively and non-significantly associated. In the rest of trees these two traits were positively and non-significantly associated.

Number of seeds endocarp^{-1} showed positive and significant association with seed weight : endocarp weight in two trees, positive and non-significant association in seven trees; negative and non-significant association in two trees. In the rest of trees these two traits were not associated at all.

Number of seeds endocarp^{-1} showed negative and significant association with seed length : endocarp length in one tree, positive and non-significant association in seven trees; negative and non-significant association in nine trees. In the rest of trees these two traits were not associated at all. Seed weight : endocarp weight was significantly and negatively associated with endocarp length : endocarp breadth in one tree In 14 trees these two traits were positively and non-significantly associated. In the rest of trees these two traits were negatively and non-significantly associated.

Table 4.3.2.32. Association of endocarp morphological characteristics of *Azadirachta indica* in HSR03

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	0.4696	0.6002	0.4658	0.8893*	0.0000	0.3149	0.2412
Endocarp breadth	-	1.0000	0.1983	0.1325	0.1805	0.0000	0.0164	-0.7434
Endocarp weight	-	-	1.0000	0.9872**	0.3764	0.0000	0.9444*	0.2408
Seed weight	-	-	-	1.0000	0.2325	0.0000	0.9836**	0.2120
Endocarp coat weight	-	-	-	-	1.0000	0.0000	0.0801	0.4686
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	0.2268
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.33. Association of endocarp morphological characteristics of *Azadirachta indica* in HSR04

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	0.7860	0.9235*	0.9215*	0.8414	0.0000	0.8197	0.6138
Endocarp breadth	-	1.0000	0.9176*	0.8103	0.9890**	0.0000	0.6553	-0.0052
Endocarp weight	-	-	1.0000	0.9744**	0.9453*	0.0000	0.8858*	0.3168
Seed weight	-	-	-	1.0000	0.8476	0.0000	0.9643**	0.4492
Endocarp coat weight	-	-	-	-	1.0000	0.0000	0.6905	0.0958
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	0.4800
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.34. Association of endocarp morphological characteristics of *Azadirachta indica* in HSR05

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	-0.2378	0.1726	-0.0645	0.7386	0.0000	-0.2392	0.8811*
Endocarp breadth	-	1.0000	0.8316	0.8146	0.4224	0.0000	0.7414	-0.6669
Endocarp weight	-	-	1.0000	0.9559*	0.5741	0.0000	0.8595	-0.2821
Seed weight	-	-	-	1.0000	0.3083	0.0000	0.9713**	-0.4608
Endocarp coat weight	-	-	-	-	1.0000	0.0000	0.0762	0.3708
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	-0.5637
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.35. Association of endocarp morphological characteristics of *Azadirachta indica in* SHS-0

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	-0.4280	0.1206	-01736.	-0.0516	0.0000	-0.2562	0.7569
Endocarp breadth	-	1.0000	0.8879*	0.9504*	0.7686	0.0000	0.7495	-0.9124*
Endocarp weight	-	-	1.0000	0.9803**	0.9723**	0.0000	0.4495	-0.6808
Seed weight	-	-	-	1.0000	0.9070*	0.0000	0.6165	-0.7559
Endocarp coat weight	-	-	-	-	1.0000	0.0000	0.2287	-0.5572
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	-0.6784
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.36. Association of endocarp morphological characteristics of *Azadirachta indica* in H100

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	-0.4568	-0.5996	-0.4403	-0.4618	0.0000	-0.0189	0.8494
Endocarp breadth	-	1.0000	0.8116	0.1666	0.9974**	0.0000	-0.6172	-0.8575
Endocarp weight	-	-	1.0000	0.7097	0.7911	0.0000	-0.0430	-0.8286
Seed weight	-	-	-	1.0000	0.1306	0.0000	0.6726	-0.3553
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.6446	-0.8578
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	0.3535
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.37. Association of endocarp morphological characteristics of *Azadirachta indica* in H082

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	-0.0573	0.6045	0.3958	0.6616	0.0000	-0.6125	0.7818
Endocarp breadth	-	1.0000	0.7510	0.8410	0.6249	0.0000	-0.0678	-0.6667
Endocarp weight	-	-	1.0000	0.8846*	0.9648**	0.0000	-0.5460	-0.0194
Seed weight	-	-	-	1.0000	0.7307	0.0000	-0.0931	-0.2279
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.7467	0.1002
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	-0.4073
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.38. Association of endocarp morphological characteristics of *Azadirachta indica* in H084

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	0.7154	0.7862	0.9036*	0.6243	0.0000	0.4987	0.0382
Endocarp breadth	-	1.0000	0.9505*	0.8686	0.9664**	0.0000	-0.2442	-0.6696
Endocarp weight	-	-	1.0000	0.9648**	0.9686**	0.0000	-0.0886	-0.5358
Seed weight	-	-	-	1.0000	0.8690	0.0000	0.1745	-0.2951
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.3317	-0.7289
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	0.8819*
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.39. Association of endocarp morphological characteristics of *Azadirachta indica* in H085

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	0.5010	0.0866	-0.2207	0.7640	0.0000	-0.4541	0.5278
Endocarp breadth	-	1.0000	0.5867	0.3638	0.5153	0.0000	0.1270	-0.4705
Endocarp weight	-	-	1.0000	0.9185*	0.1301	0.0000	0.7346	-0.4830
Seed weight	-	-	-	1.0000	-0.2727	0.0000	0.9430*	-0.5829
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.5772	0.2864
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	-0.5928
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.40. Association of endocarp morphological characteristics of *Azadirachta indica* in T33

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds $endocarp^{-1}$	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	0.8825*	0.9337*	0.9462*	0.8830*	0.0000	0.4635	0.1893
Endocarp breadth	-	1.0000	0.9908**	0.9129*	0.9889**	0.0000	0.1608	-0.2940
Endocarp weight	-	-	1.0000	0.9476*	0.9832**	0.0000	0.2469	-0.1732
Seed weight	-	-	-	1.0000	0.8734	0.0000	0.5361	0.0065
Endocarp coat weight	-	-	-	-	1.0000	0.0000	0.0703	-0.2677
Number of seeds $endocarp^{-1}$	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	0.5899
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.41. Association of endocarp morphological characteristics of *Azadirachta indica* in TGN3

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds $endocarp^{-1}$	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	0.4500	0.3657	0.0373	0.5759	0.0000	0.3453	0.3516
Endocarp breadth	-	1.0000	0.8611	0.7092	0.8183	0.0000	0.8777*	-0.6776
Endocarp weight	-	-	1.0000	0.8737	0.9071*	0.0000	0.8158	-0.6149
Seed weight	-	-	-	1.0000	0.5877	0.0000	0.8738	-0.7270
Endocarp coat weight	-	-	-	-	1.0000	0.0000	0.6006	-0.3935
Number of seeds $endocarp^{-1}$	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	-0.6399
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.42. Association of endocarp morphological characteristics of *Azadirachta indica* in NMN4

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	-0.1492	-0.0389	-0.0229	-0.0457	0.0000	0.0760	0.7124
Endocarp breadth	-	1.0000	0.9785**	0.8119	0.9542*	0.0000	-0.5133	-0.8003
Endocarp weight	-	-	1.0000	0.8904*	0.9241*	0.0000	-0.3947	-0.7186
Seed weight	-	-	-	1.0000	0.6488	0.0000	0.0664	-0.5919
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.7154	-0.7042
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	0.4075
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.43. Association of endocarp morphological characteristics of *Azadirachta indica* in TER2

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	0.9092*	0.2927	0.0272	0.6500	0.0000	-0.5731	0.9915**
Endocarp breadth	-	1.0000	0.6337	0.3978	0.8666	0.0000	-0.2761	0.8477
Endocarp weight	-	-	1.0000	0.9468*	0.8582	0.0000	0.4969	0.1744
Seed weight	-	-	-	1.0000	0.6474	0.0000	0.7471	-0.0877
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.0149	0.5530
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	-0.6396
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.2.44. Association of endocarp morphological characteristics of *Azadirachta indica* in NGN1

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
Endocarp length	1.0000	0.2225	0.8463	0.8387	0.7695	0.0000	0.3105	0.4299
Endocarp breadth	-	1.0000	0.3951	0.2345	0.5351	0.0000	-0.2981	-0.7826
Endocarp weight	-	-	1.0000	0.9576*	0.9465*	0.0000	0.2788	0.2023
Seed weight	-	-	-	1.0000	0.8135	0.0000	0.5423	0.3440
Endocarp coat weight	-	-	-	-	1.0000	0.0000	-0.0445	0.0230
Number of seeds endocarp^{-1}	-	-	-	-	-	1.0000	0.0000	0.0000
Seed weight : Endocarp weight	-	-	-	-	-	-	1.0000	0.4790
Endocarp length : Endocarp breadth	-	-	-	-	-	-	-	1.0000

**Significant at 1% level; *Significant at 5% level of significance

4.3.3. Genetic parameters with respect to endocarp morphological characteristics

Genetic parameters for the endocarp length were studied in 11 provenances (**Table** 4.3.3.1.).

Looking to the range of the character under study, it is grouped under low, medium and high category as follows:

Range	PCV (%)	GCV (%)	Heritability(%)	Genetic Advance (as % of mean)
Low	0-15	0-15	0-30	0-20
Medium	16-40	16-40	31-50	21-40
High	41-80	41-80	51-100	41-100

It was observed that PCV was higher than GCV for all the provenances. PCV ranged from 6.14 to 19.97%.Lowest value of PCV was observed in provenance 2 (Bikaner) and highest was observed for Provenance 8 (Alwar). PCV for all the provenances combined was 16.67%.

GCV ranged from zero to 14.49%.Lowest value of GCV was observed in provenance 4 (Bhatinda) and highest was observed for Provenance 8 (Alwar). GCV for all the provenances. combined was 15.10%.Heritability values for this character exhibited a wide range (low to high) of 14.40 to 82.76%.Lowest value of heritability was observed in provenance 4 (Bhatinda) and highest was observed for Provenance 1 (Hisar-I). Heritability for all the provenances. combined was 82.07%.Genetic advance (as per cent of mean) values for this character exhibited a wide range (low to medium) of 2.44 to 25.53. Lowest value was observed in provenance 4 (Bhatinda) and highest was observed for Provenance 6 (Kalka). Genetic advance for all the provenances combined was 28.18.

Genetic parameters for the endocarp breadth were studied in 11 provenances. (**Table** 4.3.3.2.). It was observed that PCV was higher than GCV for all the provenances. PCV ranged from 5.66 to 19.23%.Lowest value of PCV was observed in provenance 4 (Bhatinda) and highest was observed for Provenance 8 (Alwar). PCV for all the provenances combined was 14.99%. GCV ranged from 2.93 to 12.26%.Lowest value of GCV was observed in provenance 4 (Bhatinda) and highest was observed for Provenance 7 (Delhi). GCV for all the provenances combined was 12.52%.

Heritability values for this character exhibited a wide range (low to high) of 5.09 to 86.72%. Lowest value of heritability was observed in provenance 8 (Alwar) and highest was observed for Provenance 1 (Hisar-I). Heritability for all the provenances combined was 69.81%.Genetic advance (as per cent of mean) values for this character exhibited a wide range (low to medium) of 2.01 to 23.49. Lowest value was observed in provenance 8 (Alwar) and highest was observed for Provenance 1 (Hisar-I). Genetic advance for all the provenances combined was 21.55.

Table 4.3.3.1. Genetic parameters for endocarp length in different provenance collections of *Azadirachta indica*

Provenance	P CV	G CV	Heritability	G A	G A as %age of Mean
@	16.6693	15.1008	82.0667	3.3981	28.1806
1	11.7903	10.7262	82.7648	2.5449	20.1019
2	6.1389	3.5355	33.1677	0.5005	4.1944
3	10.1939	8.1765	64.3355	1.9692	13.5101
4	8.2402	0.0000	14.3953	0.3090	2.4436
5	11.3798	10.2010	80.3550	1.7790	18.8372
6	15.0998	13.6904	82.2040	3.0960	25.5299
7	11.3411	4.9532	19.0750	0.5054	4.4564
8	19.9711	14.4907	52.6469	2.2309	21.6592
9	12.9436	10.1452	61.4343	1.8860	16.3807
10	13.4239	11.5006	73.3987	2.8233	20.2971
11	13.7018	12.3049	80.6489	2.6785	22.7637

@ = all provenances combined

Table 4.3.3.2. Genetic parameters for endocarp breadth in different provenance collections of *Azadirachta indica*

Provenance	P CV	G CV	Heritability	G A	G A as %age of Mean
@	14.9877	12.5225	69.8092	1.3275	21.5533
1	13.1466	12.2430	86.7247	1.4832	23.4869
2	6.7732	4.0796	36.2792	0.3282	5.0619
3	10.7508	6.5772	37.4241	0.5613	8.2891
4	5.6583	2.9321	26.8529	0.2147	3.1300
5	13.8164	10.4493	57.1986	0.9169	16.2797
6	10.4156	6.7376	41.8445	0.6664	8.9782
7	15.3760	12.2563	63.5375	1.2317	20.1252
8	19.2315	4.3399	5.0925	0.1086	2.0175
9	9.0281	5.5370	37.6145	0.3768	6.9955
10	7.9002	5.3122	45.2144	0.4432	7.3584
11	10.1633	3.8667	14.4744	0.1675	3.0304

@ = all provenances combined

Table 4.3.3.3. Genetic parameters for endocarp weight in different provenance collections of *Azadirachta indica*

Provenance	P CV	G CV	Heritability	G A	G A as %age of Mean
@	36.0913	29.8653	68.4744	0.8951	50.9095
1	38.9532	36.8593	89.5382	1.4988	71.8487
2	14.4231	7.8806	29.8536	0.1706	8.8700
3	25.9785	16.9635	42.6386	0.5718	22.8184
4	15.1681	0.0000	0.0000	0.0000	0.0000
5	26.4888	21.7431	67.3804	0.4225	36.7668
6	32.2513	20.7277	41.3054	0.5788	27.4424
7	40.2674	19.6330	23.7722	0.3348	19.7192
8	50.4464	20.1609	15.9720	0.1849	16.5980
9	28.4561	17.7538	38.9255	0.3024	22.8179
10	9.8889	3.9159	15.6808	0.0601	3.1943
11	24.9869	17.7556	50.4947	0.3932	25.9911

@ = all provenances combined

Genetic parameters for the endocarp weight were studied in 11 provenances. (**Table** 4.3.3.3.). It was observed that PCV was higher than GCV for all the provenances. PCV was low to high, ranging from 9.89 to 50.45%.Lowest value of PCV was observed in provenance 10 (Pune) and highest was observed for Provenance 8 (Alwar). PCV for all the provenances. combined was 36.09%.GCV ranged from zero to 36.86%.Lowest value of GCV was observed in provenance 4 (Bhatinda) and highest was observed for Provenance 1 (Hisar-I). GCV for all the provenances. combined was 29.87%.

Heritability values for this character exhibited a wide range (low to high) of zero to 89.54%. Lowest value of heritability was observed in provenance 4 (Bhatinda) and highest was observed for Provenance 1 (Hisar-I). Heritability for all the provenances combined was 68.47%. Genetic advance (as per cent of mean) values for this character exhibited a wide range (low to medium) of zero to 71.85%.Lowest value was observed in provenance 4 (Bhatinda) and highest was observed for Provenance 1 (Hisar-I). Genetic advance for all the provenances combined was 50.91.

Genetic parameters for the endocarp seed were studied in 11 provenances. (**Table** 4.3.3.4.). It was observed that PCV was higher than GCV for all the provenances. PCV had low to high values ranging from 12.39 to 78.09%. Lowest value of PCV was observed in provenance 10 (Pune) and highest was observed for Provenance 8 (Alwar). GCV was found to have low to high range,(zero to 43.00). Lowest value of GCV was observed in provenance 7 (Delhi) and highest was observed for Provenance 8 (Alwar).

Heritability values for this character exhibited a wide range (low to high) of zero to 95.46%. Lowest value of heritability was observed in provenance 4 (Bhatinda) and 7 (Delhi) and highest was observed for Provenance 1 (Hisar-I). Genetic advance (as per cent of mean) values for this character exhibited a wide range (low to medium) of zero to 103.76. Lowest value was observed in provenance 4 (Bhatinda) and 7 (Delhi) and highest was observed for Provenance 1 (Hisar-I).

Genetic parameters for the endocarp coat weight were studied in 11 provenances. (**Table** 4.3.3.5.). It was observed that PCV was higher than GCV for all the provenances. PCV sowed a value of low to medium (13.97 to 37.33). Lowest value of PCV was observed in provenance 10 (Pune) and highest was observed for Provenance 5 (Jodhpur). PCV for all the provenances. combined was 32.39%.GCV ranged from zero to 33.22%.Lowest value of GCV was observed in provenance 2 (Bikaner). 4 (Bhatinda) and 8 (Alwar) and highest was observed for Provenance 5 (Jodhpur). GCV for all the provenances combined was 25.70%.

Heritability values for this character exhibited a wide range of low to high (zero to 79.20%). Lowest value of heritability was observed in provenance 2 (Bikaner) and 4 (Bhatinda) and 8 (Alwar) and highest was observed for Provenance 5 (Jodhpur). Heritability for all the provenances combined was 62.99%.

Table 4.3.3.4. Genetic parameters for seed weight in different provenance collections of *Azadirachta indica*

Provenance	P CV	G CV	Heritability	G A	G A as %age of Mean
@	NA	NA	NA	NA	NA
1	52.7611	51.5510	95.4655	1.1559	103.7594
2	25.3700	21.5775	72.3368	0.3987	37.8049
3	28.0012	13.0544	21.7350	0.1693	12.5373
4	32.2251	0.0000	0.0000	0.0000	0.0000
5	39.2446	32.5947	68.9817	0.2933	55.7676
6	42.8419	22.1635	26.7633	0.2281	23.6197
7	49.1942	0.0000	0.0000	0.0000	0.0000
8	78.0947	43.0039	30.3229	0.2198	48.7821
9	41.9407	15.5607	13.7654	0.0692	11.8930
10	12.3859	1.8103	2.1362	0.0054	0.5451
11	NA	NA	NA	NA	NA

@ = all provenances combined

Table 4.3.3.5. Genetic parameters for endocarp coat weight in different provenance collections of *Azadirachta indica*

Provenance	P CV	G CV	Heritability	G A	G A as %age of Mean
@	32.3888	25.7049	62.9855	0.3744	42.0246
1	25.8761	19.1355	54.68870	0.2833	29.15088
2	30.4010	0.0000	0.0000	0.0000	0.0000
3	26.5651	20.9310	62.0809	0.3927	33.9733
4	19.4557	0.0000	0.0000	0.0000	0.0000
5	37.3267	33.2184	79.1987	0.3795	60.8983
6	27.7315	20.2747	53.4516	0.3492	30.5352
7	36.0449	27.8957	59.8947	0.3896	44.4733
8	34.0383	0.0000	0.0000	0.0000	0.0000
9	27.8519	20.3830	53.5582	0.2290	30.7289
10	13.9710	9.5804	47.0227	0.1205	13.5333
11	19.9290	12.3044	38.1200	0.1258	15.6497

@ = all provenances combined

Table 4.3.3.6. Genetic parameters for number of seeds endocarp^{-1} in different provenance collections of *Azadirachta indica*

Provenance	P CV	G CV	Heritability	G A	G A as %age of Mean
@	24.3881	3.2912	1.8212	0.0970	0.9150
1	0.0000	0.0000	0.0000	0.0000	0.0000
2	32.2190	16.1095	25.0000	1.8805	16.5928
3	32.2557	0.0000	0.0000	0.0000	0.0000
4	24.2061	0.0000	0.0000	0.0000	0.0000
5	26.1891	0.0000	0.0000	0.0000	0.0000
6	19.2308	0.0000	0.0000	0.0000	0.0000
7	28.7480	0.0000	0.0000	0.0000	0.0000
8	45.5645	0.0000	0.0000	0.0000	0.0000
9	0.0000	0.0000	0.0000	0.0000	0.0000
10	0.0000	0.0000	0.0000	0.0000	0.0000
11	0.0000	0.0000	0.0000	0.0000	0.0000

@ = all provenances combined

Genetic advance (as per cent of mean) values for this character exhibited a wide range (low to high) of zero to 60.90. Lowest value was observed in provenance 2 (Bikaner), 4 (Bhatinda) and 8 (Alwar) and highest was observed for Provenance 5 (Jodhpur). Genetic advance for all the provenances combined was 42.02.

Genetic parameters for the number of seeds endocarp^{-1} were studied in 11 provenances. (**Table** 4.3.3.6.). It was observed that PCV was higher than GCV for all the provenances. PCV ranged from low to high (zero to 45.56). Lowest value of PCV was observed in provenance 1 (Hisar-I), 9 (Hisar-II), 10 (Pune) and 11 (Kaithal) and highest was observed for Provenance 8 (Alwar). PCV for all the provenances. combined was 24.39%. All the provenance collections showed a value of GCV equal to zero except Provenance 2 (Bikaner) which showed a value of 16.11. GCV for all the provenances combined was 3.29%.

[illegible] All the provenance collections showed a value equal to zero except Provenance 2 (Bikaner) which showed a value of 25.00%. Heritability for all the provenances combined was 1.82%. Genetic advance (as per cent of mean) values for this character exhibited a range low to medium. All the provenance collections showed a value of equal to zero except Provenance 2 (Bikaner) which showed a value of 16.59. Genetic advance for all the provenances. combined was 0.92.

Genetic parameters for the seed weight : endocarp weight were studied in 11 provenances. (**Table** 4.3.3.7.). It was observed that PCV was higher than GCV for all the provenances. PCV showed a range of low to medium (6.99 to 30.80). Lowest value of PCV was observed in provenance 3 (Raipur) and highest was observed for Provenance 9 (Hisar-II). PCV for all the provenances combined was 23.45%. GCV showed a range of low to medium (zero to 25.96). Lowest value of GCV was observed in provenance 4 (Bhatinda) and 9 (Hisar-II) and highest was observed for Provenance 5 (Jodhpur). GCV for all the provenances. combined was 13.26%.

Heritability values for this character exhibited a wide range (low to high) of zero to 79.67. Lowest value of heritability was observed in provenance 4 (Bhatinda) and 9 (Hisar-II) and highest was observed for Provenance 1 (Hisar-I). Heritability for all the provenances combined was 31.99%. Genetic advance (as per cent of mean) values for this character exhibited a wide range (low to high) of zero to 45.56. Lowest value was observed in provenance 4 (Bhatinda) and 9 (Hisar-II) and highest was observed for Provenance 5 (Jodhpur). Genetic advance for all the provenances. combined was 15.45.

Genetic parameters for the endocarp length : endocarp breadth were studied in 11 provenances. (**Table** 4.3.3.8.). It was observed that PCV was higher than GCV for all the provenances. PCV showed a range of low to medium (4.36 to 20.93). Lowest value of PCV was observed in provenance 1 (Hisar-I) and highest was observed for Provenance 6 (Kalka). PCV for all the provenances. combined was 16.84%. GCV showed a range of low to medium

Table 4.3.3.7. **Genetic parameters for seed weight : endocarp weight in different provenance collections of *Azadirachta indica***

Provenance	P CV	G CV	Heritability	G A	G A as %age of Mean
@	23.4512	13.2632	31.9865	0.7339	15.4526
1	17.8987	15.9759	79.6684	1.5269	29.3749
2	20.6869	11.5898	31.3878	0.7345	13.3759
3	6.9937	1.9059	7.4265	0.0576	1.0699
4	28.6333	0.0000	0.0000	0.0000	0.0000
5	30.4748	25.9612	72.5716	2.0850	45.5591
6	27.5633	5.7474	4.3480	0.1080	2.4688
7	15.5213	4.9688	10.2483	0.1563	3.2768
8	26.2282	18.6655	50.6456	1.0320	27.3638
9	30.8031	0.0000	0.0000	0.0000	0.0000
10	9.0346	4.3290	22.9595	0.2220	4.2730
11	10.9428	4.6463	18.0284	0.1886	4.0640

@ = all provenances combined

Table 4.3.3.8. **Genetic parameters for endocarp length : endocarp breadth (8) in different provenance collections of *Azadirachta indica***

Provenance	P CV	G CV	Heritability	G A	G A as %age of Mean
@	16.8384	15.1614	81.0732	5.5652	28.1219
1	4.3550	0.0000	0.0000	0.0000	0.0000
2	6.1250	2.7542	20.2201	0.4714	2.5513
3	12.2106	9.9495	66.3943	3.6182	16.7007
4	7.9291	0.0000	0.0000	0.0000	0.0000
5	13.8542	11.4518	68.3262	3.3014	19.5001
6	20.9287	19.4191	86.0944	6.1302	37.1179
7	9.0208	4.9972	30.6872	1.0648	5.7026
8	13.8847	11.6483	70.3807	3.8695	20.1306
9	12.3047	9.0532	54.1326	2.9422	13.7214
10	19.4564	17.8675	84.3344	7.8847	33.8014
11	15.0978	12.6596	70.3084	4.6760	21.8670

@ = all provenances combined

(zero to 19.42). Lowest value of GCV was observed in provenances 1 (Hisar-I) and 4 (Bhatinda) and highest was observed for Provenance 6 (Kalka). GCV for all the provenances combined was 15.16%.

Heritability values for this character exhibited a wide range (low to high) of zero to 86.09%. Lowest value of heritability was observed in provenance 1 (Hisar-I), and 4 (Bhatinda) and highest was observed for Provenance 6 (Kalka). Heritability for all the provenances combined was 81.07%.Genetic advance (as per cent of mean) values for this character exhibited a wide range (low to medium) of zero to 37.12. Lowest value was observed in provenance 1 (Hisar-I), and 4 (Bhatinda) and highest was observed for Provenance 6 (Kalka). Genetic advance for all the provenances combined was 28.12.

4.3.4. Genotypic and phenotypic correlations for endocarp morphological characteristics

Genotypic and phenotypic correlations for endocarp length are presented in **Table** 4.3.4.1. for 11 provenances. When all the provenances. were pooled, the genotypic association of endocarp breadth, endocarp weight, endocarp coat weight, number of seeds endocarp^{-1}, seed weight : endocarp weight and endocarp length : endocarp breath with endocarp length was found to be positive and significant. The values of genotypic correlations were higher than phenotypic correlations for endocarp weight, endocarp coat weight, seed weight : endocarp weight and endocarp length : endocarp breadth. Genotypic correlation of endocarp breadth with endocarp length was positive and significant in provenance ~~2 (Bikaner),~~ 5 (Jodhpur), ~~7 (Delhi), 8 (Alwar),~~ and 9 (Hisar-II) while it was negative and significant in provenance 6 (Kalka) and 10 (Pune).

Genotypic correlation of endocarp weight with endocarp length was positive and significant in provenance ~~1 (Hisar-I), 2 (Bikaner),~~ 3 (Raipur), 5 (Jodhpur), 6 (Kalka), ~~7 (Delhi), 8 (Alwar),~~ 9 (Hisar-II), 10 (Pune), and 11 (Kaithal). Genotypic correlation of seed weight with endocarp length was positive and significant in provenance ~~1 (Hisar-I), 2 (Bikaner),~~ 3 (Raipur), ~~4 (Bhatinda),~~ 6 (Kalka), ~~8 (Alwar),~~ 9 (Hisar-II), and 10 (Pune), Genotypic correlation of endocarp coat weight with endocarp length was positive and significant in provenance ~~1 (Hisar-I),~~ 3 (Raipur), 5 (Jodhpur), 6 (Kalka), ~~7 (Delhi),~~ 9 (Hisar-II), 10 (Pune), and 11 (Kaithal), ~~while it was negative and significant in provenance 4 (Bhatinda).~~

~~Genotypic correlation of number of seeds endocarp^{-1} with endocarp length was positive and significant in Provenance 2 (Bikaner).~~ Genotypic correlation of seed weight : endocarp weight with endocarp length was positive and significant in provenance ~~1 (Hisar-I), 2 (Bikaner), 8 (Alwar) and~~ 11 (Kaithal), while it was negative and significant in provenance 3 (Raipur), 5 (Jodhpur), ~~7 (Delhi),~~ and 10 (Pune). Genotypic correlation of endocarp length : endocarp breadth with endocarp length was positive and significant in provenance 3 (Raipur), 5

Table 4.3.4.1. Genotypic and phenotypic correlations of Endocarp length with other endocarp morphological characteristics in trees of *Azadirachta indica* in different geographical areas

Provenance		Number of trees (Number of observations)	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
global	G	43(215)	1.0000	0.3745*	0.8114**	NA	0.7701**	0.1551	0.4255**	0.6932**
	P		1.0000	0.4122**	0.7438**		0.7304**	0.1790	0.1842	0.6323**
3	G	5(25)	1.0000	0.1971	0.7676	0.8160	0.7542	0.0000	-1.2970**	0.7074
	P		1.0000	0.4142	0.7418	0.6563	0.7647	0.4623	-0.1436	0.4801
5	G	5(25)	1.0000	0.4516	0.7694	0.2301	0.7381	0.0000	-0.4580	0.5080
	P		1.0000	0.4123	0.6904	0.2156	0.7122	-0.2105	-0.3848	0.4405
6	G	5(25)	1.0000	-0.7057	0.7620	0.6333	0.8523	0.0000	0.2919	0.9758**
	P		1.0000	-0.2779	0.5047	0.2973	0.6947	-0.0191	-0.0766	0.8945*
9	G	6(30)	1.0000	0.4762	0.8628*	0.5836	0.9698**	0.0000	0.0000	0.8558*
	P		1.0000	0.4166	0.7509	0.4000	0.8857**	0.0000	-0.0470	0.7501
10	G	4(20)	1.0000	-1.1424**	0.5668	1.9243**	0.8949	0.0000	-1.1488**	1.0197**
	P		1.0000	-0.5363	0.3872	-0.1976	0.7745	0.0000	-0.4945	0.9288

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.4.2. Genotypic and phenotypic correlations of Endocarp breadth with other endocarp characteristics in trees of *Azadirachta indica* in different geographical areas

Provenance		Number of trees (Number of observations)	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
global	G	43(215)	0.3745*	1.0000	0.7961**	NA	0.7665**	0.1556	0.3823*	-0.3978**
	P		0.4122**	1.0000	0.7766**		0.7622**	0.3336*	0.1858	-0.4296**
3	G	5(25)	0.1971	1.0000	0.8313	0.8744	0.8237	0.0000	-1.2141**	-0.5544
	P		0.4142	1.0000	0.8184	0.7414	0.8224	0.4754	0.0965	-0.5888
5	G	5(25)	0.4516	1.0000	0.8685	0.3233	0.7805	0.0000	-0.4147	-0.5414
	P		0.4123	1.0000	0.7710	0.2910	0.7507	0.0386	-0.3371	-0.6305
6	G	5(25)	-0.7057	1.0000	-0.0090	0.2747	-0.2704	0.0000	1.7439**	-0.8451
	P		-0.2779	1.0000	0.3798	0.4056	0.2856	0.5304	0.2236	-0.6735
9	G	6(30)	0.4762	1.0000	0.9024**	1.1761**	0.6771	0.0000	0.0000	-0.0470
	P		0.4166	1.0000	0.7656	0.6599	0.6019	0.0000	0.2839	-0.2857
10	G	4(20)	-1.1424**	1.0000	-0.4639	2.5546**	-0.9385	0.0000	1.2121**	-1.0508**
	P		-0.5363	1.0000	0.1176	0.3192	-0.1392	0.0000	0.5566	-0.8069

**Significant at 1% level; *Significant at 5% level of significance

(Jodhpur), 6 (Kalka), ~~8 (Alwar),~~ 9 (Hisar-II), 10 (Pune), and 11 (Kaithal), ~~while it was negative and significant in provenance 4 (Bhatinda) and 7 (Delhi).~~

Genotypic and phenotypic correlations for endocarp breadth are presented in **Table** 4.3.4.2. for 11 provenances. When all the provenances. were pooled, the genotypic association of endocarp length, endocarp weight, endocarp coat weight, number of seeds endocarp^{-1}, and seed weight : endocarp weight with endocarp breadth was found to be positive and significant while endocarp length : endocarp breadth was found to be negatively and significantly associated with endocarp breadth. The values of genotypic correlations were higher than phenotypic correlations for endocarp weight, endocarp coat weight, seed weight : endocarp weight and endocarp length : endocarp breadth.

Genotypic correlation of endocarp length with endocarp breadth was positive and significant in provenance ~~1 (Hisar-I), 2 (Bikaner),~~ 5 (Jodhpur), ~~7 (Delhi), 8 (Alwar),~~ and 9 (Hisar-II) while it was negative and significant in provenance 6 (Kalka) and 10 (Pune). Genotypic correlation of endocarp weight with endocarp breadth was positive and significant in provenance 1 (Hisar-I), 3 (Raipur), 5 (Jodhpur), ~~7 (Delhi), 8 (Alwar);~~ 9 (Hisar-II), and 11 (Kaithal), while it was negative and significant in provenance 10 (Pune).

Genotypic correlation of seed weight with endocarp breadth was positive and significant in provenance ~~1 (Hisar-I),~~ 3 (Raipur), ~~8 (Alwar),~~ 9 (Hisar-II) and 10 (Pune). Genotypic correlation of endocarp coat weight with endocarp breadth was positive and significant in provenance ~~1 (Hisar-I),~~ 3 (Raipur), 5 (Jodhpur), ~~7 (Delhi),~~ 9 (Hisar-II), and 11 (Kaithal), while it was negative and significant in provenance 10 (Pune).

~~Genotypic correlation of number of seeds endocarp^{-1} with endocarp breadth was positive and significant in Provenance 2 (Bikaner).~~ Genotypic correlation of seed weight : endocarp weight with endocarp breadth was positive and significant in provenance ~~1 (Hisar-I),~~ 6 (Kalka), ~~8 (Alwar),~~ 10 (Pune), and 11 (Kaithal), while it was negative and significant in provenance 3 (Raipur), 5 (Jodhpur), ~~and 7 (Delhi).~~ Genotypic correlation of seed weight : endocarp weight with endocarp breadth was ~~positive and significant in provenance 8 (Alwar) while it was~~ negative and significant in provenance 3 (Raipur), 5 (Jodhpur), 6 (Kalka), ~~7 (Delhi),~~ and 10 (Pune).

Genotypic and phenotypic correlations for endocarp weight are presented in **Table** 4.3.4.3. for 11 provenances. When all the Provenances. were pooled, the genotypic association of endocarp length, endocarp breadth, endocarp coat weight, number of seeds endocarp^{-1}, seed weight : endocarp weight and endocarp length : endocarp breadth with endocarp weight was found to be positive and significant. The values of genotypic correlations were generally higher than phenotypic correlations. Genotypic correlation of endocarp length with endocarp weight was positive and significant in provenance ~~1 (Hisar-I), 2 (Bikaner),~~ 3 (Raipur), 5 (Jodhpur), 6 (Kalka), ~~7 (Delhi), 8 (Alwar),~~ 9 (Hisar-II), 10 (Pune), and 11 (Kaithal).

Table 4.3.4.3. Genotypic and phenotypic correlations of Endocarp weight with other endocarp morphological characteristics in trees of *Azadirachta indica* in different geographical areas

Provenance		Number of trees (Number of observations)	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
global	G	43(215)	0.8114**	0.7961**	1.0000	NA	0.9367**	0.2643	0.5563**	0.1606
	P		0.7438**	0.7766**	1.0000		0.8715**	0.3329*	0.4426**	0.0622
3	G	5(25)	0.7676	0.8313	1.0000	1.0226**	1.0121**	0.0000	-1.7845**	0.0528
	P		0.7418	0.8184	1.0000	0.9598**	0.9385**	0.4967	0.0702	-0.1112
5	G	5(25)	0.7694	0.8686	1.0000	0.5716	0.7336	0.0000	-0.2665	-0.1022
	P		0.6904	0.7710	1.0000	0.6458	0.7355	-0.0058	-0.0750	-0.1936
6	G	5(25)	0.7620	-0.0090	1.0000	0.9789**	0.9820**	0.0000	1.1666**	0.5726
	P		0.5047	0.3798	1.0000	0.9475**	0.9089*	0.3660	0.6116	0.2072
9	G	6(30)	0.8628*	0.9024*	1.0000	0.9222**	0.9735**	0.0000	0.0000	0.4558
	P		0.7508	0.7656	1.0000	0.8579**	0.7995	0.0000	0.4301	0.2306
10	G	4(20)	0.5668	-0.4639	1.0000	-0.5747	0.9851*	0.0000	-0.3490	0.5616
	P		0.3872	0.1176	1.0000	0.7492	0.7564	0.0000	0.1730	0.2270

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.4.4. Genotypic and phenotypic correlations of Seed weight with other endocarp morphological characteristics in trees of *Azadirachta indica* A. Juss.in different geographical areas

Provenance		Number of trees (Number of observations)	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
global	G	43(215)	NA	NA	NA	1.0000	NA	NA	NA	NA
	P					1.0000				
3	G	5(25)	0.8160	0.8744	1.0226**	1.0000	1.0683**	0.0000	-2.1424**	0.0717
	P		0.6563	0.7414	0.9598**	1.0000	0.8038	0.5317	0.3444	-0.0978
5	G	5(25)	0.2301	0.3233	0.5716	1.0000	-0.1383	0.0000	0.6459	-0.0726
	P		0.2156	0.2910	0.6458	1.0000	-0.0423	0.0291	0.6969	-0.1091
6	G	5(25)	0.6333	0.2747	0.9789**	1.0000	0.9227*	0.0000	1.2560**	0.3835
	P		0.2973	0.4056	0.9475**	1.0000	0.7280	0.4593	0.8160	0.3888
9	G	6(30)	0.5836	1.1761**	0.9221**	1.0000	0.8088	0.0000	0.0000	-0.0092
	P		0.3999	0.6599	0.8579*	1.0000	0.3780	0.0000	0.7932	-0.0612
10	G	4(20)	-1.9243**	2.5546**	-0.5747	1.0000	-0.7071	0.0000	2.1424**	-2.0143**
	P		-0.1976	0.3192	0.7492	1.0000	0.1335	0.0000	0.6310	-0.2709

**Significant at 1% level *Significant at 5% level of significance

Genotypic correlation of endocarp breadth with endocarp weight was positive and significant in provenance ~~1 (Hisar-I),~~ 3 (Raipur), 5 (Jodhpur), ~~7 (Delhi), 8 (Alwar),~~ 9 (Hisar-II), ad 11 (Kaithal), while it was negative and significant in provenance 10 (Pune). Genotypic correlation of seed weight with endocarp weight was positive and significant in provenance ~~1 (Hisar-I), 2 (Bikaner),~~ 3 (Raipur), 5 (Jodhpur), 6 (Kalka), ~~8 (Alwar),~~ and 9 (Hisar-II) while it was negative and significant in provenance 10 (Pune).

Genotypic correlation of endocarp coat weight with endocarp weight was positive and significant in provenance ~~1 (Hisar-I),~~ 3 (Raipur), 5 (Jodhpur), 6 (Kalka), ~~7 (Delhi),~~ 10 (Pune), and 11 (Kaithal), ~~while it was negative and significant in provenance 4 (Bhatinda). Genotypic correlation of number of seeds endocarp^{-1} with endocarp weight was positive and significant in Provenance 2 (Bikaner).~~ Genotypic correlation of seed weight : endocarp weight with endocarp weight was positive and significant in provenance ~~1 (Hisar-I), 2 (Bikaner) and~~ 6 (Kalka), ~~8 (Alwar),~~ and 11 (Kaithal), while it was negative and significant in provenance 3 (Raipur) ~~and 7 (Delhi).~~

Genotypic correlation of endocarp length : endocarp breadth with endocarp weight was positive and significant in provenance ~~2 (Bikaner),~~ 6 (Kalka), ~~8 (Alwar),~~ 9 (Hisar-II) and 10 (Pune), ~~while it was negative and significant in provenance 4 (Bhatinda) and 7 (Delhi).~~

Genotypic and phenotypic co-efficients of variation for seed weight are presented in **Table** 4.3.4.4. for 11 Provenance. Genotypic correlation of endocarp length with seed weight was positive and significant in provenance ~~1 (Hisar-I), 2 (Bikaner),~~ 3 (Raipur), ~~4 (Bhatinda),~~ 6 (Kalka), ~~8 (Alwar),~~ and 9 (Hisar-II) while it was negative and significant in provenance 10 (Pune). Genotypic correlation of endocarp breadth with seed weight was positive and significant in provenance ~~1 (Hisar-I),~~ 3 (Raipur), ~~8 (Alwar),~~ 9 (Hisar-II) and 10 (Pune).

Genotypic correlation of endocarp weight with seed weight was positive and significant in provenance ~~1 (Hisar-I), 2 (Bikaner),~~ 3 (Raipur), 5 (Jodhpur), 6 (Kalka), ~~8 (Alwar),~~ and 9 (Hisar-II) while it was negative and significant in provenance 10 (Pune). Genotypic correlation of endocarp coat weight with seed weight was positive and significant in provenance ~~1 (Hisar-I),~~ 3 (Raipur), ~~4 (Bhatinda),~~ 6 (Kalka), and 9 (Hisar-II) while it was negative and significant in provenance 10 (Pune). ~~Genotypic correlation of number of seeds endocarp^{-1} with seed weight was positive and significant in Provenance 2 (Bikaner).~~

Genotypic correlation of seed weight : endocarp weight with seed weight was positive and significant in provenance ~~1 (Hisar-I), 2 (Bikaner),~~ 5 (Jodhpur), 6 (Kalka), ~~8 (Alwar)~~ and 10 (Pune), while it was negative and significant in provenance 3 (Bikaner) ~~and 4 (Bhatinda).~~ Genotypic correlation of endocarp length : endocarp breadth with seed weight was negative and significant in provenance 10 (Pune).

Table 4.3.4.5. Genotypic and phenotypic correlations of Endocarp coat weight with other endocarp morphological characteristics in trees of *Azadirachta indica* in different geographical areas

Provenance		Number of trees (Number of observations)	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
global	G	43(215)	0.7701**	0.7665**	0.9367**	NA	1.0000	-0.2175	0.2412	0.1581
	P		0.7304**	0.7622**	0.8715**		1.0000	0.1980	-0.0094	0.0720
3	G	5(25)	0.7542	0.8237	1.0121**	1.0683**	1.0000	0.0000	-1.5748**	0.0406
	P		0.7647	0.8224	0.9385*	0.8038	1.0000	0.3986	-0.2851	-0.1155
5	G	5(25)	0.7381	0.7805	0.7336	-0.1383	1.0000	0.0000	-0.8566	-0.0632
	P		0.7122	0.7507	0.7355	-0.0423	1.0000	-0.0334	0.7166	-0.1564
6	G	5(25)	0.8523	-0.2704	0.9820**	0.9227**	1.0000	0.0000	1.0403**	0.7256
	P		0.6947	0.2856	0.9089*	0.7280	1.0000	0.1860	0.2475	0.3936
9	G	6(30)	0.9698**	0.6770	0.9735**	0.8088	1.0000	0.0000	0.0000	0.7018**
	P		0.8857*	0.6019	0.7995	0.3780	1.0000	0.0000	-0.1483	0.4913**
10	G	4(20)	0.8949	-0.9385	0.9850*	-0.7071	1.0000	0.0000	-0.7525	0.9093
	P		0.7744	-0.1392	0.7564	0.1335	1.0000	0.0000	-0.3644	0.6073

**Significant at 1% level *Significant at 5% level of significance

Table 4.3.4.6. Genotypic and phenotypic correlations of Number of seeds endocarp^{-1} with other endocarp morphological characteristics in trees of *Azadirachta indica* in different geographical areas

Site		Number of trees (Number of observations)	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
global	G	43(215)	0.1551	0.1556	0.2643	NA	-0.2175	1.0000	1.1992**	-0.0107
	P		0.1790	0.3336*	0.3329*		0.1980	1.0000	0.2194	-0.1066
3	G	5(25)	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000
	P		0.4623	0.4754	0.4967	0.5317	0.3986	1.0000	-0.1811	-0.0202
5	G	5(25)	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000
	P		-0.2105	0.0386	-0.0058	0.0291	-0.0334	1.0000	0.0591	-0.2380
6	G	5(25)	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000
	P		-0.0191	0.5304	0.3660	0.4593	0.1860	1.0000	0.3769	-0.2354
9	G	6(30)	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000
	P		0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000
10	G	4(20)	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000
	P		0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000

**Significant at 1% level; *Significant at 5% level of significance

Genotypic and phenotypic correlation for endocarp coat weight for 11 provenances are presented in **Table** 4.3.4.5. When all the provenances were pooled, the genotypic association of endocarp length, endocarp breadth, endocarp weight seed weight : endocarp weight and endocarp length : endocarp breadth with endocarp coat weight was found to be positive and significant, while number of seeds endocarp^{-1} was negatively and significantly associated with the endocarp coat weight. The values of genotypic correlations were generally higher than phenotypic correlations.

Genotypic correlation of endocarp length with endocarp coat weight was positive and significant in provenance ~~1 (Hisar-I),~~ 3 (Raipur), 5 (Jodhpur), 6 (Kalka), ~~7 (Delhi),~~ 9 (Hisar-II), 10 (Pune), and 11 (Kaithal), ~~while it was negative and significant in provenance 4 (Bhatinda)~~.

Genotypic correlation of endocarp breadth with endocarp coat weight was positive and significant in provenance ~~1 (Hisar-I),~~ 3 (Raipur), 5 (Jodhpur), ~~7 (Delhi),~~ 9 (Hisar-II) and 11 (Kaithal), while it was negative and significant in provenance 10 (Pune).

Genotypic correlation of endocarp weight with endocarp coat weight was positive and significant in provenance ~~1 (Hisar-I),~~ 3 (Raipur), 5 (Jodhpur), 6 (Kalka), ~~7 (Delhi),~~ 9 (Hisar-II), and 10 (Pune), ~~while it was negative and significant in provenance 4 (Bhatinda).~~ Genotypic correlation of seed weight with endocarp coat weight was positive and significant in provenance ~~1 (Hisar-I),~~ 3 (Raipur), ~~4 (Bhatinda),~~ 6 (Kalka) and 9 (Hisar-II) while it was negative and significant in provenance 10 (Pune), .

Genotypic correlation of seed weight : endocarp weight with endocarp coat weight was positive and significant in provenance ~~1 (Hisar-I), 4 (Bhatinda),~~ 6 (Kalka), and 11 (Kaithal), while it was negative and significant in provenance 3 (Raipur), 5 (Jodhpur), ~~7 (Delhi),~~ and 10 (Pune). Genotypic correlation of endocarp length : endocarp breadth with endocarp coat weight was positive and significant in provenance 6 (Kalka), 9 (Hisar-II), 10 (Pune), and 11 (Kaithal), ~~while it was negative and significant in provenance 4 (Bhatinda) and 7 (Delhi)~~.

Genotypic and phenotypic correlations for number of seeds endocarp^{-1} are presented in **Table** 4.3.4.6. for 11 sites. When all the sites were pooled, the genotypic association of endocarp length, endocarp breadth, endocarp weight and seed weight : endocarp weight with number of seeds endocarp^{-1} was found to be positive and significant, while endocarp coat weight was negatively and significantly associated with the number of seeds endocarp^{-1}. The values of genotypic correlations were generally less than phenotypic correlations.

~~Genotypic correlation of endocarp length with number of seeds endocarp^{-1} was positive and significant in site 2 (Bikaner). Genotypic correlation of endocarp breadth with number of seeds endocarp^{-1} was positive and significant in site 8 (Alwar) while it was negative and significant in site 2 (Bikaner). Genotypic correlation of endocarp weight with number of seeds endocarp^{-1} was positive and significant in site 2 (Bikaner). Genotypic correlation of seed weight with~~

Table 4.3.4.7. Genotypic and phenotypic correlations of Seed weight : Endocarp weight with other endocarp morphological characteristics in trees of *Azadirachta indica* in different geographical areas

Provenance		Number of trees (Number of observations)	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
global	G	43(215)	0.4255**	0.3823*	0.5563**	NA	0.2412	1.1992**	1.0000	0.0841
	P		0.1842	0.1858	0.4426**		-0.0094	0.2194	1.0000	-0.0005
3	G	5(25)	-1.2970**	-1.2141**	-1.7845**	-2.1424**	-1.5748**	0.0000	1.0000	-0.1765
	P		-0.1436	-0.0965	0.0702	0.3444	-0.2751	0.1811	1.0000	0.0149
5	G	5(25)	-0.4580	-0.4147	-0.2665	0.6459	-0.8566	0.0000	1.0000	-0.0139
	P		-0.3849	-0.3371	-0.0750	0.6969	-0.7166	0.0591	1.0000	0.0143
6	G	5(25)	0.2919	1.7439**	1.1665**	1.2560**	1.0403**	0.0000	1.0000	-0.3491
	P		-0.0766	0.2236	0.6116**	0.8160	0.2475	0.3769	1.0000	-0.1591
9	G	6(30)	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000
	P		-0.0469	0.2839	0.4301	0.7932**	-0.1483	0.0000	1.0000	-0.2638
10	G	4(20)	-1.1488**	1.2121**	-0.3489	2.1424	-0.7525	0.0000	1.0000	-1.1464**
	P		-0.4945	0.5566	0.1730	0.6310	0.3644	0.0000	1.0000	-0.5754

**Significant at 1% level; *Significant at 5% level of significance

Table 4.3.4.8. Genotypic and phenotypic and environmental correlations of Endocarp length : Endocarp breadth in trees of *Azadirachta indica* in different geographical areas

Provenance		Number of trees (Number of observations)	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
global	G	43(215)	0.6932*	-0.3978**	0.1606	NA	0.1581	-0.0107	0.0841	1.0000
	P		0.6323*	-0.4296**	0.0622		0.0720	-0.1066	-0.0005	1.0000
3	G	5(25)	0.7074**	-0.5544**	0.0528	0.0717	0.0406	0.0000	-0.1765	1.0000
	P		0.4801*	-0.5888**	-0.1113	-0.0978	-0.1155	-0.0202	0.0149	1.0000
5	G	5(25)	0.5080*	-0.5414**	-0.1022	-0.0726	-0.0632	0.0000	-0.0139	1.0000
	P		0.4405*	-0.6305**	-0.1936	-0.1092	-0.1564	0.2380	0.0143	1.0000
6	G	5(25)	0.9758**	-0.8451**	0.5726**	0.3835	0.7256**	0.0000	0.3491	1.0000
	P		0.8945**	-0.6735**	0.2072	0.0389	0.3936	-0.2354	-0.1591	1.0000
9	G	6(30)	0.8558**	-0.0470	0.4558*	-0.0092	0.7018**	0.0000	0.0000	1.0000
	P		0.7501**	-0.2857	0.2306	-0.0612	0.4913**	0.0000	-0.2638	1.0000
10	G	4(20)	1.0197**	-1.0508**	0.5617	-2.0143**	0.9093	0.0000	-1.1464**	1.0000
	P		0.9288	-0.8069	0.2270	-0.2709	0.6073	0.0000	-0.5754	1.0000

**Significant at 1% level; *Significant at 5% level of significance

~~number of seeds endocarp^{-1} was positive and significant in site 2 (Bikaner). Genotypic correlation of seed weight : endocarp weight with number of seeds endocarp^{-1} was positive and significant in site 2 (Bikaner). Genotypic correlation of endocarp length : endocarp breadth with number of seeds endocarp^{-1} was positive and significant in site 2 (Bikaner).~~

Genotypic and phenotypic correlations for seed weight : endocarp weight are presented in **Table** 4.3.4.7. for 11 provenances. When all the provenances were pooled, the genotypic association of endocarp length, endocarp breadth, endocarp weight, endocarp coat weight and number of seeds endocarp^{-1} with seed weight : endocarp weight was found to be positive and significant.

Genotypic correlation of endocarp length with seed weight : endocarp weight was positive and significant in provenance ~~2 (Bikaner), 4 (Bhatinda), 8 (Alwar), and~~ 11 (Kaithal), while it was negative and significant in provenance 3 (Raipur), 5 (Jodhpur), ~~7 (Delhi)~~ and 10 (Pune). Genotypic correlation of endocarp breadth with seed weight : endocarp weight was positive and significant in provenance ~~1 (Hisar-I),~~ 6 (Kalka), ~~8 (Alwar),~~ 10 (Pune), and 11 (Kaithal), while it was negative and significant in provenance 3 (Raipur), 5 (Jodhpur), and ~~7 (Delhi).~~

Genotypic correlation of endocarp weight with seed weight : endocarp weight was positive and significant in provenance ~~1 (Hisar-I), 2 (Bikaner),~~ 6 (Kalka), ~~8 (Alwar),~~ and 11 (Kaithal), while it was negative and significant in provenance 3 (Raipur), ~~7 (Delhi),~~ and 10 (Pune). Genotypic correlation of seed weight with seed weight : endocarp weight was positive and significant in provenance ~~1 (Hisar-I), 2 (Bikaner),~~ 5 (Jodhpur), 6 (Kalka) ~~and 8 (Alwar)~~ while it was negative and significant in provenance 3 (Raipur) ~~and 4 (Bhatinda).~~ Genotypic correlation of endocarp coat weight with seed weight : endocarp weight was positive and significant in provenance ~~1 (Hisar-I), 4 (Bhatinda),~~ 6 (Kalka) and 11 (Kaithal), while it was negative and significant in provenance 3 (Raipur), 5 (Jodhpur), ~~7 (Delhi)~~ and 10 (Pune). ~~Genotypic correlation of number of seeds endocarp^{-1} with seed weight : endocarp weight was positive and significant in provenance 2 (Bikaner).~~

Genotypic correlation of endocarp length : endocarp breadth with seed weight : endocarp weight was ~~positive and significant in provenance 2 (Bikaner) and 7 (Delhi) while it was~~ negative and significant in provenance 10 (Pune).

Genotypic and phenotypic correlations for endocarp length : endocarp breadth are presented in **Table** 4.3.4.8. for 11 provenances. When all the provenances were pooled, the genotypic association of endocarp length, endocarp weight and endocarp coat weight with endocarp length : endocarp breadth was found to be positive and significant, while endocarp breath was negatively and significantly associated with the endocarp length : endocarp breadth. Genotypic correlation of endocarp length with endocarp length : endocarp breadth was positive and significant in provenance 3 (Raipur), 5 (Jodhpur), 6 (Kalka), ~~8 (Alwar),~~ 9 (Hisar-II), 10 (Pune), and 11 (Kaithal), ~~while it was negative and significant in provenance 4 (Bhatinda).~~

Table 4.3.5.1. Direct and indirect effects of various characters on Seed weight in *Azadirachta indica* provenance 3 (Raipur)

	Endocarp length	Endocarp breadth	Endocarp weight	Endocarp coat weight	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth	Genotypic correlation
Endocarp length	0.0000	0.0000	1.8515	-1.0355	0.0000	0.0000	0.8160
Endocarp breadth	0.0000	0.0000	2.0053	-1.1309	0.0000	0.0000	0.8744
Endocarp weight	0.0000	0.0000	2.4122	-1.3895	0.0000	0.0000	1.0226
Endocarp coat weight	0.0000	0.0000	2.4413	-1.3730	0.0000	0.0000	1.0683
Seed weight : Endocarp weight	0.0000	0.0000	-4.3046	2.1622	0.0000	0.0000	-2.1424
Endocarp length : Endocarp breadth	0.0000	0.0000	0.1275	-0.056	0.0000	0.0000	0.0717

Residual effect = 0.0000

Table 4.3.5.2. Direct and indirect effects of various characters on Seed weight in *Azadirachta indica* provenance 5 (Jodhpur)

	Endocarp length	Endocarp breadth	Endocarp weight	Endocarp coat weight	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth	Genotypic correlation
Endocarp length	0.0000	0.0000	1.1214	-0.8912	0.0000	0.0000	0.2301
Endocarp breadth	0.0000	0.0000	1.2658	-0.9425	0.0000	0.0000	0.3233
Endocarp weight	0.0000	0.0000	1.4574	-0.8858	0.0000	0.0000	0.5716
Endocarp coat weight	0.0000	0.0000	1.0692	-1.2075	0.0000	0.0000	-0.1383
Seed weight : Endocarp weight	0.0000	0.0000	-0.3884	1.0343	0.0000	0.0000	0.6459
Endocarp length : Endocarp breadth	0.0000	0.0000	-0.1489	0.0763	0.0000	0.0000	-0.0726

Residual effect = 0.0000

Genotypic correlation of endocarp breadth with endocarp length : endocarp breadth was ~~positive and significant in provenance 8 (Alwar) while it was~~ negative and significant in provenance 3 (Raipur), 5 (Jodhpur), 6 (Kalka), ~~7 (Delhi),~~ and 10 (Pune). Genotypic correlation of endocarp weight with endocarp length : endocarp breadth was positive and significant in provenance ~~2 (Bikaner),~~ 6 (Kalka), ~~8 (Alwar),~~ 9 (Hisar-II), and 10 (Pune)~~, while it was negative and significant in provenance 4 (Bhatinda), and 7 (Delhi).~~

Genotypic correlation of seed weight with endocarp length : endocarp breadth was positive and significant in provenance 2 (Bikaner), while it was negative and significant in provenance 4 (Bhatinda) and 10 (Pune). Genotypic correlation of endocarp coat weight with endocarp length : endocarp breadth was positive and significant in provenance 6 (Kalka), 9 (Hisar-II), 10 (Pune), and 11 (Kaithal), ~~while it was negative and significant in provenance 4 (Bhatinda) and 7 (Delhi). Genotypic correlation of number of seeds endocarp^{-1} with endocarp length : endocarp breadth was positive and significant in provenance 2 (Bikaner).~~ Genotypic correlation of seed weight : endocarp weight with endocarp length : endocarp breadth was ~~positive and significant in provenance 2 (Bikaner) and 7 (Delhi) while it was~~ negative and significant in provenance 10 (Pune).

4.3.5. Path analysis with respect to endocarp morphological characteristics

Path analysis was performed in different provenance collections on the basis of genotypic correlations.

Path analysis (**Table** 4.3.5.1.), in provenance 3 (Raipur), showed that endocarp length had a positive indirect effect (1.8515) on seed weight via endocarp weight and negative indirec effect (-1.0355) via endocarp coat weight. But the positive indirect was more than the negative indirect effect resulting into positive (0.8160) correlation with seed weight. Endocarp breadth had a positive indirect effect (1.8515) on seed weight via endocarp weight and negative indirect effect (-1.1309) via endocarp coat weight. But the positive indirect effect was more than th negative indirect effect resulting into positive (0.8744) correlation with seed weight.

Endocarp weight had a positive direct effect (2.4122) on seed weight and negative indirec effect (-1.3895) via endocarp coat weight. But the positive direct was more than the negativ indirect effect resulting into positive (0.1.0226) correlation with seed weight. Endocarp coa weight had a negative direct effect (-1.3730) on seed weight and positive indirect effe (2.4413) via endocarp weight. But the positive indirect was more than the negative indire effect resulting into positive (1.0683) correlation with seed weight.

Seed weight : endocarp weight had a positive indirect effect (2.1622) on seed weight v endocarp weight and negative indirect effect (-4.3046) via endocarp coat weight. But tl negative indirect was more than the positive indirect effect resulting into negative (-2.142 correlation with seed weight. Endocarp length : endocarp breadth had a positive indirect effe

Genotypic correlation of endocarp breadth with endocarp length : endocarp breadth was ~~positive and significant in provenance 8 (Alwar) while it was~~ negative and significant in provenance 3 (Raipur), 5 (Jodhpur), 6 (Kalka), ~~7 (Delhi),~~ and 10 (Pune). Genotypic correlation of endocarp weight with endocarp length : endocarp breadth was positive and significant in provenance ~~2 (Bikaner),~~ 6 (Kalka), ~~8 (Alwar),~~ 9 (Hisar-II), and 10 (Pune)~~, while it was negative and significant in provenance 4 (Bhatinda), and 7 (Delhi).~~

Genotypic correlation of seed weight with endocarp length : endocarp breadth was positive and significant in provenance 2 (Bikaner), while it was negative and significant in provenance 4 (Bhatinda) and 10 (Pune). Genotypic correlation of endocarp coat weight with endocarp length : endocarp breadth was positive and significant in provenance 6 (Kalka), 9 (Hisar-II), 10 (Pune), and 11 (Kaithal), ~~while it was negative and significant in provenance 4 (Bhatinda) and 7 (Delhi). Genotypic correlation of number of seeds endocarp^{-1} with endocarp length : endocarp breadth was positive and significant in provenance 2 (Bikaner).~~ Genotypic correlation of seed weight : endocarp weight with endocarp length : endocarp breadth was ~~positive and significant in provenance 2 (Bikaner) and 7 (Delhi) while it was~~ negative and significant in provenance 10 (Pune).

4.3.5. Path analysis with respect to endocarp morphological characteristics

Path analysis was performed in different provenance collections on the basis of genotypic correlations.

Path analysis (**Table** 4.3.5.1.), in provenance 3 (Raipur), showed that endocarp length had a positive indirect effect (1.8515) on seed weight via endocarp weight and negative indirect effect (-1.0355) via endocarp coat weight. But the positive indirect was more than the negative indirect effect resulting into positive (0.8160) correlation with seed weight. Endocarp breadth had a positive indirect effect (1.8515) on seed weight via endocarp weight and negative indirect effect (-1.1309) via endocarp coat weight. But the positive indirect effect was more than the negative indirect effect resulting into positive (0.8744) correlation with seed weight.

Endocarp weight had a positive direct effect (2.4122) on seed weight and negative indirect effect (-1.3895) via endocarp coat weight. But the positive direct was more than the negative indirect effect resulting into positive (0.1.0226) correlation with seed weight. Endocarp coat weight had a negative direct effect (-1.3730) on seed weight and positive indirect effect (2.4413) via endocarp weight. But the positive indirect was more than the negative indirect effect resulting into positive (1.0683) correlation with seed weight.

Seed weight : endocarp weight had a positive indirect effect (2.1622) on seed weight via endocarp weight and negative indirect effect (-4.3046) via endocarp coat weight. But the negative indirect was more than the positive indirect effect resulting into negative (-2.1424) correlation with seed weight. Endocarp length : endocarp breadth had a positive indirect effect

Table 4.3.5.3. Direct and indirect effects of various characters on Seed weight in *Azadirachta indica* provenance 6 (Kalka)

	Endocarp length	Endocarp breadth	Endocarp weight	Endocarp coat weight	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth	Genotypic correlation
Endocarp length	0.0000	0.0000	1.5567	-0.9234	0.0000	0.0000	0.6333
Endocarp breadth	0.0000	0.0000	-0.0183	0.2930	0.0000	0.0000	0.2747
Endocarp weight	0.0000	0.0000	2.0428	-1.0640	0.0000	0.0000	0.9789
Endocarp coat weight	0.0000	0.0000	2.0061	-1.0834	0.0000	0.0000	0.9227
Seed weight : Endocarp weight	0.0000	0.0000	2.3830	-1.1270	0.0000	0.0000	1.2560
Endocarp length : Endocarp breadth	0.0000	0.0000	1.1697	-0.7862	0.0000	0.0000	0.3835

Residual effect = 0.0000

Table 4.3.5.4. Direct and indirect effects of various characters on Seed weight in *Azadirachta indica* provenance 9 (Hisar)

	Endocarp length	Endocarp breadth	Endocarp weight	Endocarp coat weight	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth	Genotypic correlation
Endocarp length	-7.3152	0.4676	2.6723	1.5187	0.0000	3.2403	0.5836
Endocarp breadth	-3.4832	0.9821	2.7949	1.0603	0.0000	-0.1780	1.1761
Endocarp weight	-6.3116	0.8862	3.0972	1.5245	0.0000	1.7259	0.9221
Endocarp coat weight	-7.0945	0.6649	3.0152	1.5659	0.0000	2.6572	0.8088
Seed weight : Endocarp weight	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Endocarp length : Endocarp breadth	-6.2601	-0.0462	1.4117	1.0989	0.0000	3.7864	-0.0092

Residual effect = 0.0266

(0.1275) on seed weight via endocarp weight and negative indirect effect (-0.056) via endocarp coat weight. But the positive indirect was more than the negative indirect effect resulting into positive (0.0717) correlation with seed weight.

Path analysis (**Table** 4.3.5.2.), in provenance 5 (Jodhpur), showed that endocarp length had a positive indirect effect (1.1214) on seed weight via endocarp weight and negative indirect effect via endocarp coat weight (-0.8912). This resulted into positive (0.2301) correlation with seed weight. Endocarp breadth had a positive indirect effect (1.2658) on seed weight via endocarp weight and negative indirect effect via endocarp coat weight (-0.9425). This resulted into positive (0.3233) correlation with seed weight. Endocarp weight had a positive direct effect (1.4574) on seed weight and negative indirect effect via endocarp coat weight (-0.8858). This resulted into positive (0.5716) correlation with seed weight.

Endocarp coat weight had a negative direct effect (-1.2075) on seed weight via endocarp coat weight and positive indirect effect via endocarp weight (1.0692). This resulted into negative(-0.1383) correlation with seed weight. Seed weight : endocarp weight had a positive indirect effect (1.0343) on seed weight via endocarp coat weight and negative indirect effect via endocarp weight (-0.3884). This resulted into positive (0.6459) correlation with seed weight. Endocarp length : endocarp breath had a positive indirect effect (0.0763) on seed weight via endocarp coat weight and negative indirect effect via endocarp weight (-0.1489). This resulted into negative (-0.0726) correlation with seed weight.

Path analysis (**Table** 4.3.5.3.), when the data from provenance 6 (Kalka) was pooled, showed that endocarp length had a positive indirect effect (1.5567) on seed weight via endocarp weight and negative indirect effect (-0.9234) via endocarp coat weight. This resulted into positive (0.6333) correlation with seed weight. Endocarp breadth had a negative indirect effect (-0.0183) on seed weight via endocarp weight and positive indirect effect (0.2930) via endocarp coat weight. This resulted into positive (0.2747) correlation with seed weight.

Endocarp weight had a positive direct effect (2.0428) on seed weight and negative indirect effect via endocarp coat weight (-1.0640). This resulted into positive (0.9789) correlation with seed weight. Endocarp coat weight had a negative direct effect (-1.0834) on seed weight via endocarp coat weight and positive indirect effect via endocarp weight (2.0061). This resulted into positive (0.9227) correlation with seed weight. Seed weight : endocarp weight had a positive indirect effect (2.3830) on seed weight via endocarp weight and negative indirect effect via endocarp weight (-1.1270). This resulted into positive (1.25609) correlation with seed weight. Endocarp length : endocarp breath had a negative indirect effect (-0.7862) on seed weight via endocarp coat weight and positive indirect effect via endocarp weight (1.1697). This resulted into positive (0.3835) correlation with seed weight.

Path analysis, (**Table** 4.3.5.4) when the data from provenance 9 (Hisar) was pooled, showed that endocarp length had a negative direct effect (-7.3152) on seed weight. However, this

negative effect was neutralized by positive indirect effect via endocarp breadth (0.4676), endocarp weight (2.6723), endocarp coat weight (1.5187), and endocarp length : endocarp breadth (3.2403). But the positive indirect effect was more than the negative direct effect resulting into positive (0.5836) correlation with seed weight.

Endocarp breadth had a positive direct effect (0.9821) on seed weight alongwith indirect positive effect via endocarp weight (2.7949), and endocarp coat weight (1.0603). This character also had negative indirect effect via endocarp length (-3.4832) and endocarp length : endocarp breadth (-0.1780). But the positive direct and indirect effect was more than the negative indirect effect resulting into positive (1.1761) correlation with seed weight. Endocarp weight had a positive direct effect (3.0972) on seed weight alongwith direct positive effect via and endocarp breadth (0.8862) endocarp coat weight (1.5245) and endocarp length : endocarp breadth (1.7259). This character also had negative indirect effect via endocarp length (-6.3116). But the positive direct and indirect effect was more than the negative indirect effect resulting into positive (0.9221) correlation with seed weight.

Endocarp coat weight had a positive direct effect (1.5659) on seed weight alongwith indirect positive effect of endocarp breadth (0.6649), endocarp weight (3.0152) and endocarp length : endocarp breadth (2.6572). This character also had negative indirect effect via endocarp length (-7.0945). But the positive direct and indirect effect was more than the negative indirect effect resulting into positive (0.9221) correlation with seed weight. Seed weight : endocarp weight had no direct or indirect effect on seed weight. Consequently there was no association of this character with seed weight.

Endocarp length : endocarp breadth had a positive direct effect (3.7864) on seed weight alongwith direct positive effect of endocarp weight (1.4117), endocarp coat weight (1.0989), This character also had negative indirect effect via endocarp length (-6.2601), and endocarp breadth (-0.0462). But the negative indirect effect was less than the positive direct and indirect effect resulting into positive correlation (-0.0092) with seed weight.

Path analysis (**Table** 4.3.5.5.), when the data from provenance 10 (Pune) was pooled, showed that endocarp length had no direct effect on seed weight. However, there was a positive effect (2.3268) via endocarp weight and negative indirect effect via endocarp coat weight (-4.2511). But the negative indirect effect was more than the positive indirect effect and resulting into negative (-1.9243) correlation with seed weight.

Endocarp breadth had no direct effect on seed weight. However, there was a positive effect (4.5867) via endocarp coat weight and negative indirect effect via endocarp weight (-1.9041). But the positive indirect effect was more than the negative indirect effect resulting into positive (2.5546) correlation with seed weight. Endocarp weight had a positive direct effect (4.1049) on seed weight alongwith indirect negative effect via endocarp coat weight (-4.6796). But the

Table 4.3.5.5. Direct and indirect effects of various characters on Seed weight in *Azadirachta indica* provenance 10 (Pune)

	Endocarp length	Endocarp breadth	Endocarp weight	Endocarp coat weight	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth	Genotypic correlation
Endocarp length	0.0000	0.0000	2.3268	-4.2511	0.0000	0.0000	-1.9243
Endocarp breadth	0.0000	0.0000	-1.9041	4.4587	0.0000	0.0000	2.5546
Endocarp weight	0.0000	0.0000	4.1049	-4.6796	0.0000	0.0000	-0.5747
Endocarp coat weight	0.0000	0.0000	4.0435	-4.7506	0.0000	0.0000	-0.7071
Seed weight : Endocarp weight	0.0000	0.0000	-1.4325	3.5749	0.0000	0.0000	2.1424
Endocarp length : Endocarp breadth	0.0000	0.0000	2.3055	-4.3198	0.0000	0.0000	-2.0143

Residual effect =0.0000

Table 4.3.5.6. Direct and indirect effects of various characters on Seed weight : Endocarp weight in *Azadirachta indica* in all the 11 provenances combined

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Endocarp length : Endocarp breadth	Genotypic correlation
Endocarp length	-2.606	0.3160	3.4355	-0.1750	-1.9106	1.3660	0.4255
Endocarp breadth	-0.9761	0.8437	3.3705	-0.1702	-1.9017	-0.7839	0.3823
Endocarp weight	-2.1149	0.6717	4.2338	-0.2269	-2.3239	0.3165	0.5563
Seed weight	-1.9636	0.6182	4.1360	-0.2323	-2.0817	0.2729	0.7495
Endocarp coat weight	-2.0072	0.6467	3.9659	-0.1949	-2.4809	0.3115	0.2412
Endocarp length : Endocarp breadth	-1.8066	-0.3356	0.6800	-0.0322	-0.3922	1.9707	0.0841

Residual effect = 0.0379

positive direct effect was less than the negative indirect effect resulting into negative (-0.5747) correlation with seed weight.

Endocarp coat weight had a negative direct effect (-4.7506) on seed weight alongwith indirect positive effect via endocarp weight (4.0435). But the positive indirect effect was less than the negative direct effect resulting into negative (-0.7071) correlation with seed weight.

Seed weight : endocarp weight had a positive indirect effect (3.5749) on seed weight via endocarp coat weight alongwith indirect negative effect via endocarp weight (-1.4325) But the positive indirect effect was more than the negative indirect effect resulting into positive (2.1424) correlation with seed weight. Endocarp length : endocarp breadth had a positive indirect effect (2.3055) on seed weight via endocarp weight alongwith indirect negative effect via endocarp coat weight (-4.3198). But the positive indirect effect was less than the negative indirect effect resulting into negative (-2.0143) correlation with seed weight.

Path analysis (**Table** 4.3.5.6.), when the data from provenance 1 (Hisar) was pooled, showed that endocarp length had a negative direct effect (-0.6038) on seed weight : endocarp weight alongwith negative indirect effect via endocarp breadth (-0.0352), seed weight (-7.2712) and endocarp coat weight (-3.3500). However, this negative effect was neutralized by positive indirect effect via endocarp weight (12.3252). But the positive indirect effect was more than the negative direct and indirect effect and resulting into positive (1.0816) correlation with seed weight : endocarp weight.

Endocarp breadth had negative direct effect (-0.0347) on seed weight : endocarp weight alongwith negative indirect effect via endocarp length (-0.6128), seed weight (-7.1869) and endocarp coat weight (-3.3978). However, this negative effect was neutralized by positive indirect effect via endocarp weight (12.2607). Positive indirect effect was more than the negative direct and indirect effect and resulting into positive (1.0450) correlation with seed weight : endocarp weight. Endocarp weight had a positive direct effect (12.2188) on seed weight : endocarp weight This character also had negative indirect effect via endocarp length (-0.6091), endocarp breadth (-0.0348), seed weight (-7.1671), endocarp coat weight (-3.3794). But the positive direct effect was more than the negative indirect effect resulting into positive (1.0448) correlation with seed weight : endocarp weight.

Seed weight had a negative direct effect (-7.1406) on seed weight : endocarp weight. This character also had negative indirect effect via endocarp length (-0.6149), endocarp breadth (-0.050), and endocarp coat weight (-3.4669). However, this negative effect was neutralized by the positive indirect effect via endocarp weight (12.2641). Positive indirect effect was more than the negative direct and indirect effect resulting into positive (1.0233) correlation with seed weight : endocarp weight.

Endocarp coat weight had a negative direct effect (-3.2656) on seed weight : endocarp weight alongwith indirect positive effect of endocarp weight (12.6444). This character also had negative indirect effect via endocarp length (-0.6194), endocarp breadth (-0.0361), seed weight (-7.5807) and endocarp coat (-3.2656). But the positive indirect effect was more than the negative direct and indirect effect resulting into positive (0.1597) correlation with seed weight : endocarp weight. Endocarp length : endocarp breadth had no direct or indirect effect on seed weight : endocarp weight.

Path analysis (**Table** 4.3.5.7.), when the data from provenance 3 (Raipur) was pooled, showed that endocarp length had a positive direct effect (7.1915) on seed weight : endocarp weight alongwith indirect positive effect via endocarp weight (7.1831). This character also had indirect negative effect via endocarp breadth (-1.4221), seed weight (-1.3746), endocarp coat weight (-6.2922), and endocarp length : endocarp breadth (-6.5782). But the positive direct and indirect effect was less than the negative indirect effect and resulting into negative (-1.2970) correlation with seed weight : endocarp weight.

Endocarp breadth had a negative direct effect (-7.2164) on seed weight : endocarp weight alongwith indirect positive effect via endocarp length (1.4172), endocarp weight (7.7799) and endocarp length : endocarp breadth (-0.5024). , This character also had negative indirect effect via seed weight (-1.4730) and endocarp coat weight (-6.8719). But the positive indirect effect was less than the negative direct indirect effect resulting into negative (-1.2141) correlation with seed weight : endocarp weight.

Endocarp weight had a positive direct effect (9.3584) on seed weight : endocarp weight alongwith direct positive effect of endocarp length (5.5199). This character also had negative indirect effect via endocarp breadth (-5.9991), seed weight (-1.7227), endocarp coat weight (-8.4437), and endocarp length : endocarp breadth (0.2028). But the positive direct and indirect effect was less than the negative indirect effect resulting into negative (-1.7845) correlation with seed weight : endocarp weight. Seed weight had a negative direct effect (-1.6846) on seed weight : endocarp weight alongwith indirect negative effect of endocarp breadth (-6.3102), seed weight (-1.6846), endocarp coat weight (-8.9129), and endocarp length : endocarp breadth (-0.6670). This character also had positive indirect effect via endocarp length (5.8683). But the positive indirect effect was less than the negative direct and indirect effect resulting into negative (-2.1424) correlation with seed weight : endocarp weight.

Endocarp coat weight had a positive direct effect (-8.3430) on seed weight : endocarp weight alongwith indirect negative effect of endocarp breadth (-5.9439), seed weight (-1.7997) and endocarp length : endocarp breadth (-0.3776). This character also had positive indirect effect via endocarp length (5.4238) and endocarp weight (9.4713). But the positive indirect effect was more than the negative direct and indirect effect resulting into negative (-1.5748) correlation with seed weight : endocarp weight.

Table 4.3.5.7. Direct and indirect effects of various characters on Seed weight : Endocarp weight in *Azadirachta indica* in provenance 3 (Raipur)

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Endocarp length : Endocarp breadth	Genotypic correlation
Endocarp length	7.1915	-1.4221	7.1831	-1.3746	-6.2922	-6.5782	-1.2970
Endocarp breadth	1.4172	-7.2164	7.7799	-1.4730	-6.8719	5.1549	-1.2141
Endocarp weight	5.5199	-5.9991	9.3584	-1.7227	-8.4437	-0.4914	-1.7845
Seed weight	5.8683	-6.3102	9.5702	-1.6846	-8.9129	-0.6670	-2.1424
Endocarp coat weight	5.4238	-5.9439	9.4713	-1.7997	-8.3430	-0.3776	-1.5748
Endocarp length : Endocarp breadth	5.0874	4.0004	0.4946	-0.1208	-0.3388	-9.2990	-0.1765

Residual effect = -0.1138

Table 4.3.5.8. Direct and indirect effects of various characters on Seed weight : Endocarp weight in *Azadirachta indica* in provenance 5 (Jodhpur)

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Endocarp length : Endocarp breadth	Genotypic correlation
Endocarp length	13.0015	-1.7862	-166.4418	33.4791	126.1055	-4.8255	-0.4580
Endocarp breadth	5.8715	-3.9552	-187.8798	47.0403	133.3533	5.1434	-0.4147
Endocarp weight	10.0035	-3.4352	-216.3222	83.1620	125.3376	0.9705	-0.2665
Seed weight	2.9918	-1.2788	-123.6490	145.4907	-23.6215	0.6900	0.6459
Endocarp coat weight	9.5966	-3.0872	-158.6994	-20.1157	170.8470	0.5999	-0.8566
Endocarp length : Endocarp breadth	6.6042	2.1415	22.0986	-10.5673	-10.7891	-9.4998	-0.0139

Residual effect = -0.0839

Endocarp length : endocarp breadth had a negative direct effect (-9.2990) on seed weight : endocarp weight alongwith indirect negative effect of seed weight (-0.1208), endocarp coat weight (-0.3388). This character also had positive indirect effect via endocarp length (5.0874), and endocarp breadth (4.0004), endocarp weight (0.4946). Positive indirect effect was less than the negative direct and indirect effect resulting into negative (-0.1765 correlation with seed weight : endocarp weight.

Path analysis (**Table** 4.3.5.8.), when the data from all the provenance 5 (Jodhpur)was pooled, showed that endocarp length had a positive direct effect (13.0015) on seed weight : endocarp weight alongwith indirect positive effect via seed weight (33.4791), and endocarp coat weight (126.1055). This character also had negative effect via endocarp breadth (-1.7862), endocarp weight (166.4418) and endocarp length : endocarp breadth (-4.8255). But the positive direct and indirect effect was less than the negative indirect effect and resulting into negative (-0.4580) correlation with seed weight : endocarp weight.

Endocarp breadth had a positive direct effect (-3.9552) on seed weight : endocarp weight alongwith indirect negative effect via endocarp weight (187.8798), endocarp coat weight (0.0689). This character also had positive indirect effect via seed weight (47.0403), endocarp coat weight (133.3533) and endocarp length : endocarp breadth (5.1434). But the positive indirect effect was less than the negative direct indirect effect resulting into negative (-0.4147) correlation with seed weight : endocarp weight.

Endocarp weight had a negative direct effect (-216.3222) on seed weight : endocarp weight alongwith indirect negative effect of endocarp breadth (-3.4352). seed weight : endocarp weight (0.1655) and endocarp length : endocarp breadth (0.2028). This character also had positive indirect effect via endocarp length (10.0035), seed weight (83.1620), endocarp coat weight (125.3376) and endocarp length : endocarp breadth (0.9705). But the positive indirect effect was less than the negative direct and indirect effect resulting into negative (-0.2665) correlation with seed weight : endocarp weight.

Seed weight had a positive direct effect (145.4907) on seed weight : endocarp weight alongwith indirect positive effect of endocarp length (2.9918), and endocarp length : endocarp breadth (0.6900). This character also had negative indirect effect via endocarp breadth (-1.2788), endocarp weight (-123.6490) and endocarp coat weight (-23.6215). But the positive direct and indirect effect was more than the negative indirect effect resulting into positive (0.6459) correlation with seed weight : endocarp weight.

Endocarp coat weight had a positive direct effect (170.8470) on seed weight : endocarp weight alongwith indirect positive effect of endocarp length (9.5966), endocarp coat weight (170.8470),and endocarp length : endocarp breadth (0.5999). This character also had negative indirect effect via endocarp breadth (-3.0872), endocarp weight (-158.6994) and seed weight (-

20.1157). But the positive direct and indirect effect was less than the negative indirect effect resulting into negative (-0.8566) correlation with seed weight : endocarp weight.

Endocarp length : endocarp breadth had a negative direct effect (-9.4998) on seed weight : endocarp weight alongwith indirect negative effect of seed weight (-10.7891), and endocarp coat weight (-10.7891). This character also had positive indirect effect via endocarp length (6.6042), endocarp breadth (2.1415) and endocarp weight (22.0986). But the positive indirect effect was less than the negative direct indirect effect resulting into negative (-0.0139) correlation with seed weight : endocarp weight.

Path analysis (**Table** 4.3.5.9.), when the data from provenance 6 (Kalka) was pooled, showed that endocarp length had a positive direct effect (7.2417) on seed weight : endocarp weight alongwith positive indirect effect via endocarp breadth (3.8115), seed weight (4.0413) and endocarp coat weight (0.0592). This character also had a negative indirect effect via endocarp weight (-2.7506) and endocarp length : endocarp breadth (-12.1134). But the positive direct and indirect effect was more than the negative indirect effect and resulting into positive (0.2919) correlation with seed weight : endocarp weight.

Endocarp breadth had a negative direct effect (-5.4011) on seed weight : endocarp weight alongwith negative indirect effect of endocarp length (-5.1104), and endocarp coat weight (-0.0188). This negative effect was neutralized by positive indirect effect of endocarp weight (0.0324), seed weight (1.7527) and endocarp length : endocarp breadth (10.4908). Positive indirect effect was more than the negative indirect effect resulting into positive (1.7439) correlation with seed weight : endocarp weight.

Endocarp weight had a negative direct effect (-3.6096) on seed weight : endocarp weight alongwith negative indirect effect of endocarp length : endocarp breadth (-7.1075). This negative effect was neutralized by indirect positive effect via endocarp length (5.5183), endocarp breadth (0.0484), seed weight (6.2466) and endocarp coat weight (0.0842). Positive indirect effect was more than the negative indirect effect resulting into positive (1.1665) correlation with seed weight : endocarp weight.

Seed weight had a positive direct effect (6.3813) on seed weight : endocarp weight alongwith indirect positive effect of endocarp length (4.5861), and endocarp coat weight (0.0641). This character also had negative indirect effect via endocarp breadth (-1.4835), endocarp weight (-3.5334) and endocarp length : endocarp breadth (-4.7604). But the positive direct and indirect effect was more than the negative indirect effect resulting into positive (1.2560) correlation with seed weight : endocarp weight.

Endocarp coat weight had a positive direct effect (0.0694) on seed weight : endocarp weight alongwith indirect positive effect of endocarp length (6.1721), endocarp breadth (1.4605), seed weight (5.8883). This character also had negative indirect effect via endocarp weight (-3.5448)

Table 4.3.5.9. **Direct and indirect effects of various characters on Seed weight : Endocarp weig . *Azadirachta indica* in provenance 6 (Kalka)**

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Endocarp length : Endocarp breadth	Genotypic correlation
Endocarp length	7.2417	3.8115	-2.7506	4.0413	0.0592	-12.1134	0.2919
Endocarp breadth	-5.1104	-5.4011	0.0324	1.7527	-0.0188	10.4908	1.7439
Endocarp weight	5.5183	0.0484	-3.6096	6.2466	0.0682	-7.1075	1.1665
Seed weight	4.5861	-1.4835	-3.5334	6.3813	0.0641	-4.7604	1.2560
Endocarp coat weight	6.1721	1.4605	-3.5448	5.8883	0.0694	-9.0076	1.0403
Endocarp length : Endocarp breadth	7.0666	4.5646	-2.0667	2.4472	0.0504	-12.4134	-0.3491

Residual effect = 0.0967

Table 4.3.5.10. **Direct and indirect effects of various characters on Seed weight : Endocarp weight in *Azadirachta indica* in provenance 10 (Pune)**

	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Endocarp length : Endocarp breadth	Genotypic correlation
Endocarp length	-99.3632	-68.8909	41.4910	36.5286	-89.5110	178.5961	-1.1488
Endocarp breadth	113.5134	60.3032	-33.9529	-48.4926	93.8806	-184.0391	1.2121
Endocarp weight	-56.3228	-27.9719	73.1970	10.9097	-98.5328	98.3711	-0.3489
Seed weight	191.2078	154.0502	-42.0682	-18.9825	70.7300	-352.7945	2.1424
Endocarp coat weight	-88.9159	-56.5971	72.1032	13.4225	-100.0281	159.2625	-0.7525
Endocarp length : Endocarp breadth	-101.3200	-63.3647	41.1112	38.2360	-90.9564	175.1469	-1.1464

Residual effect = 5.4881

and endocarp length : endocarp breadth (-9.0076). Positive direct and indirect effect was more than the negative indirect effect resulting into positive (1.0403) correlation with seed weight : endocarp weight.

Endocarp length : endocarp breadth had a positive direct effect (-12.4134) on seed weight : endocarp weight alongwith indirect negative effect of endocarp weight (-2.0667). This character also had positive indirect effect via endocarp length (7.0666), and endocarp breadth (4.5646), seed weight (2.4472) and endocarp coat weight (0.0504). Positive indirect effect was less than the negative direct effect resulting into negative (-0.03491) correlation with seed weight : endocarp weight. Path analysis, when the data from provenance 9 (Hisar) was pooled, showed that no endocarp character considered had direct or indirect effect on seed weight : endocarp weight.

Path analysis (**Table** 4.3.5.10.), when the data from provenance 10 (Pune) was pooled, showed that endocarp length had a negative direct effect (-99.3632) on seed weight : endocarp weight alongwith the negative i direct effect via endocarp breadth (-89.5110), and endocarp coat weight (178.5961). However, this negative effect was neutralized in part by positive indirect effect via endocarp weight (41.4910), seed weight (36.5286), and endocarp length : endocarp breadth (178.5961). But the negative direct and indirect effect was more than the negative indirect effect resulting into negative (-1.1488) correlation with seed weight : endocarp weight. Endocarp breadth had a positive direct effect (60.3032) on seed weight : endocarp weight alongwith direct positive effect via endocarp length (113.5134), and endocarp coat weight (93.8806). This character also had negative indirect effect via endocarp weight (-33.9529), seed weight (-48.4926) and endocarp length : endocarp breadth (-184.0391). But the positive direct and indirect effect was more than the negative indirect effect resulting into positive (1.2121) correlation with seed weight : endocarp weight.

Endocarp weight had a positive direct effect (73.1970) on seed weight : endocarp weight alongwith direct positive effect via seed weight (10.9097), and endocarp length : endocarp breadth (98.3711). This character also had negative indirect effect via endocarp length (56.3228), endocarp breadth (-27.9719) and endocarp coat weight (-98.5328). But the negative indirect effect was more than the positive direct and indirect effect resulting into negative (-0.3489) correlation with seed weight : endocarp weight.

Seed weight had a negative direct effect (-18.9825) on seed weight : endocarp weight alongwith negative indirect effect of endocarp weight (-42.0682), and endocarp length : endocarp breadth (-352.7945). This character also had positive indirect effect via endocarp length (191.2078), endocarp breadth (154.050), and endocarp coat weight (70.7300). But the positive direct and indirect effect was more than the negative indirect effect resulting into positive (2.1424) correlation with seed weight : endocarp weight.

Endocarp coat weight had a positive direct effect (-100.0281) on seed weight : endocarp weight alongwith indirect positive effect via endocarp weight (72.1032), seed weight (13.4225), and endocarp length : endocarp breadth (159.2625). This character also had negative indirect effect via endocarp length (-88.9159), and endocarp breadth (-56.5971). But the negative direct and indirect effect was more than the negative indirect effect resulting into negative (-0.7525) correlation with seed weight : endocarp weight.

Endocarp length : endocarp breadth had a positive direct effect (175.1469) on seed weight : endocarp weight alongwith direct positive effect via endocarp weight (41.1112), seed weight (38.2360) and endocarp length: endocarp breadth (175.1469). This character also had negative indirect effect via endocarp length (-101.3200), and endocarp breadth (-63.3647). But the positive direct and indirect effect was less than the negative indirect effect resulting into negative (-1.1464) correlation with seed weight : endocarp weight.

4.4. Leaf morphology

4.4.1. Variation with respect to leaf morphological characteristics

Leaf morphological characteristics of some trees of Hisar provenance were studied (**Table** 4.4.1.1.). Leaf length showed a range of 10.0 to 39.0 cm. Highest value for Leaf length (39.0 cm) was observed in BAC-3 and the same tree exhibited the highest value for mean Leaf length (30.5 cm). Lowest mean Leaf length (16.4 cm) was found in BSB-0. Highest coefficient of variation (32.10) for this character was observed in HSR-06, while HSR-03 exhibited a lowest value of 6.98. Number of Leaflets showed a range of six to 22. Highest value for and number of Leaflets (22) was observed in HSR-05. Highest mean number of Leaflets (18.30) was found in HSR-05. Lowest mean number of Leaflets (9.9) was found in BSB-0. Highest coefficient of variation (37.40) for this character was observed in BGS-0, while HSR-03 exhibited a lowest value of 8.40.

Leaflet length showed a range of 2.3 to 8.5 cm. Highest value for Leaf length (8.5 cm) was observed in HSR-04 and the same tree exhibited the highest value for mean Leaflet length (5.79 cm). Lowest value for this character (2.2 cm) was observed in HSR-05. Lowest mean Leaflet length (3.75) was found in SHS-0. Highest coefficient of variation for this character was observed in HSR-06, while FTR-1 exhibited a lowest value of 9.73.

Number of serrations towards rachis showed a range of 0 to 12. Highest value for number of serrations towards rachis (12) was observed in BAC-3. Lowest value for and number of serrations towards rachis (0) was observed in two genotypes namely, HSR-02 and BGS-0. BGS-0 exhibited the highest value for mean number of serrations towards rachis (6.50). Lowest mean number of serrations towards rachis (0.70) was found in HSR-02. Highest coefficient of variation (74.21) for this character was observed in GPN-0, while HSR-03 exhibited a lowest value of 16.98. Number of serrations away from rachis showed a range of five to 19. Highest

Table 4.4.1.1. Variability with respect to leaf morphological characteristics in some trees of *Azadirachta indica* of Hisar provenance

Acc. Number	Leaf length (cm)			Number of leaflets			Leaflet length (cm)		
	Range	Mean	C.V.	Range	Mean	C.V.	Range	Mean	C.V.
HSR-03	18.5-22.4	20.49 (±1.43)	6.98	10-14	11.90 (±1.37)	11.51	4.2-7.1	5.79 (±0.90)	15.79
HSR-02	17.6-26.0	21.93 (±3.06)	13.95	12-14	13.10 (±1.10)	8.40	3.7-6.6	5.05 (±1.04)	20.59
SHS-0	18.0-26.0	20.56 (±2.59)	12.60	12-16	13.20 (±1.87)	14.17	2.3-5.0	3.75 (±0.91)	24.27
HSR-04	14.5-35.2	28.73 (±3.20)	11.14	16-20	17.55 (±2.17)	12.36	2.3-8.5	6.13 (±1.52)	24.80
HSR-01	14.5-29.8	20.7 (±5.88)	28.41	8-15	12.00 (±2.21)	18.42	3.6-6.4	5.29 (±0.95)	17.96
BSB-O	13.1-24.5	16.4 (3.67)	22.38	7-14	9.9 (2.28)	23.03	3.8-5.8	5.08 (0.64)	12.60
HSR-06	10.0-25.0	16.42 (±5.27)	32.10	10-19	13.90 (±3.11)	22.37	2.9-6.0	4.33 (±1.14)	26.33
BAC-3	22.5-39.0	30.5 (±6.50)	21.31	13-19	15.4 (±2.37)	15.39	3.2-6.8	5.16 (±1.16)	22.48
BAS-2	10.0-15.5	12.72 (±1.55)	12.19	7-12	10.50 (±1.90)	18.10	3.5-5.2	4.59 (±0.60)	13.07
BGS-0	13.0-32.5	20.94 (±6.33)	30.23	6-18	10.40 (±3.89)	37.40	4.0-6.0	5.26 (±0.71)	13.50
GPN-0	12.8-27.0	20.16 (±4.62)	22.92	9-17	14.10 (±2.56)	18.16	3.3-7.1	4.85 (±1.24)	25.57
HSR-05	15.0-29.4	20.75 (±4.94)	23.81	13-22	18.30 (±2.98)	16.28	2.2-5.6	4.00 (±0.96)	24.00
FTR-1	17.5-28.7	23.38 (±4.14)	17.71	12-17	14.70 (±1.95)	13.27	3.8-5.0	4.42 (±0.43)	9.73
C.D. (5%)		3.9959			2.18833			0.8848	

Table 4.4.1.1. (continued)

Acc. Number	Number of serrations towards rachis			Number of serrations away from rachis			TL
	Range	Mean	C.V.	Range	Mean	C.V.	
HSR-03	3-7	2.50 (±2.54)	-	6-16	10.50 (±2.76)	26.29	10
HSR-02	0-2	0.70 (±0.82)	-	5-11	7.90 (±2.02)	25.57	0
SHS-0	2-11	4.60 (±2.63)	57.17	6-12	9.30 (±1.49)	16.02	0
HSR-04	2-7	2.50 (±2.54)	-	7-16	11.56 (±3.06)	26.47	0
HSR-01	1-8	4.70 (±1.89)	40.21	10-19	13.6 (±3.13)	23.01	10
BSB-O	1-5	2.60 (±1.26)	48.46	5-12	8.60 (2.32)	26.98	70
HSR-06	1-5	3.40 (±1.07)	31.47	8-11	8.90(±1.10)	12.36	60
BAC-3	3-12	5.30 (±2.71)	51.13	11-18	13.0(±2.67)	20.54	50
BAS-2	2-9	5.50 (±2.27)	41.27	6-14	11.50 (±2.22)	19.30	10
BGS-0	0-10	6.50 (±2.68)	41.23	9-18	14.60 (±2.84)	19.45	0
GPN-0	2-8	3.80 (±2.82)	74.21	8-12	9.80 (±2.82)	28.78	0
HSR-05	2-7	3.80 (±1.69)	44.47	8-16	10.80 (±2.97)	27.50	10
FTR-1	1-3	1.80 (±0.92)	51.11	5-10	6.80 (±1.40)	20.59	60
C.D. (5%)		1.9062			2.1313		

TL = Number of leaves showing terminal leaflet (%)

Table 4.4.2.1. Association of different morphological characteristics of leaf in different trees of Hisar provenance (Accession No. of tree HSR-01)

	No. of leaflets	Leaflet length	No. of serrations away from rachis	No. of serrations towards rachis
Leaf length	0.89**	0.45	0.30	0.37
No. of leaflets	-	0.36	0.19	0.32
Leaflet length	-	-	0.31	0.65*
No. of serrations away from rachis	-	-	-	0.78**

** significant at 1% level of significance * significant at 5% level of significance

Table 4.4.2.2. Association of different morphological characteristics of leaf in different trees of Hisar provenance (Accession No. of tree BSB-0)

	No. of leaflets	Leaflet length	No. of serrations away from rachis	No. of serrations towards rachis
Leaf length	0.89**	0.39	-0.14	-0.12
No. of leaflets	-	0.05	-0.13	-0.05
Leaflet length	-	-	-0.48	0.50
No. of serrations away from rachis	-	-	-	0.17

** significant at 1% level of significance * significant at 5% level of significance

Table 4. 4.2.3. Association of different morphological characteristics of leaf in different trees of Hisar provenance (Accession No. of tree HSR-06)

	No. of leaflets	Leaflet length	No. of serrations away from rachis	No. of serrations towards rachis
Leaf length	0.71*	0.83**	0.72*	0.69*
No. of leaflets	-	0.33	0.35	0.39
Leaflet length	-	-	0.67*	0.71*
No. of serrations away from rachis	-	-	-	0.41

** significant at 1% level of significance * significant at 5% level of significance

Table 4.4.2.4. Association of different morphological characteristics of leaf in different trees of Hisar provenance (Accession No. of tree BAC-3)

	No. of leaflets	Leaflet length	No. of serrations away from rachis	No. of serrations towards rachis
Leaf length	0.82**	0.40	-0.25	0.60
No. of leaflets	-	0.06	-0.32	0.67*
Leaflet length	-	-	0.16	0.15
No. of serrations away from rachis	-	-	-	-0.60

** significant at 1% level of significance * significant at 5% level of significance

value for and number of serrations away from rachis (19) was observed in HSR-01. Highest mean number of serrations away from rachis (14.60) was found in BGS-0. Lowest mean number of serrations away from rachis (7.90) was found in HSR-02 and HSR-05. Highest coefficient of variation (28.78) for this character was observed in GPN-0, while HSR-03 exhibited a lowest value of 7.35.

Number of leaves showing terminal leaflet showed a range of 0 to 70 per cent. Highest value for this character (70 per cent) was observed in BSB-0. Lowest and number of leaves showing terminal leaflet (0) was found in five genotypes.

4.4.2. Association of leaf morphological characteristics

Association of different morphological characteristics of leaf in tree HSR-01 of Hisar provenance was studied (**Table** 4.4.2.1.). There was positive association amongst all the leaf characteristics though only some of the associations were significant. The association between Leaf length and number of leaflets was significant (0.89) at one per cent level of significance. Leaflet length and number of serrations were found to be significantly associated with and number of serrations towards rachis at one per cent (0.65) and five per cent (0.78) level of significance. In addition to this, association between Leaf length and Leaflet length was found to be high but non-significant away from rachis was found to be very high (0.64) but non-significant.

Association of different morphological characteristics of leaf in tree with accession number BSB-0 of Hisar provenance was studied (**Table** 4.4.2.2.). There was negative association of number of serration away from rachis with all the characters except number of serrations towards rachis, with which it was positively correlated. Only the association between Leaf length and number of leaflets was found to be significant (0.89) at one per cent level of significance. Association of different morphological characteristics of leaf in tree with accession number HSR-06 of Hisar provenance was studied (**Table** 4.4.2.3.). There was positive association amongst all the leaf characteristics and many of the associations were significant. Leaf length was positively and significantly associated with all other characters of leaf. Association of Leaflet length with and number of serration away from rachis and number of serrations towards rachis was found to be significant, i.e. 0.67 and 0.71 respectively, at five per cent level of significance. In addition to this, association between number of leaflets and number of serrations away from rachis was found to be very high (0.64) but non-significant.

Association of different morphological characteristics of leaf in tree with accession number BAC-3 of Hisar provenance was studied (**Table** 4.4.2.4.). There was positive association amongst most of the leaf characteristics though most of the associations were non-significant. Only the association between Leaf length and number of leaflets was found to be significant (0.82) at one per cent level of significance and association between number of leaflets and number of serrations towards rachis was found to be significant (0.67) at five per cent level of

Table 4.4.2.5. Association of different morphological characteristics of leaf in different trees of Hisar provenance (Accession No. of tree BAS-2)

	No. of leaflets	Leaflet length	No. of serrations away from rachis	No. of serrations towards rachis
Leaf length	0.10	0.21	0.51	-0.20
No. of leaflets	-	-0.12	-0.17	0.07
Leaflet length	-	-	0.67*	0.72*
No. of serrations away from rachis	-	-	-	0.27

**significant at 1% level of significance; * significant at 5% level of significance

Table 4.4.2.6. Association of different morphological characteristics of leaf in different trees of Hisar provenance (Accession No. of tree BGS-0)

	No. of leaflets	Leaflet length	No. of serrations away from rachis	No. of serrations towards rachis
Leaf length	0.97**	-0.07	0.27	0.30
No. of leaflets	-	-0.15	0.19	0.19
Leaflet length	-	-	0.34	0.51
No. of serrations away from rachis	-	-	-	0.83**

** significant at 1% level of significance; * significant at 5% level of significance

Table 4.4.2.7. Association of different morphological characteristics of leaf in different trees of Hisar provenance (Accession No. of tree GPN-0)

	No. of leaflets	Leaflet length	No. of serrations away from rachis	No. of serrations towards rachis
Leaf length	0.74*	0.84**	0.26	0.16
No. of leaflets	-	0.38	0.14	-0.38
Leaflet length	-	-	0.14	0.24
No. of serrations away from rachis	-	-		0.48

** significant at 1% level of significance; * significant at 5% level of significance

Table 4.4.2.8 Association of different morphological characteristics of leaf in different trees of Hisar provenance (Accession No. of tree HSR-05)

	No. of leaflets	Leaflet length	No. of serrations away from rachis	No. of serrations towards rachis
Leaf length	0.55	0.69*	-0.17	-0.14
No. of leaflets		-0.05	-0.41	-0.58
Leaflet length			0.46	0.48
No. of serrations away from rachis				0.77**

** significant at 1% level of significance; * significant at 5% level of significance

significance. In addition to this, association of number of serrations towards rachis with Leaf length and number of serrations away from rachis was found to be very high (i.e., 0.60 and -0.60 respectively) but the values were non-significant.

Association of different morphological characteristics of leaf in tree with accession number BAS-2 of Hisar provenance was studied (**Table** 4.4.2.5.). There was positive association amongst most the leaf characteristics though most of the associations were non-significant. Only the association of Leaflet length with number of serration away from rachis and number of serrations towards rachis was found to be significant (i.e., 0.67 and 0.72) at five per cent level of significance. In addition to this, association between Leaflet length and number of serrations away from rachis was found to be very high (0.51) but non-significant.

Association of different morphological characteristics of leaf in tree with accession number of tree B-4, old campus of Hisar provenance was studied (**Table** 4.4.2.6.). There was positive association amongst all the leaf characteristics though most of the associations were non-significant. Only the association between number of leaflets and number of serrations towards rachis was found to be significant (0.64) at five per cent level of significance. In addition to this, association between number of leaflets and number of serrations away from rachis was found to be very high (0.64) but non-significant.

Association of different morphological characteristics of leaf in tree with accession number of tree B-4, old campus of Hisar provenance was studied (**Table** 4.4.2.7.). There was positive association amongst all the leaf characteristics though most of the associations were non-significant. Only the association between number of leaflets and number of serrations towards rachis was found to be significant (0.64) at five per cent level of significance. In addition to this, association between number of leaflets and number of serrations away from rachis was found to be very high (0.64) but non-significant.

Association of different morphological characteristics of leaf in tree with accession number of tree B-4, old campus of Hisar provenance was studied (**Table** 4.4.2.8.). There was positive association amongst all the leaf characteristics though most of the associations were non-significant. Only the association between number of leaflets and number of serrations towards rachis was found to be significant (0.64) at five per cent level of significance. In addition to this, association between number of leaflets and number of serrations away from rachis was found to be very high (0.64) but non-significant.

Association of different morphological characteristics of leaf in tree with accession number of tree B-4, old campus of Hisar provenance was studied (**Table** 4.4.2.9.). There was positive association amongst all the leaf characteristics though most of the associations were non-significant. Only the association between number of leaflets and number of serrations towards rachis was found to be significant (0.64) at five per cent level of significance. In addition to

Table 4.4.2.9. Association of different morphological characteristics of leaf in different trees of Hisar provenance (Accession No. of tree FTR-1)

	No. of leaflets	Leaflet length	No. of serrations away from rachis	No. of serrations towards rachis
Leaf length	0.91**	0.32	-0.50	-0.72*
No. of leaflets		0.22	-0.53	-0.68*
Leaflet length			0.04	0.23
No. of serrations away from rachis				0.57

** significant at 1% level of significance; * significant at 5% level of significance

Table 4.4.2.10. Association of different morphological characteristics of leaf in different trees of Hisar provenance (Accession No. of tree HSR03)

	No. of leaflets	Leaflet length	No. of serrations away from rachis	No. of serrations towards rachis
Leaf length	0.07	-0.47	-0.23	0.38
No. of leaflets		0.36	0.57	0.51
Leaflet length			0.60	-0.02
No. of serrations away from rachis				0.43

** significant at 1% level of significance; * significant at 5% level of significance

Table 4.4.2.11. Association of different morphological characteristics of leaf in different trees of Hisar provenance (Accession No. of tree HSR02)

	No. of leaflets	Leaflet length	No. of serrations away from rachis	No. of serrations towards rachis
Leaf length	0.61	0.69*	0.20	0.46
No. of leaflets		0.05	-0.21	0.15
Leaflet length			0.20	0.13
No. of serrations away from rachis				0.78**

** significant at 1% level of significance; * significant at 5% level of significance

Table 4.4.2.12. Association of different morphological characteristics of leaf in different trees of Hisar provenance (Accession No. of tree Sp. Hos.)

	No. of leaflets	Leaflet length	No. of serrations away from rachis	No. of serrations towards rachis
Leaf length	0.70*	0.30	0.62	-0.32
No. of leaflets		0.35	0.51	-0.50
Leaflet length			0.69*	0.05
No. of serrations away from rachis				-0.42

** significant at 1% level of significance; * significant at 5% level of significance

Table 4.4.2.13. Association of different morphological characteristics of leaf in different trees of Hisar provenance (Accession No. of tree HSR04-B-4, old campus)

	No. of leaflets	Leaflet length	No. of serrations away from rachis	No. of serrations towards rachis
Leaf length	0.18	0.15	0.36	0.28
No. of leaflets		0.30	0.25	0.64*
Leaflet length			0.60	0.02
No. of serrations away from rachis				0.43

** significant at 1% level of significance; * significant at 5% level of significance

this, association between number of leaflets and number of serrations away from rachis was found to be very high (0.64) but non-significant.

Association of different morphological characteristics of leaf in tree with accession number of tree B-4, old campus of Hisar provenance was studied (**Table** 4.4.2.10.). There was positive association amongst all the leaf characteristics though most of the associations were non-significant. Only the association between number of leaflets and number of serrations towards rachis was found to be significant (0.64) at five per cent level of significance. In addition to this, association between number of leaflets and number of serrations away from rachis was found to be very high (0.64) but non-significant.

Association of different morphological characteristics of leaf in tree with accession number of tree B-4, old campus of Hisar provenance was studied (**Table** 4.4.2.11.). There was positive association amongst all the leaf characteristics though most of the associations were non-significant. Only the association between number of leaflets and number of serrations towards rachis was found to be significant (0.64) at five per cent level of significance. In addition to this, association between number of leaflets and number of serrations away from rachis was found to be very high (0.64) but non-significant.

Association of different morphological characteristics of leaf in tree with accession number of tree B-4, old campus of Hisar provenance was studied (**Table** 4.4.2.12.). There was positive association amongst all the leaf characteristics though most of the associations were non-significant. Only the association between number of leaflets and number of serrations towards rachis was found to be significant (0.64) at five per cent level of significance. In addition to this, association between number of leaflets and number of serrations away from rachis was found to be very high (0.64) but non-significant.

Association of different morphological characteristics of leaf in tree with accession number of tree B-4, old campus of Hisar provenance was studied (**Table** 4.4.2.13.). There was positive association amongst all the leaf characteristics though most of the associations were non-significant. Only the association between number of leaflets and number of serrations towards rachis was found to be significant (0.64) at five per cent level of significance. In addition to this, association between number of leaflets and number of serrations away from rachis was found to be very high (0.64) but non-significant.

When all the trees were compared then it was found that leaf length was positively associated with and number of leaflets in all the trees. Highest incidence of negative association (46.15 per cent) was seen in the case of correlation coefficients between number of leaflets and number of serration away from rachis. No negative association was found to be significant while 31.37 per cent (13.24 per cent significant at one per cent level of significance and 18.13 per cent significant at five per cent level of significance) positive associations were significant (**Table** 4.4.2.14.).

Table 4.4.2.14. Break-up of correlation coefficients of leaf morphological characteristics of 13 neem trees of Hisar Provenance

Pairs of leaf morphological characteristics	Number of trees showing positive correlations (significant at 1%) [significant at 5%]	Number of trees showing negative correlations (significant at 1%) [significant at 5%]
Leaf length : Number of leaflets	13 (5) [3]	0 (0) [0]
Leaf length : Leaflet length	11(3) [2]	2 (0) [0]
Leaf length : Number of serration away from rachis	8 (0) [1]	5 (0) [0]
Leaf length : Number of serrations towards rachis	9 (0) [2]	4(0) [0]
Number of leaflets : Leaf length	13 (5) [3]	0 (0) [0]
Number of leaflets : Leaflet length	10 (0) [0]	3 (0) [0]
Number of leaflets: Number of serration away from rachis	7 (0) [0]	6 (0) [0]
Number of leaflets : Number of serrations towards rachis	9 (0) [3]	4 (0) [0]
Leaflet length : Leaf length	11 (3) [2]	2 (0) [0]
Leaflet length : Number of leaflets	10 (0) [0]	3 (0) [0]
Leaflet length : Number of serration away from rachis	12 (0) [3]	1 (0) [0]
Leaflet length : Number of serrations towards rachis	12 (0) [3]	1 (0) [0]
Number of serrations away from rachis : Leaf length Number of leaflets	8 (0) [1]	5 (0) [0]
Number of serrations away from rachis : Number of leaflets	7 (0) [0]	6 (0) [0]
Number of serrations away from rachis : Leaflet length	12 (0) [3]	1 (0) [0]
Number of serrations away from rachis : Number of serrations towards rachis	11 (4) [0]	2 (0) [0]
Number of serrations towards rachis : Leaf length	9 (0) [2]	4 (0) [0]
Number of serrations towards rachis : Number of leaflets	9 (0) [3]	4 (0) [0]
Number of serrations towards rachis : Leaflet length	12 (0) [3]	1 (0) [0]
Number of serrations towards rachis : Number of serrations towards rachis	11 (4) [0]	2 (0) [0]

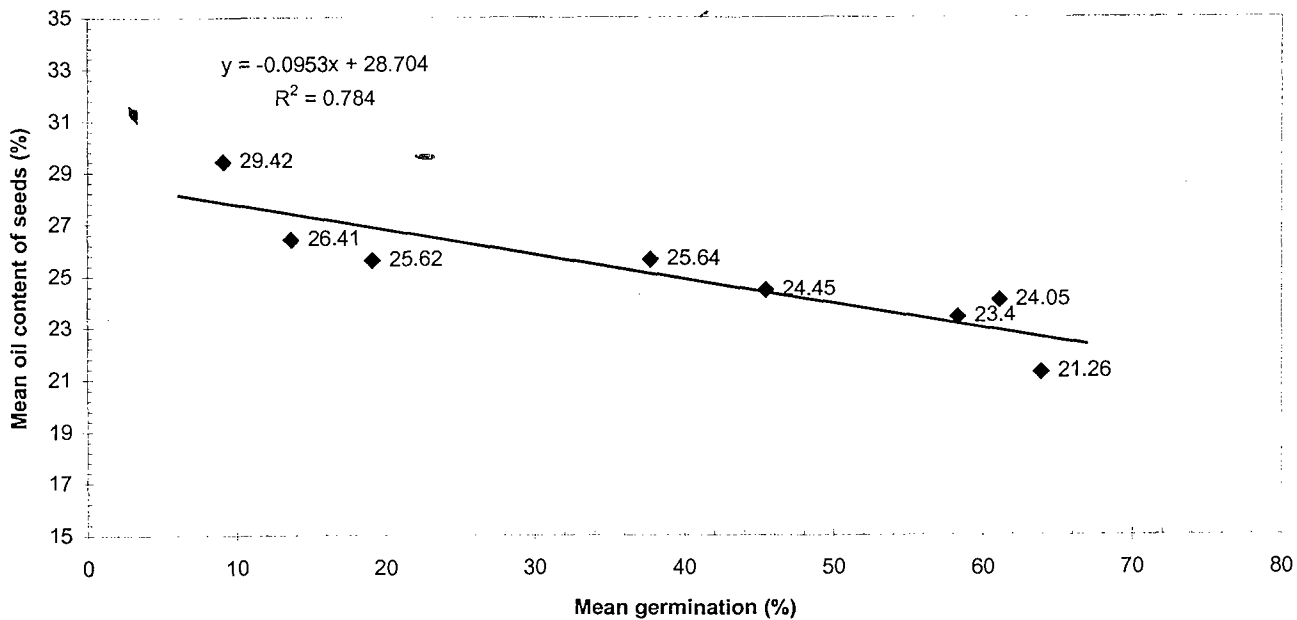
Table 4.5.1.1. Variation w.r.t. seed viability in different provenance collections of *Azadirachta indica*

Accession Number	Provenance	Number of endocarps sown	Number of endocarps germinated	Mean germination	Coefficient of variation	Radicle length (mm)	
						Range	Mean ($\pm$S.D.)
T5	Delhi	30	11	37.72^{b} ($\pm$17.37)	46.05	2-5	3.55^{a}($\pm$1.21)
T7	Delhi	52	14	24.89^{g} ($\pm$14.98)	60.19	3-8	4.93^{b}($\pm$1.83)
Mean of the provenance				**31.35**			**4.24**
T30	Alwar	5	1	20.00@	-	3-3	-
T31	Alwar	24	11	44.16^{h} ($\pm$1.83)	4.14	4-16	8.30^{d}($\pm$7.65)
Mean of the provenance				**32.08**			-
H083	Kaithal	30	1	3.13^{a} ($\pm$4.42)	-	1-1	-
H084	Kaithal	21	9	37.73^{b}($\pm$10.92)	28.94	2-9	4.75^{b}($\pm$2.8)
Mean of the provenance				**20.43**			-
T20	Jodhpur	58	1	2.28^{a} ($\pm$3.22)	-	1-1	-
T21	Jodhpur	27	2	6.67^{a} ($\pm$9.43)	-	6-8	7.00^{d}($\pm$1.41)
T22	Jodhpur	41	31	74.02^{c}($\pm$13.17)	17.79	4-16	11.9^{e}($\pm$4.91)
T23	Jodhpur	10	6	60@	-	12-21	17.17^{f}($\pm$3.54)
T24	Jodhpur	18	14	77.78^{c}($\pm$15.71)	20.20	9-20	14.0^{g}($\pm$6.08)
Mean of the provenance				**44.15**			**12.52**
H099	Kalka	20	2	9.09^{f} ($\pm$12.86)	-	4-5	4.50^{b}($\pm$0.71)
H101	Kalka	21	12	58.34^{d} ($\pm$11.79)	20.21	2-9	4.50^{b}($\pm$3.03)
H102	Kalka	18	11	61.12^{d}($\pm$7.86)	12.86	6-20	12.8^{e}($\pm$4.57)
H107	Kalka	11	5	45.45@	-	9-16	10.2^{e}($\pm$4.00)
Mean of the provenance				**43.5**			**8.00**
HSR01	Hisar	21	4	19.09^{e} ($\pm$1.29)	6.76	4-11	6.25^{d}($\pm$3.30)
HSR02	Hisar	26	3	13.64^{fe} ($\pm$19.28)	-	9-12	10.67^{e}($\pm$1.53)
HSR03	Hisar	60	0	0.00@	-	-	-
HSR04	Hisar	27	16	63.89^{d}($\pm$19.71)	30.85	2-37	14.00^{g}($\pm$10.83)
HSR05	Hisar	12	3	25.00@	-	NA	7.33^{d}($\pm$4.51)
SHS-0	Hisar	34	33	96.43^{p} ($\pm$5.05)	5.24	2-25	6.39^{d}($\pm$5.55)
Mean of the provenance				**36.34**			**8.928**
Mean of all the provenance				**37.16**			**8.72**

Values with different superscripts are significantly different at P = 0.5 according to *t* test

@ t-test not performed

Fig. 4.5.1.1. Relationship of mean germination with oil content of seed in some provenance collections of *Azadirachta indica*



4.5. Provenance evaluation for seed viability/growth performance

4.5.1. *In vitro* evaluation for endocarp viability

Within and between provenance variation with respect to seed viability in different provenance collections of neem was studied (**Table** 4.5.1.1.). The endocarps were stored for a period of 6 months at a temperature of 25(±2° C). Overall mean germination was 37.16 per cent. Eleven genotypes exhibited above average (>37.16) seed viability. While one genotype from Hisar provenance (HSR03) was found to have lost viability. Six genotypes SHS-0, HSR02 (Hisar), H099 (Kalka), T20, T21 (Jodhpur), H083 (Kaithal) were found to have low average viability (<15 per cent). T31 (Alwar), T22, T23 (Jodhpur), H101, H102, H107 (Kalka), HSR04 and SHS-0 (Hisar) showed high level (>40 per cent) of viability. Highest mean germination percentage was observed in SHS-0 (Hisar) followed by T24 (Jodhpur).

Amongst the provenances, Jodhpur showed highest mean germination percentage of 44.15, closely followed by Kalka (43.5 per cent). Kaithal showed lowest mean germination percentage of 20.43. Highest coefficient of variation for mean germination was recorded for T7D (Delhi) and lowest for T31 (Alwar). Highest value of mean radicle length was observed in T23 (Jodhpur). Eleven genotypes exhibited above average (>8.72) seed viability. Association (Karl Pearson Correlation Coefficient) between mean germination and radicle length was found to be positive and non-significant (0.452) at five per cent level of significance but it was significant at 10 per cent level of significance. When the extreme value of germination (96.43 per cent of SHS-0 from Hisar provenance) was excluded from the data the association between mean germination and radicle length was found to be positive and significant (0.621) at 2 per cent level of significance.

Association (Karl Pearson Correlation Coefficient) between mean germination and oil content was found to be negative and non-significant (-0.503) at five per cent level, when the extreme value of germination (96.43 per cent of SHS-0 from Hisar provenance) was excluded from the data. The association between mean germination and oil content was found to be negative and significant (-0.885) at one per cent level of significance, when the extreme value of germination (0 per cent of HSR-3 and 3.13 per cent of KTL5) were excluded from the data. Regression equation (Fig. 4.5.1.1.) for linear regression of seed germination on seed oil content gives an equation for linear regression $y = -0.0953x + 28.704$, with a very high value of coefficient of determination ($R^2 = 0.784$) pointing to very high level of goodness of fitness of the regression line.

4.5.2. Nursery evaluation for growth performance

Provenance evaluation of different provenance collections was done in nursery. Three months old seedlings were studied. Data on seedling height (**Table** 4.5.2.1.) showed a wide

Table 4.5.2.1. Provenance evaluation of seedling growth in different genotypes of neem under field condition at Hisar w.r.t. seedling height

Treatment Number	Tree Id. Number	Provenance	Seedling height (cm)			
			Mean	Range	S D	C V
17	UAS Dharwad-1	Dharwad	13.53	11.0-15.8	1.73	12.76
1	UAS Dharwad -2	Dharwad	13.40	10.1-19.0	4.12	30.71
2	UAS Dahrwad-3	Dharwad	13.92	5.0-22.0	4.98	35.78
3	Andipatti Udumal Pet	Andipatti Udumal Pet	15.28	9.3-20.3	3.29	21.49
4	Thuckianpalam (Valapaddy)	Thuckianpalam (Valapaddy)	11.65	6.9-15.8	2.89	24.79
5	Chitoor	Chitoor	14.83	12.2-18.0	2.10	14.19
16	Adu.	Aduthurai	17.58	6.5-22.0	5.42	30.82
18	Uthu[illegible]	Uthupatty	16.30	11.0-22.0	3.42	21.00
8	H[illegible]	Kaithal	10.28	9.9-12.9	1.47	14.29
12	H10[illegible]	Pinjore	12.90	9.9-16.2	2.39	18.53
7	H106	Pinjore	15.08	7.0-23.0	4.87	32.28
20	H101	Kalka	16.83	13.5-20.0	2.53	15.02
15	H108	Kalka	13.37	9.0-18.3	3.25	24.32
14	H104	Patiala	17.20	14.0-18.2	2.28	13.26
10	H080	Kurukshetra	13.11	8.0-16.5	2.81	21.39
13	H048	Rohtak	12.85	7.2-17.3	3.60	28.01
22	H049	Rohtak	8.70	5.5-15.2	3.93	45.23
21	H056	Gurgaon	9.03	5.0-11.5	2.23	24.68
6	H004	Barwala	16.52	14.0-21.0	2.73	16.53
27	H006	Barwala	14.67	12.0-17.0	2.05	14.01
23	BAC-01	Hisar	13.57	10.0-19.0	2.75	20.24
9	HSR03	Hisar	10.48	7.5-16.0	2.86	27.30
11	HSR01	Hisar	13.03	7.8-17.0	3.16	24.23
19	HSR04	Hisar	10.50	7.5-12.0	1.47	14.02
25	H011	Fatehabad	11.33	10.5-12.5	0.55	4.88
24	T22	Jodhpur	10.83	7.5-12.5	1.70	15.69
26	T20	Jodhpur	6.83	2.5-11.5	3.72	54.37

C.D. (0.05) = 4.009

Table 4.5.2.2. Provenance evaluation of different genotypes of *Azadirachta indica* in field conditions w.r.t. to collar diameter

Treatment Number	Tree Id. Number	Provenance	Seedling Collar Diameter (cm)			
			Mean	Range	S D	C V
17	UAS Dharwad-1	Dharwad	0.180	0.17-0.20	0.025	13.981
1	UAS Dharwad -2	Dharwad	0.212	0.20-0.25	0.022	10.357
2	UAS Dahrwad-3	Dharwad	0.185	0.14-0.21	0.024	12.772
3	Andipatti Udumal Pet	Andipatti Udumal Pet	0.230	0.20-0.27	0.026	11.226
4	Thuckianpalam (Valapaddy)	Thuckianpalam (Valapaddy)	0.233	0.20-0.29	0.034	14.569
5	Chitoor	Chitoor	0.230	0.20-0.30	0.033	14.420
16	Adu.	Aduthurai	0.233	0.20-0.25	0.034	14.777
18	Uthupatty	Uthupatty	0.315	0.26-0.39	0.047	14.862
8	H083	Kaithal	0.203	0.16-0.25	0.026	12.908
12	H105	Pinjore	0.197	0.16-0.25	0.027	13.977
7	H106	Pinjore	0.220	0.20-0.26	0.023	10.497
20	H101	Kalk[illegible]	0.353	0.31-0.43	0.038	10.673
15	H108	Kalka	0.203	0.20-0.21	0.005	2.318
14	H104	Patiala	0.228	0.20-0.28	0.025	10.851
10	H080	Kurukshetra	[illegible].182	0.16-0.20	0.015	08.050
13	H048	Rohtak	0.260	0.22-0.27	0.024	9.421
22	H049	Rohtak	0.195	0.13-0.25	0.040	20.672
21	H056	Gurgaon	0.247	0.16-0.29	0.043	17.463.
6	H004	Barwala	0.270	0.25-0.30	0.018	6.762
27	H006	Barwala	0.243	0.20-0.32	0.044	18.173.
23	BAC-01	Hisar	0.215	0.15-0.26	0.033	15.131
9	HSR03	Hisar	0.162	0.10-0.18	0.036	22.135
11	HSR01	Hisar	0.202	0.13-0.27	0.041	20.125
19	HSR04	Hisar	0.225	0.16-0.29	0.047	20.964
25	H011	Fatehabad	0.215	0.13-0.25	0.041	19.503
24	T22	Jodhpur	0.172	0.14-0.20	0.025	14.433
26	T20	Jodhpur	0.190	0.14-0.26	0.041	21.700

C.D. (0.05) = 0.004

Table 4.5.2.3. Association among endocarp morphological characteristics and seedling growth parameters in some provenance collections of *Azadirachta indica*

	Seedling length	Seedling collar diameter	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : endocarp weight	Endocarp length : endocarp breadth
Seedling length	1.0000	0.7812*	0.5569	0.4174	0.6405	0.8339*	0[illegible]87	-0.0612	0.4529	0.2183
Seedling collar diameter	-	1.0000	0.7929*	0.7773*	0.8968**	0.74[illegible]5	0.8438*	-0.2973	-0.1367	0.2125

**Significant at 1% level *Significant at 5% level of significance

variation (2.5 to 23.0 cm). Highest value of mean seedling height was observed in Adu. (Aduthurai) with a value of 17.58 followed by H101 (Kalka) (16.83 cm). Lowest value of mean seedling height was observed in tree 20 (Jodhpur) with a value of 6.83 cm followed by tree H049 (Rohtak) (6.83 cm). Highest coefficient of variation (54.37) was observed in tree 20 (Jodhpur) followed by UAS Dharwad-3 (Dharwad) (35.78). Least coefficient of variation was observed in tree H011 (Fatehabad) (4.88).

Data on seedling collar diameter (**Table** 4.5.2.2.) showed a wide variation (0.1 to 0.43 cm). Highest value of mean seedling collar diameter was observed in H101 (Kalka) with a value of 0.353 followed by Uthupatty (0.315 cm). Lowest value of mean seedling collar diameter was observed in tree taken from Campus School, Hisar with a value of 0.162 cm followed by tree 22 (0.172 cm). Highest coefficient of variation (22.135) was observed in tree taken from Campus School, Hisar followed by HSR04 (20.964). Significant positive correlation (0.52 at one per centsignificance) was found between seedling height and collar diameter when Karl Pearson correlation coefficient was calculated with the help of pooled mean values for both the characters for 26 genotypes. Seven gerotypes were further analysed for possible association of seedling growth parameters with endocarp morphological characteristics.

Seedling length was found to be positively associated with all the endocarp morphological characteristics except a slight negative association with and the association with number of seeds endocarp^{-1}. Only the association with seed weight was significant. Similarly collar diameter was found to be positively associated with all the endocarp morphological characteristics except a non-significant negative association with number of seeds endocarp^{-1} and seed weight : endocarp weight. Only the association with seed weight was significant. Except for a non-significant association with endocarp length : endocarp breadth all the associations were significant (**Table** 4.5.2.3.).

4.6. Biochemical parameters

4.6.1. Seed oil content

Seed oil content and endocarp morphology of neem trees in five provenances of northern and western India were investigated to identify ecotypes with high oil yield which could be selected for mass multiplication and/or incorporated into tree improvement programs. Whether oil yield was related to endocarp and seed morphology was also determined.

In the present study, trees with GBH ranging from 55.6 to 280cm from different provenances were represented (**Table** 4.6.1.1.). It was observed that young trees with a GBH between 55.6 and 65cm in some provenances start producing seeds rich in oil (over 20 per cent). Maximum average oil production potential was observed in seeds from the Hisar provenance from which a value of 27.59 percent oil was noted. Seeds from the same provenance exhibited maximum variability in oil content. The highest oil yield (32.61 per cent)

Table 4.6.1.1. Oil content of seeds of *Azadirachta indica* collected from five provenances from north-western India

Provenance	Latitude	Longitude	Agro-ecological region*	Number of trees studied	Oil content (%) Range	Oil content (%) Mean (Standard Error)
Bikaner	28.01N	73.22E	(2)	5	23.87-29.06	$25.80^{b}(\pm 1.95)$
Hisar	29.10N	75.46E	(2)	7	21.26-32.61	$27.59^{b}(\pm 4.07)$
Kaithal	29.48N	78.26E	(4)	6	20.03-25.64	$21.51^{a}(\pm 2.11)$
Pune	18.31N	73.55E	(6)	4**	23.25-27.79	$26.37^{b}(\pm 2.15)$
Kalka	30.42N	76.54E	(15)	5	20.00-29.42	$25.42^{b}(\pm 2.40)$

* As suggested by Sehgal *et al.* (1990)
(2) Western plain, hot arid eco-region with desert and saline soils; (4) Northern plain and central highlands, hot semi-arid eco-region with alluvium-derived soils; (6) Deccan plateau, hot semi-arid eco-region with shallow and medium (including deep) black soils; (15) Western Himalayas warm sub-humid (including humid) eco-region with brown forest and podzolic soils;

** Trees from different villages of Pune district; In 5th column, values with different superscripts are significantly different at 5% level of significance.

Table 4.6.1.2. Association of dbh and endocarp morphology with seed oil content in five provenance collections from northern and western India in *Azadirachta indica*

Characters	Oil content (%)				
	Bikaner	**Hisar**	**Kaithal**	**Pune**	**Kalka**
20 endocarp weight	0.28	-0.26	0.48	-0.70	0.16
Endocarp length	-0.03	-0.65	0.56	-0.97*	-0.62
G.B.H.	-0.52	-0.52	0.64	ND	-0.27

*significant at 5% level of significance; ND = Not determined.

was observed from a tree of the same provenance. Least potential of average oil production was exhibited in the trees grown in the Kaithal provenance with a value of 21.51 per cent.

GBH and morphological parameters of seeds were not consistently correlated with oil yield. In some provenances, one or more characteristics were positively correlated, while in others these were negatively correlated though most of the correlations were non-significant. Only in the case of samples from Pune provenance was endocarp length significantly and negatively correlated to oil content. The endocarps from trees in this provenance had the maximum average endocarp length and unique morphology with an elongated body and pointed tip. Furthermore, only in samples from the Kaithal provenance, were endocarp length and GBH positively correlated with oil content while in others these were negatively correlated. On the other hand, only in case of endocarp samples from the Hisar provenance was endocarp weight negatively correlated with oil content while in others it was positively correlated (**Table** 4.6.1.2.).

4.6.2. Fatty acid profile of seeds

Investigations revealed the presence of two saturated fatty acids namely, palmitic acid (16:0) and stearic acid (18:0) and two unsaturated fatty acids namely, oleic acid (18:1) and linoleic acid (18:2). Though already reported, myristic acid, arachidic acid and behenic acid could not be detected in the present study. This may be due to differences in instrument resolution or source of seeds. The mean and range of variation for different fatty acids is presented in **Table** 4.6.2.1., which indicates that there is a considerable potential in the genotypes from different provenances to offer various combinations of fatty acid composition which can exploited as such or this information may be used for further genetic improvement work of neem.

Neem oil is found to completely devoid of erucic acid and linolenic acid in the samples studied so far, but rich in linoleic acid (3.35-19.64 per cent) and oleic acid (44.84-67.81 per cent). Ratio of unsaturated fatty acids to saturated fatty acids exhibited considerable variation ranging from 1.62 (Kalka) to 2.96 (Bikaner). Thus it offers great scope to further manipulate the content of unsaturated fatty acids by studying samples from diverse agro-ecological regions and /or genetic manipulations involving tissue culture techniques(**Table** 4.6.2.2.).

Another interesting trend observed was the content of essential unsaturated fatty acid, linoleic acid, which was lowest (mean=5.77 per cent) in arid regions (Bikaner), followed by semiarid region (Hisar) with a mean of 8.40 per cent and mesic and sub-temperate regions (Kaithal , Pune and Kalka) with a mean ranging from 14.19 to 18.88 per cent. This could be explained by the lower activity of desaturases in regions with relatively higher atmospheric temperature during the fruit setting season.

Correlation studies amongst different fatty acids showed significant as well as non-significant associations. Palmitic acid was associated with stearic acid positively, in two

Table 4.6.2.1. Fatty acid profile of seeds of *Azadirachta indica* from five provenance collections of India

Provenance	Latitude	Longitude	Number of Trees	Palmitic Acid		Stearic Acid		Oleic Acid		Linoleic acid	
				Range	Mean (S.D.)	Range	Mean (S.D.)	Range	Mean (S.D.)	Range	Mean (S.D.)
Bikaner	28.01N	73.22E	5	13.88-19.41	16.51^{a} ($\pm$1.97)	10.53-15.06	12.59^{a} ($\pm$1.84)	61.29-67.81	65.13^{p} ($\pm$3.17)	3.35-7.42	5.77^{x} ($\pm$1.70)
Hisar	29.10N	75.46E	7	16.87-19.43	18.10^{a} ($\pm$0.93)	12.45-16.47	14.44^{ac} ($\pm$1.38)	56.28-65.10	59.06^{q} ($\pm$3.16)	4.43-11.39	8.40^{x} ($\pm$3.14)
Kaithal	29.48N	7826E	6	16.30-16.84	16.93^{a} ($\pm$0.45)	13.77-17.64	14.99^{c} ($\pm$1.46)	51.47-57.26	53.86^{r} ($\pm$2.24)	10.36-16.46	14.19^{z} ($\pm$2.24)
Kalka	30.42N	76.54E	5	6.87-19.33	18.27^{a} ($\pm$1.12)	15.41-20.94	17.75^{bc} ($\pm$2.22)	44.84-48.06	47.07^{s} ($\pm$1.30)	13.93-18.93	16.91^{z} ($\pm$1.85)
Pune	18.31N	73.55E	4	15.68-19.95	18.15^{a} ($\pm$1.84)	14.28-17.72	15.85^{c} ($\pm$1.45)	45.27-48.24	46.94^{s} ($\pm$1.26)	17.62-19.64	18.88^{z} ($\pm$0.88)
Pooled Mean					17.59		15.12		54.41		12.83

Values with different superscripts are significantly different at P = 0.5 according to *t* test

Table 4.6.2.2. **Ratio of unsaturated to saturated fatty acids in seeds of *Azadirachta indica* from five provenances collections of India**

Provenance	Number of Trees	Range	Mean (S.D.)
Bikaner	5	2.01-2.96	2.47^{a} ($\pm$0.40)
Hisar	7	1.95-2.28	2.14^{a} ($\pm$0.11)
Kaithal	6	1.62-1.88	1.78^{x} ($\pm$0.10)
Kalka	5	1.85-2.01	1.94^{y} ($\pm$0.07)
Pune	4	1.79-2.29	2.08^{a} ($\pm$0.16)
Pooled mean			2.08

Values with different superscripts are significantly different at P = 0.5 according to *t* test

Table 4.6.2.3. Association of seed biochemical and endocarp morphological characteristics of trees of *Azadirachta indica* in six provenance collections

	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Unsaturated fatty acids	saturated fatty acids	Ratio of unsaturated and saturated fatty acids	20 endocarp weight	Endocarp length	Oil percent
Palmitic acid	1.0000	0.1950	-0.4134*	0.2367	-0.6164**	0.6316**	-0.6541**	0.2295	0.1708	0.0753
Stearic acid	-	1.0000	-0.7469**	0.5468**	-0.8915**	0.8836**	-0.8589**	0.0822	0.1096	0.0560
Oleic acid	-	-	1.0000	-0.9457**	-0.7948**	-0.7878**	0.7869**	-0.1430	-0.2723	0.1631
Linoleic acid	-	-	-	1.0000	-0.5542**	0.5452**	-0.5480**	0.0991	0.2702	-0.2616
Unsaturated fatty acids	-	-	-	-	1.0000	-0.9991**	0.9917**	-0.1813	-0.1927	-0.0708
Saturated fatty acids	-	-	-	-	-	1.0000	-0.9913**	0.1746	0.1682	0.0802
Ratio of unsaturated and saturated fatty acids	-	-	-	-	-	-	1.0000	-0.1959	-0.2100	-0.0357
20 endocarp wt.	-	-	-	-	-	-	-	1.0000	0.7003**	0.2523
Endocarp length	-	-	-	-	-	-	-	-	1.0000	-0.0168

**Significant at 1% level *Significant at 5% level of significance

Table 4.6.2.4. Association of seed biochemical and endocarp morphological characteristics of trees of *Azadirachta indica* in five provenances (except ALD1)

	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	unsaturated fatty acids	saturated fatty acids	Ratio of unsaturated and saturated fatty acids	20 endocarp weight	Endocarp length	Oil percent
Palmitic acid	1.0000	0.1950	-0.4133*	0.2367	-0.6162**	0.6316**	-0.6541**	0.2295	0.1708	0.0753
Stearic acid	-	1.0000	-0.7469**	0.5468**	-0.8914**	0.8836**	-0.8589**	0.0822	0.1096	0.0560
Oleic acid	-	-	1.0000	-0.9457**	0.7947**	-0.7878**	0.7869**	-0.1430	-0.2723	0.1631
Linoleic acid	-	-	-	1.0000	-0.5542**	0.5452**	-0.5479**	0.0991	0.2702	-0.2616
Unsaturated fatty acids	-	-	-	-	1.0000	-0.9989**	0.9916**	-0.1812	-0.1927	-0.0708
Saturated fatty acids	-	-	-	-	-	1.0000	-0.9912**	0.1746	0.1682	0.0803
Ratio of unsaturated and saturated fatty acids	-	-	-	-	-	-	1.0000	-0.1959	-0.2100	-0.0357
20 endocarp wt.	-	-	-	-	-	-	-	1.0000	0.7003**	0.2523
Endocarp length	-	-	-	-	-	-	-	-	1.0000	-0.0168

**Significant at 1% level *Significant at 5% level of significance

Table 4.6.2.5. Association of seed biochemical and endocarp morphological characteristics of trees of *Azadirachta indica* in Bikaner provenance

	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Unsaturated fatty acids	Saturated fatty acids	Ratio of unsaturated and saturated fatty acids	20 endocarp weight	Endocarp length	Oil percent
Palmitic acid	1.0000	0.5454	-0.6264	-0.5807	-0.8883*	0.8881*	-0.8879*	0.4482	0.5312	-0.5258
Stearic acid	-	1.0000	-0.9038*	-0.0290	-0.8698	0.8696	-0.8659	0.7794	0.6564	-0.0684
Oleic acid	-	-	1.0000	-0.1598	0.8645	-0.8646	0.8458	-0.9016*	-0.9100*	0.1336
Linoleic acid	-	-	-	1.0000	0.3580	-0.3580	0.3886	0.3171	0.3689	0.4335
Unsaturated fatty acids	-	-	-	-	1.0000	-1.0000	0.9979**	-0.6915	-0.6730	0.3471
Saturated fatty acids	-	-	-	-	-	1.0000	-0.9978**	0.6914	0.6729	-0.3472
Ratio of unsaturated and saturated fatty acids	-	-	-	-	-	-	1.0000	-0.6485	-0.6358	0.3929
20 endocarp wt.	-	-	-	-	-	-	-	1.0000	0.9145*	0.2756
Endocarp length	-	-	-	-	-	-	-	-	1.0000	-0.0243

**Significant at 1% level *Significant at 5% level of significance

Table 4.6.2.6. Association of seed biochemical and endocarp morphological characteristics of trees of *Azadirachta indica* in Kaithal provenance

	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Unsaturated fatty acids	Saturated fatty acids	Ratio of unsaturated and saturated fatty acids	20 endocarp weight	Endocarp length	Oil percent
Palmitic acid	1.0000	-0.6951	0.2018	0.0455	0.4674	-0.4737	0.4254	-0.0661	-0.3328	-0.6812
Stearic acid	-	1.0000	-0.2731	-0.2346	-0.9608**	0.9624**	-0.9459**	0.2016	0.7223	0.8016
Oleic acid	-	-	1.0000	-0.8603*	0.2602	-0.2585	0.2621	0.5672	0.3735	-0.3332
Linoleic acid	-	-	-	1.0000	0.2683	-0.2703	0.2656	-0.6748	-0.7707	-0.0431
Unsaturated fatty acids	-	-	-	-	1.0000	-1.0000**	0.9984**	-0.2062	-0.7543	-0.7116
Saturated fatty acids	-	-	-	-	-	1.0000	-0.9980**	0.2219	0.7592	0.7245
Ratio of unsaturated and saturated fatty acids	-	-	-	-	-	-	1.0000	-0.1848	-0.7468	-0.6786
20 endocarp wt.	-	-	-	-	-	-	-	1.0000	0.6669	0.4782
Endocarp length	-	-	-	-	-	-	-	-	1.0000	0.5603

**Significant at 1% level *Significant at 5% level of significance

provenances (with arid and semi-arid climate) and negatively in three provenances (with mesic climate). However, a reverse trend was observed in case of association of palmitic acid with linoleic acid, wherein, positive correlation was obtained in mesic agro-climate and negative in case of arid and semi-arid agro-climate. The two unsaturated fatty acids were invariably negatively (though not significantly in every case) associated with each other.

Association of seed and endocarp morphological and biochemical characteristics of trees of *Azadirachta indica* A. Juss.in six provenances was studied (**Table** 4.6.2.3.).

Palmitic acid and stearic acids were positively and non-significantly associated with 20 endocarp weight; oil content and endocarp length. Oleic acid was negatively and non-significantly associated with 20 endocarp weight and endocarp length; and positively and non-significantly associated with oil content. Linoleic acid was positively and non-significantly associated with 20 endocarp weight and endocarp length; and negatively and significantly associated with oil content.

Proportion of unsaturated fatty acids was negatively and non-significantly associated with 20 endocarp weight, oil content and endocarp length. Proportion of saturated fatty acids was positively and non-significantly associated with 20 endocarp weight, oil content and endocarp length. Ratio of unsaturated and saturated fatty acids was negatively and non-significantly associated with 20 endocarp weight, oil content and endocarp length.

Twenty endocarp weight was positively and non-significantly associated with oil content; and positively and significantly associated with endocarp length. Endocarp length was negatively and non-significantly associated with oil content. Association of endocarp morphological and biochemical characteristics of trees of *Azadirachta indica* A. Juss. in five provenances (excluding Allahabad) provenance was studied (**Table** 4.6.2.4.).

Palmitic acid and stearic acid was positively and non-significantly associated with 20 endocarp weight, oil content and endocarp length. Oleic acid was positively and non-significantly associated with oil content; negatively and non-significantly associated with; and positively and non-significantly associated with endocarp length and 20 endocarp weight.

Linoleic acid was negatively and non-significantly associated with oil content; positively and non-significantly associated with endocarp length and 20 endocarp weight. Proportion of unsaturated fatty acids was negatively and non-significantly associated with 20 endocarp weight, oil content and endocarp length. Proportion of saturated fatty acids was positively and non-significantly associated with 20 endocarp weight, oil content and endocarp length. Ratio of unsaturated and saturated fatty acids was negatively and non-significantly associated with 20 endocarp weight, oil content and endocarp length. Twenty endocarp weight was positively and non-significantly associated with oil content; and positively and significantly associated with

endocarp length. Endocarp length was negatively and non-significantly associated with oil content.

Association of seed and endocarp morphological and biochemical characteristics of trees of *Azadirachta indica* A. Juss. in Bikaner provenance was studied (**Table** 4.6.2.5.). Palmitic acid and stearic acid were negatively and non-significantly associated oil content; positively and non-significantly associated with 20 endocarp weight and endocarp length. Oleic acid was positively and non-significantly associated with oil content; negatively and significantly associated with 20 endocarp weight and endocarp length. Linoleic acid was positively significantly associated with 20 endocarp weight, oil content and endocarp length. Proportion of unsaturated fatty acids was positively and non-significantly associated with oil content; negatively and non-significantly associated with 20 endocarp weight and endocarp length. Proportion of saturated fatty acids was negatively and non-significantly associated with oil content; positively and non-significantly associated with 20 endocarp weight and endocarp length. Ratio of unsaturated and saturated fatty acids was positively and non-significantly associated with oil content; negatively and non-significantly associated with 20 endocarp weight and endocarp length. Twenty endocarp weight was positively and non-significantly associated with oil content; and positively and significantly associated with endocarp length. Endocarp length was negatively and non-significantly associated with oil content.

Association of endocarp morphological and biochemical characteristics of trees of *Azadirachta indica* A. Juss. in Kaithal provenance was studied (**Table** 4.6.2.6.). Palmitic acid, linoleic acid, Proportion of unsaturated fatty acids and Ratio of unsaturated and saturated fatty acids were negatively and non-significantly associated with 20 endocarp weight, oil content and non significantly associated with endocarp length. Stearic acid and Proportion of saturated fatty acids were positively and non-significantly associated with 20 endocarp weight, oil content and endocarp length. Oleic acid was positively and non-significantly associated with 20 endocarp weight and endocarp length; and negatively and non-significantly associated with oil content. Twenty endocarp weight positively and non-significantly associated with endocarp length and oil content. Endocarp length was positively and significantly associated with oil content.

Association of endocarp morphological and biochemical characteristics of trees of *Azadirachta indica* A. Juss. in Kalka provenance was studied (**Table** 4.6.2.7.). Palmitic acid was positively and non-significantly associated with 20 endocarp weight and endocarp length; negatively and non-significantly associated with oil content. Stearic acid was negatively and significantly associated with 20 endocarp weight; positively and non-significantly associated with oil content; and negatively and non-significantly associated with endocarp length. Oleic acid and Linoleic acid were positively and non-significantly associated with 20 endocarp weight, oil content and endocarp length. Proportion of unsaturated fatty acids was positively

Table 4.6.2.7. Association of seed biochemical and endocarp morphological characteristics of trees of *Azadirachta indica* in Kalka provenance

	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Unsaturated fatty acids	Saturated fatty acids	Ratio of unsaturated and saturated fatty acids	20 endocarp weight	Endocarp length	Oil percent
Palmitic acid	1.0000	-0.8756	-0.2345	0.6109	0.6114	-0.6116	0.6277	0.7021	0.8163	-0.5473
Stearic acid	-	1.0000	0.1034	-0.7430	-0.9176*	0.9177*	-0.9251*	-0.9445*	-0.6943	0.0941
Oleic acid	-	-	1.0000	-0.6828	0.0235	-0.0237	0.0536	0.1627	0.1173	0.2319
Linoleic acid	-	-	-	1.0000	0.7143	-0.7146	0.6925	0.5945	0.2580	0.0542
Unsaturated fatty acids	-	-	-	-	1.0000	-1.0000**	0.9991**	0.9694**	0.4654	0.2962
Saturated fatty acids	-	-	-	-	-	1.0000	-0.9992**	-0.9695**	-0.4657	-0.2965
Ratio of unsaturated and saturated fatty acids	-	-	-	-	-	-	1.0000	0.9795**	0.4867	0.2765
20 endocarp wt.	-	-	-	-	-	-	-	1.0000	0.5799	0.1619
Endocarp length	-	-	-	-	-	-	-	-	1.0000	-0.6188

**Significant at 1% level *Significant at 5% level of significance

Table 4.6.2.8. Association of seed biochemical and endocarp morphological characteristics of trees of *Azadirachta indica* in Hisar provenance

	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Unsaturated fatty acids	Saturated fatty acids	Ratio of unsaturated and saturated fatty acids	20 endocarp weight	Endocarp length	Oil percent
Palmitic acid	1.0000	0.1950	0.0912	-0.4371	-0.6240	0.6233	-0.6019	-0.3367	-0.4818	-0.0039
Stearic acid	-	1.0000	-0.4196	-0.0482	-0.8493*	0.8491*	-0.8591*	0.1877	-0.0247	0.4211
Oleic acid	-	-	1.0000	-0.8476*	0.2850	-0.2820	0.3403	-0.6156	-0.7310	0.4987
Linoleic acid	-	-	-	1.0000	0.2669	-0.2704	-0.2094	0.6378	0.8868**	-0.6807
Unsaturated fatty acids	-	-	-	-	1.0000	-1.0000**	0.9962**	0.0340	0.2743	-0.3238
Saturated fatty acids	-	-	-	-	-	1.0000	-0.9965**	-0.0312	-0.2757	0.3295
Ratio of unsaturated and saturated fatty acids	-	-	-	-	-	-	1.0000	-0.0519	0.1976	-0.2866
20 endocarp wt.	-	-	-	-	-	-	-	1.0000	0.8727*	-0.3175
Endocarp length	-	-	-	-	-	-	-	-	1.0000	-0.6547

**Significant at 1% level *Significant at 5% level of significance

Table 4.6.2.9. Association of seed biochemical and endocarp morphological characteristics of trees of *Azadirachta indica* in Pune provenance

	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Unsaturated fatty acids	Saturated fatty acids	Ratio of unsaturated and saturated fatty acids	20 endocarp weight	Endocarp length	Oil percent
Palmitic acid	1.0000	-0.8944	-0.7179	0.7903	-0.2801	0.6381	-0.5265	-0.9762*	-0.8208	0.7797
Stearic acid	-	1.0000	0.3609	-0.6585	-0.1726	-0.2265	0.0941	0.9547*	0.6587	-0.7270
Oleic acid	-	-	1.0000	-0.8173	0.7274	-0.9426	0.8720	0.5505	0.8596	-0.7062
Linoleic acid	-	-	-	1.0000	-0.1990	0.5875	-0.4377	-0.6808	-0.9961**	0.9845*
Unsaturated fatty acids	-	-	-	-	1.0000	-0.9067	0.9642*	0.1240	0.2745	-0.0276
Saturated fatty acids	-	-	-	-	-	1.0000	-0.9848*	-0.4830	-0.6536	0.4465
Ratio of unsaturated and saturated fatty acids	-	-	-	-	-	-	1.0000	0.3739	0.5109	-0.2829
20 endocarp wt.	-	-	-	-	-	-	-	1.0000	0.7061	-0.6999
Endocarp length	-	-	-	-	-	-	-	-	1.0000	-0.9688*

**Significant at 1% level; *Significant at 5% level of significance

Table 4.6.2.10. Association of Latitude with biochemical profile of seeds of some provenance collections of *Azadirachta indica* from north-western India

	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Saturated fatty acids	Unsaturated fatty acids	Ratio of unsaturated and saturated fatty acids
Latitude	0.7368	0.9849*	-0.9899*	0.9472	0.9640*	-0.9653**	-0.9392
Longitude	0.2794	0.6243	-0.7524	0.8172	0.5539	-0.5582	-0.6086

22 trees were used from four provenance collections namely Bikaner, Hisar, Kaithal and Kalka;
Pooled mean values for each provenance were used for correlation analysis; **Significant at 1% level;
*Significant at 5% level of significance

and significantly associated with 20 endocarp weight; positively and non-significantly associated with oil content and endocarp length. Proportion of saturated fatty acids was negatively and significantly associated with 20 endocarp weight; negatively and non-significantly associated with oil content and endocarp length. Ratio of unsaturated and saturated fatty acids was positively and significantly associated with 20 endocarp weight; positively and non-significantly associated with oil content and endocarp length. Twenty endocarp weight was positively and non-significantly associated with endocarp length and oil content;. Endocarp length was negatively and non-significantly associated with oil content.

Association of endocarp morphological and biochemical characteristics of trees of *Azadirachta indica* A. Juss. in Hisar provenance was studied (**Table** 4.6.2.8.). Palmitic acid was negatively and non-significantly associated with 20 endocarp weight, oil content and endocarp length. Stearic acid was positively and significantly associated with 20 endocarp weight and oil content; negatively and non-significantly associated with endocarp length. Oleic acid was negatively and non-significantly associated with 20 endocarp weight and endocarp length; and positively and non-significantly associated with oil content. Linoleic acid was positively and significantly associated with endocarp length; positively and non-significantly associated with 20 endocarp weight; and negatively and non-significantly associated with oil content. Proportion of unsaturated fatty acids was negatively and non-significantly associated with oil content; and positively and non-significantly associated with endocarp length and 20 endocarp weight. Proportion of saturated fatty acids was negatively and non-significantly associated with 20 endocarp weight and endocarp length; and positively and non-significantly associated with oil content. Ratio of unsaturated and saturated fatty acids was positively and non-significantly associated with endocarp length; negatively and non-significantly associated with oil content and 20 endocarp weight. Twenty endocarp weight was negatively and non-significantly associated with oil content; and positively and significantly associated with endocarp length. Endocarp length was negatively and significantly associated with oil content.

Association of endocarp morphological and biochemical characteristics of trees of *Azadirachta indica* A. Juss. in Pune provenance was studied (**Table** 4.6.2.9.). Palmitic acid was negatively and significantly associated with 20 endocarp weight; positively and non-significantly associated with oil content; and negatively and non significantly associated with endocarp length. Stearic acid was positively and significantly associated with 20 endocarp weight; negatively and non-significantly associated with oil content; and positively and non significantly associated with endocarp length. Oleic acid was positively and non-significantly associated with 20 endocarp weight and endocarp length; and negatively and non-significantly associated with oil content. Linoleic acid was negatively and non-significantly associated with 20 endocarp weight; positively and significantly associated with oil content; and negatively and significantly associated with endocarp length. Proportion of unsaturated fatty acids was

positively and non-significantly associated with 20 endocarp weight; negatively and non-significantly associated with oil content; and positively and non-significantly associated with endocarp length. Proportion of saturated fatty acids was negatively and non-significantly associated with 20 endocarp weight; positively and non-significantly associated with oil content; and negatively and non-significantly associated with endocarp length. Ratio of unsaturated and saturated fatty acids was positively and non-significantly associated with 20 endocarp weight; negatively and non-significantly associated with oil content; and positively and non-significantly associated with endocarp length. Twenty endocarp weight was negatively and non-significantly associated with oil content; and positively and non-significantly associated with endocarp length. Endocarp length was negatively and significantly associated with oil content.

Association of latitude and longitude with data related to fatty acids was studied in 22 trees from four provenance collections namely, Bikaner, Hisar, Kaithal and Kalka. Pooled mean values for each provenance were used for correlation analysis(**Table** 4.6.2.10.). Palmitic acid, stearic acid, linoleic acid and saturated fatty acids were found to positively associated with both latitude and longitude. Oleic acid, unsaturated fatty acids and ratio of unsaturated and saturated fatty acids were negatively associated with both latitude and longitude.

4.6.3. Seed protein content

Average protein value for the seed samples of 10 provenances is 12.91 per cent with a range of 10.06 to 16.63 per cent. Highest protein content (16.63 per cent) was observed in the seeds of a tree from Kaithal provenance (H084) followed by a tree from Delhi provenance (T7D) which showed a value of 15.53 per cent. Lowest protein content (10.06 per cent) was observed in the seeds of a tree from Hisar provenance (HSR04) followed by two trees from Delhi provenance (T5D) and Jodhpur provenance (24) which showed a value of 11.38 per cent. Correlation coefficient of protein with oil content showed a significant value (P = 0.5) of 0.686 (**Table** 4.6.3.1.).

Protein content of H084 (Kaithal) was significantly different from all other samples except that of T7D (Delhi). On the on the other extreme, samples from Alwar was significantly different from only two samples, namely, H084 (Kaithal) and HSR04 (Hisar). Similarly sample from Kalka (H107) contained protein content which significantly differed from only two samples, namely, Kaithal (H084) and Delhi (T7D).

4.6.4. Azadirachtin content of seeds

Average azadirachtin value ~~for the seed samples~~ of 7 seed samples from 5 provenance collections from Haryana is 0.3987 per cent with a range of 0.281 per cent to 0.583 per cent and a coefficient of variation of 31.85. Highest azadirachtin content (0.583 per cent) was observed in the seeds of a tree from Pinjore provenance (H105G) followed by a tree from

Table 4.6.3.1. Protein content of whole endocarps of different provenances of *Azadirachta indica*

Accession No.	Provenance	Protein content (%)	C.V.
H101	Kalka	12.03 (±0.31)	2.58
H107	Kalka	12.91 (±0.93)	7.20
H084	Kaithal	16.63 (±0.62)	3.73
H083	Kaithal	12.91 (±0.32)	2.48
T7	Delhi	15.53 (±0.31)	2.00
T5	Delhi	11.38 (±0.62)	5.45
HSR04	Hisar	10.06 (±0.00)	-
HSR03	Hisar	13.13 (±0.00)	-
31	Alwar	13.13 (±0.62)	4.72
24	Jodhpur	11.38 (±0.00)	-
cd (5%)		1.299	

Table 4.6.4.1. Azadirachtin content of seed samples of *Azadirachta indica* from provenance collections of Haryana

Sample Number	Place of collection	Date of seed collection	Azadirachtin content (%)
H105G	Pinjore	12 Aug., 1999	0.583
H106G	Pinjore	13 Aug., 1999	0.572
HSR03	Hisar	08 Sep., 1999	0.322
HSR01	Hisar	08 Sep., 1999	0.313
H048	Rohtak	25 July, 1999	0.281
H080	Kurukshetra	01 Aug, 1999	0.397
H072	Narnaund	31 Sep., 1999	0.323
Pooled mean			0.3987 (±0.127)

Fig. 4.6.5.3. Relationship between longitude and seed protein content in different provenance collections of *Azadirachta indica* from Haryana

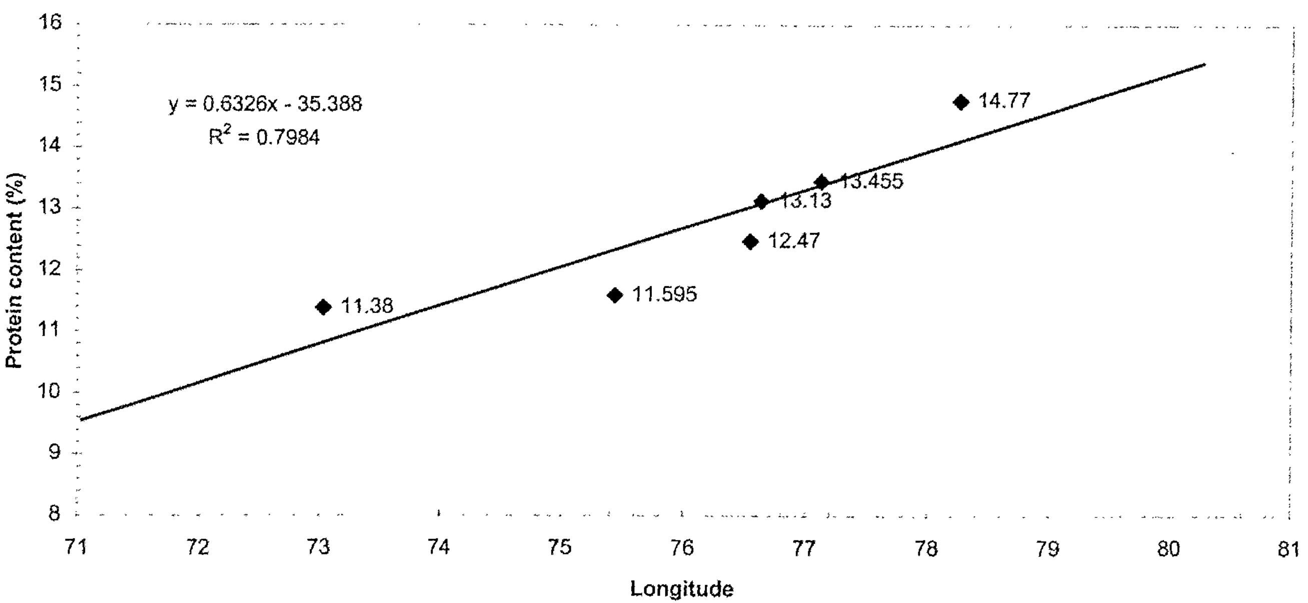


Fig. 4.6.5.4. Relationship between seed azadirachtin content and latitude in some provenance collections of *A. indica* from Haryana

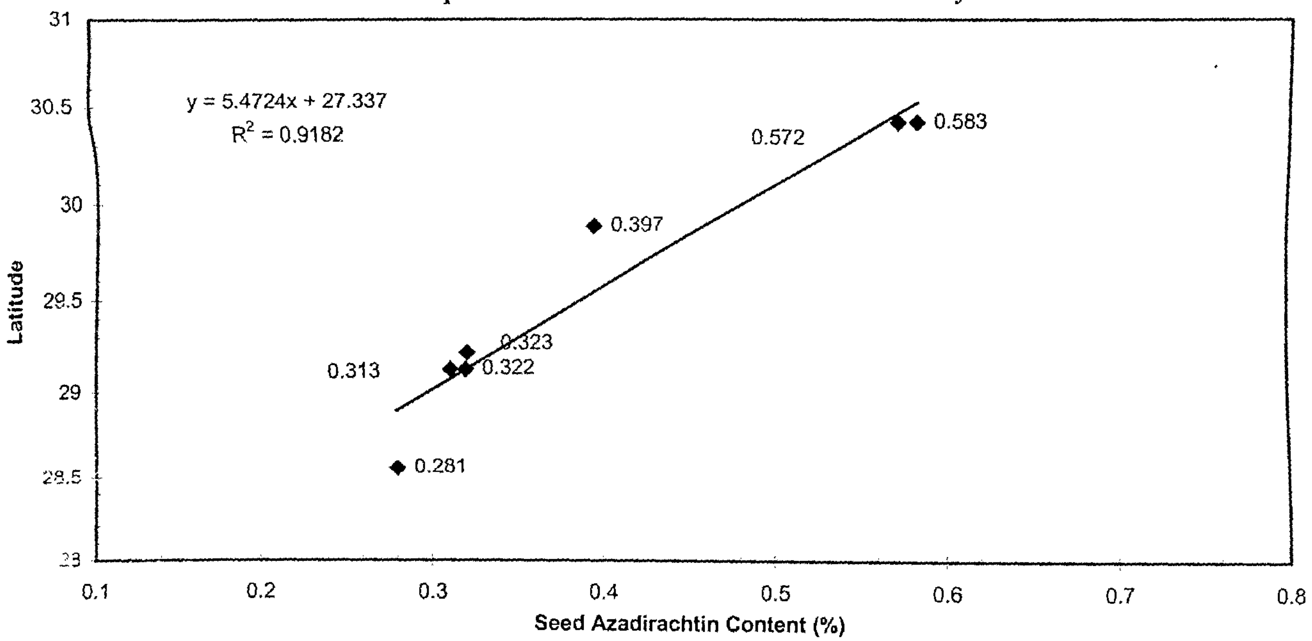
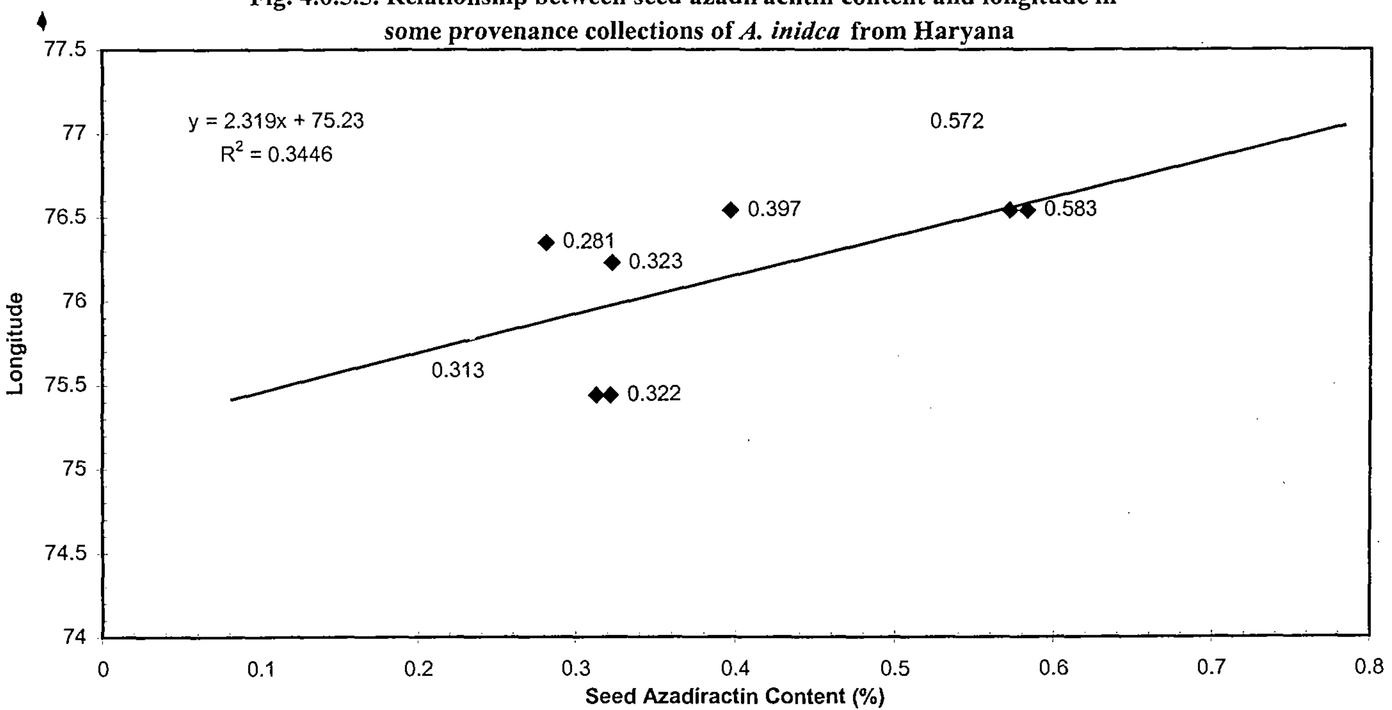


Fig. 4.6.5.5. Relationship between seed azadirachtin content and longitude in some provenance collections of *A. inidca* from Haryana



same provenance (H106G) which showed a value of 0.572 per cent. Lowest azadirachtin content (0.281 per cent) was observed in the seeds of a tree from Rohtak provenance (0.281) followed by a tree from Hisar provenance (HSR01) which showed a value of 0.313 per cent (**Table** 4.6.4.1.).

4.6.5. Regression analysis with respect to seed biochemical characteristics

Multiple regression analysis of linoleic acid on all other seed characteristics (namely, palmitic acid, stearic acid, oleic acid, unsaturated fatty acids, saturated fatty acids, ratio of unsaturated and saturated fatty acids, 20 endocarp weight, and endocarp length) in *Azadirachta indica* was done (**Table** 4.6.5.1.). Coefficient of determination (R^2) was very high (0.99), pointing to very high level of goodness of fitness of the regression line.

Multiple regression analysis of ~~character 10~~ Oil per cent on all other endocarp and seed characteristics (namely, palmitic acid, stearic acid, oleic acid, Linoleic acid, unsaturated fatty acids, Saturated fatty acids, ratio of unsaturated and saturated fatty acids, 20 endocarp wt., endocarp length,) in *Azadirachta indica* was done (**Table** 4.6.5.2.). Coefficient of determination (R^2) was very low (0.33), pointing to very low level of goodness of fitness of the regression line.

Protein content of seeds was found to be positively associated with longitude (0.8935) which was significant at 0.1 per cent level of significance, while association of latitude with the azadirachtin content (0.371) was found to be non-significant. Regression equation (Fig. 4.6.5.3.) for linear regression of seed protein content on longitude gives an equation for linear regression $y = 0.6326x - 35.388$, with a very high value of coefficient of determination ($R^2 = 0.7984$) pointing to very high level of goodness of fitness of the regression line.

Azadirachtin content of seeds was found to be positively associated with latitude (0.0958) which was significant at 0.1 per cent level of significance, while association of longitude with the azadirachtin content (0.587) was found to be non-significant. Regression equation (Fig. 4.6.5.4.) for linear regression of seed azadirachtin content on latitude gives an equation for linear regression $y = 5.4724x - 27.337$, with a very high value of coefficient of determination ($R^2 = 0.9182$) pointing to very high level of goodness of fitness of the regression line. Regression equation (Fig. 4.6.5.5.) for linear regression of seed azadirachtin content on longitude gives an equation for linear regression $y = 2.319x + 75.23$, with a low level of coefficient of determination ($R^2 = 0.3446$) pointing to very low level of goodness of fitness of the regression line.

4.7. Divergence studies

The uni-variate analysis although exhibited significant variation for all the characters yet such variation cannot be used for measuring the extent of genetic diversity. In order to

Table 4.6.5.1. **Multiple regression analysis of Linoleic acid on all other endocarp characteristics (Palmitic acid, Stearic acid, Oleic acid, Unsaturated fatty acids, Saturated fatty acids, Ratio, 20 endocarp wt., Endocarp length, Oil percent) in *Azadirachta indica***

Provenance	Constant	Regression Co-efficients									R^2
		b_1	b_2	b_3	b_4	b_5	b_6	b_7	b_8	b_9	
Global*	0.026503	-2.739942	-2.743294	-1.000000	1.000000	2.740667	0.000000	0.000000	-0.000000	0.000000	0.999437

*all the provenances taken together

Table 4.6.5.2. **Multiple regression analysis of oil percent on all other endocarp characteristics (Palmitic acid, Stearic acid, oleic acid, Linoleic acid, Unsaturated fatty acids, Saturated fatty acids, Ratio, 20 endocarp wt., Endocarp length) in *Azadirachta indica***

Provenance	Constant	Regression Co-efficients									R^2
		b_1	b_2	b_3	b_4	b_5	b_6	b_7	b_9	b_{10}	
Global*	-420.125163	3.905475	3.975021	1.494494	1.242920	1.213937	2.119086	30.152132	1.403867	0.103561	0.331662

*all the provenances taken together

overcome this problem and to quantify genetic divergence between any two groups of genotypes, D^2 analysis and Hierarchical cluster analysis were done.

4.7.1. D^2 analysis

D^2 values for all possible (903) combinations among 43 genotypes were calculated and these genotypes were grouped into ten clusters (C) by Tocher's method (Rao, 1952). The membership profile of different clusters is presented in **Table** 4.7.1.1. Cluster CI consisted of maximum number of genotypes i.e., 12. Clusters namely, CII, CIII, and CIV consisted of 10, 7, and 5 genotypes respectively. CV, CVI, and CVII had two genotypes each, whereas CVIII and CIX and CX were represented by one genotype each. The genotypes of CIX and CX were extraordinary for one or the other character that made them so divergent from others.

Provenance wise break of clusters is given in **Table** 4.7.1.2. Clusters CI consisted of maximum number of provenance collections and CII and CIII and CIV consisted of 10, 7 and 5 provenance collections respectively. Clusters CV, CVI and CVII represented 2 provenance collections each; and CVIII, CIX and CX had only one provenance collections each. Genotypes of Hisar and Kaithal were clustered into maximum numbers of clusters i.e. four, out of which two were same. Genotypes of Jodhpur, Kalka and Raipur were clustered into equal number of maximum numbers of clusters i.e. three. Only Kalka and Raipur shared Cluster IV. Genotypes belonging to Alwar and Delhi shared same clusters i.e., CII and CIII. Similarly genotypes belonging to Bikaner and Bhatinda provenance collections were clustered in single cluster i.e., CI.

Intra and inter-cluster distances were calculated and are presented in **Table** 4.7.1.3. The intra-cluster distances were relatively smaller than inter-cluster distances indicating homogeneous nature of the groups and presence of narrow genetic variation within a cluster. The minimum intra-cluster distance was observed in cluster CVI, whereas maximum intra-cluster distance was observed in CVII followed by CV.

The minimum inter-cluster distance was observed between cluster CIII and CVI followed by between CI and CIII, whereas maximum inter-cluster distance was observed between CIX and CX followed by between CVII and CX and between CVIII and CX . In general CX was found to be situated maximum apart from all the other clusters followed by CIX and CVI. In general CIII was found to be situated minimum apart from all the other clusters followed by CI.

Cluster means (**Table** 4.7.1.4.) indicated differences for all the characters among all the clusters. The differences were more pronounced for endocarp length, endocarp weight, seed weight and endocarp length : endocarp breadth. Highest mean endocarp length was observed in single genotype in CX while it was found to be least in single genotype in CIX. Maximum mean endocarp breadth was found in CVI and minimum in single genotype in CVIII. Highest

Table 4.7.1.1. Clustering of 43 genotypes of *Azadirachta indica* based on D^2 analysis of eight endocarp morphological characteristics

Cluster	Number of genotypes	Genotypes
CI	12	T3 , T4, T5, T52, T41, T43, T62, T63, T64, TGN3, NMN4, TER2
CII	10	T24, T20, T22, T7D, T31, HSR01, HSR03, HSR05, H082, T33
CIII	7	T17, T5D , H083, T30, HSR02, SHS-0, H084
CIV	5	T18, T42, T44, H101, H107
CV	2	T45, H085
CVI	2	H099, H100
CVII	2	T21, H102
CVIII	1	HSR04
CIX	1	T23
CX	1	NGN1

Table 4.7.1.2. Provenance wise break up of clusters of 43 genotypes of *Azadirachta indica* based on D^2 analysis of eight endocarp morphological characteristics

Cluster	Number of genotypes	Number of provenances	Genotypes									
			Hisar	Bikaner	Jodhpur	Alwar	Pune	Kalka	Kaithal	Delhi	Bhatinda	Raipur
CI	12	5		T3 T4 T5			TGN3 NMN4 TER2		T52		T62 T63 T64	T41 T43
CII	10	5	HSR01 HSR05 HSR03		T24 T20 T22	T31 T33			H082	T7D		
CIII	7	4	T17 HSR02 SHS-0			T30			H083 H084	T5D		
CIV	5	3	T18					H101 H107				T42 T44
CV	2	2							H085			T45
CVI	2	2						H099 H100				
CVII	2	2			T21			H102				
CVIII	1	1	HSR04									
CIX	1	1			T23							
CX	1	1					NGN1					

Fig. 4.7.1.1. Cluster memebership of differnet provenance collections of *A. indica* based on D^2 analysis

Fig. 4.7.1.1. Cluster memebership of differnet provenance collections of *A. indica* based on D^2 analysis

Table 4.7.1.3. Intra and inter-cluster average D^2 values among 43 genotypes of *Azadirachta indica* based on eight endocarp morphological characteristics

Cluster	CI	CII	CIII	CIV	CV	CVI	CVII	CVIII	CIX	CX
CI	6.40	25.67	12.11	17.01	23.70	46.66	29.75	30.35	56.43	56.19
CII	-	7.10	14.78	52.03	77.56	65.22	31.97	29.09	20.89	92.67
CIII	-	-	7.89	26.87	20.19	5.72	35.24	16.20	45.16	53.39
CIV	-	-	-	11.45	28.60	71.94	61.53	43.73	98.39	53.99
CV	-	-	-	-	13.84	94.00	73.41	13.29	97.44	17.91
CVI	-	-	-	-	-	4.31	21.19	86.12	81.01	135.30
CVII	-	-	-	-	-	-	17.72	66.081	37.41	125.33
CVII	-	-	-	-	-	-	-	0.0	81.680	26.118
CIX	-	-	-	-	-		-	-	0.00	173.70
CX	-	-	-	-	-	-	-	-	-	0.00

Table 4.7.1.4. Mean values of clusters for 8 endocarp morphological characteristics in *Azadirachta indica*

Cluster	Number of genotypes	Endocarp length	Endocarp breadth	Endocarp weight	Seed weight	Endocarp coat weight	Number of seeds endocarp^{-1}	Seed weight : Endocarp weight	Endocarp length : Endocarp breadth
		Character 1	Character 2	Character 3	Character 4	Character 5	Character 6	Character 7	Character 8
1	12	1.2625 (0.0656)	0.6491 (0.0399)	0.2004 (0.0240)	0.1079 (0.0168)	0.0925 (0.0113)	1.0667 (0.1303)	0.5323 (0.0435)	1.9564 (0.1539)
2	10	1.0046 (0.0638)	0.5320 (0.0410)	0.1116 (0.0194)	0.0475 (0.0134)	0.0642 (0.0138)	1.0200 (0.0632)	0.4126 (0.0858)	1.8992 (0.1450)
3	7	1.2191 (0.0496)	0.5907 (0.0354)	0.1659 (0.0183)	0.0769 (0.0113)	0.0890 (0.0095)	1.0857 (0.1574)	0.4549 (0.0322)	2.1541 (0.1187)
4	5	1.4488 (0.1007)	0.7012 (0.0216)	0.2654 (0.0368)	0.1335 (0.0305)	0.1319 (0.0145)	1.0400 (0.0894)	0.4929 (0.0725)	2.0679 (0.1052)
5	2	1.4260 (0.1499)	0.5750 (0.0537)	0.1946 (0.0503)	0.1018 (0.0382)	0.0928 (0.0122)	1.2000 (0.2828)	0.5094 (0.0602)	2.4886 (0.0410)
6	2	1.1430 (0.0156)	0.8015 (0.0148)	0.2264 (0.0065)	0.1103 (0.0052)	0.1161 (0.0117)	1.1000 (0.1414)	0.4861 (0.0349)	1.4330 (0.0520)
7	2	1.0110 (0.0156)	0.6795 (0.0615)	0.1413 (0.0168)	0.0681 (0.0194)	0.0732 (0.0025)	1.0000 (0.0000)	0.4504 (0.1171)	1.5007 (0.1653)
8	1	1.3000	0.5160	0.1386	0.0510	0.0876	1.0000	0.3538	2.5192
9	1	0.7780	0.5500	0.0910	0.0484	0.0426	1.2000	0.5324	2.1982
10	1	1.6280	0.5570	0.1972	0.0952	0.1020	1.0000	0.4822	2.9334

Underlined values means that these are the original mean values of the individual genotypes; Figures in parentheses are standard deviations

mean endocarp weight was observed in CIV while it was found to be least in single genotype in CIX. Maximum mean seed weight was found in CIV and minimum in single genotype in CIX.

Highest mean seed weight : endocarp weight was observed in CI and CIX while it was found to be least in single genotype in CVII. Maximum endocarp length : endocarp breadth was found in single genotype in CX and minimum in single genotype in CVI. Maximum mean number of seeds $endocarp^{-1}$ were shown by CV, least were shown by CVII and two other clusters (CVIII and CX). It is worth mentioning that genotypes of CI, CIV, CV and CVI were having most of the favourable characters viz. high seed weight and high seed weight : endocarp weight.

4.7.2. Hierarchical cluster analysis

The D^2 analysis agglomerated more than 50 per cent of the genotypes in two clusters, as the clustering process is totally arbitrary. Moreover, it utilizes variance-covariance matrix for analysis where standardisation of scales of different variable is required. Therefore, it becomes imperative to utilize some other method of clustering which overcomes the drawback of D^2 analysis.

The average linkage group method, often called UPGMA (Unweighted pair-group method using arithmetic averages), was used for further analysis. Squared euclidean measure was used to find out the relative distances between and within the different clusters. Optimum number of clusters were determined using sum of squares index, D_{jk} (Romesburg, 1988). In the agglomerative hierarchical clustering process, average linkage was kept the criterion for clustering. It defines the distance between two clusters as the average of the distances between all pairs of cases in which one member of the pair is from each of the clusters.

Hierarchical cluster analysis of endocarp morphological data:

Endocarp morphological data of 43 genotypes of neem (all 8 endocarp morphological characteristics used) was subjected to hierarchical cluster analysis with the help of squared euclidean distance and UPGMA approach. The cluster analysis identified 8 clusters (Fig. 4.7.2.1.) containing between one and 11 genotypes. Cluster CVI and CVIII were having maximum genotypes i.e., 11 genotypes. Sub-Clusters C I and CIV had least number of genotypes (one each). Clusters CII, CIII, CV, CVII comprised 9, 3, 3, and four genotypes respectively.

The association among the different genotypes is presented in the form of dendrogram prepared using rescaled distances. The genotypes which are lying nearer to each other in the dendrogram are more similar to one another than those lying apart. The resemblance coefficient between the two genotypes is the value at which their branches join. The dendrogram also showed the relative magnitude of resemblance among the different clusters. Two major clusters were visible namely, A and B.

Fig. 4.7.2.1. **Dendrogram produced from endocarp morphological data of 43 genotypes of *Azadirachta indica* with the help of hierarchical cluster analysis using squared euclidean distance and UPGMA approach (all 8 endocarp and seed morphological characteristics used)**

```
Cluster  C A S E                 0         5         10        15        20        25
         Label          Num      +---------+---------+---------+---------+---------+

         HSR05           33       -+-+
         H082            36       -+ +---+
         T7D             25       -+ I   I
         HSR01           29       -+-+   +-+
         T17              1       -+     I I
CVIII    T24             16       ---+---+ +-+
         HSR03           31       ---+     I +---+
         T22             18       ---------+ I   I
         T31             28       -------+---+   +-------+
         T33 (1998)      39       -------+       I       I
         T21             19       ---------------+       I
         H083            26       -+-+                   I
         H085            38       -+ +-+                 I
CVII     HSR02           30       ---+ +---------+       +-------+
         HSR04           32       -----+         I       I       I
         T43              9       -+             I       I       I
         NMN4            41       -+-+           I       I       I
         T5               4       -+ +---+       I       I       I
         TGN3            40       -+-+   I       +-------+       I Sub-major Cluster BII
         TER2            42       -+ I   I       I               I
CVI      T52              6       -+ I   +---+   I               +-------+
         H084            37       -+-+   I   I   I               I       I
         SHS-0           34       -+     I   I   I               I       I
         T62             12       -+-+   I   +---+               I       I Major Cluster B
         T64             14       -+ +---+   I                   I       +-------+
         T4               3       ---+       I                   I       I       I
         T5D             24       -----------+                   I       I       I
CV       T20             17       -----------+-------------------+       I       I
         H102            21       -----------+                           I       +-+
CIV      T23             15       ---------------------------------------+       I I
         T3               2       -----------------+-------+                     I I
CIII     T45             11       -----------------+       +-----------------+   I I
         T30             27       -------------------------+                 I   I I
         H099            23       -------+-----------------+                 +---+ I
         H100            35       -------+                 I                 I     I
         T41              7       -+-----+                 I                 I     I
         T63             13       -+     +---------+       +-----------------+     I
CII      T18              5       -------+         +-+     I  Sub-major Cluster BI I
         T42              8       -----+-----+     I I     I                       I
         H107            22       -----+     +-----+ +-----+                       I
         H101            20       -----------+       I                             I
         T44             10       -------------------+                             I
CI       NGN1            43       -------------------------------------------------+
                                                              Major Cluster A

             C A S E             0         5         10        15        20        25
         Label          Num      +---------+---------+---------+---------+---------+
```

Dendrogram with z-score standardisation

Major cluster A was containing only one genotype namely, NGN1 (Pune).

Major cluster B contained 42 genotypes and was further divided into two sub-major clusters. Sub-major cluster BI contained 13 genotypes namely, T3, T45, T30, H099, H100, T41, T63, T18, T42, H107, H101, T44. Sub-major cluster BII was containing 29 genotypes namely, HSR05, H082, T7, HSR01, T17, T24, HSR03, T22, T31, T33, T21, H083, H085, HSR02, HSR04, T43, NMN4, T5, TGN3, TER2, T52, H084, SHS-0, T62, T64, T4, T5D, T20, and H102, and T23.

Cluster CI comprised nine genotypes out of which 4 belonged to Kalka (Haryana) provenance, 3 were from Raipur (Madhya Pradesh), one belonged to Hisar and one to Bhatinda. CIII comprised three genotypes out of which one each belonged to Raipur, Alwar and Bikaner.

Endocarp morphological data of 43 genotypes of neem (7 endocarp morphological characteristics used i.e. without number of seeds endocarp^{-1}) was subjected to hierarchical cluster analysis with the help of squared euclidean distance and UPGMA approach. The cluster analysis identified two major clusters A (12 genotypes) and B (31 genotypes); and 11 sub-clusters (Fig. 4.7.2.2.) containing between two and eight genotypes.

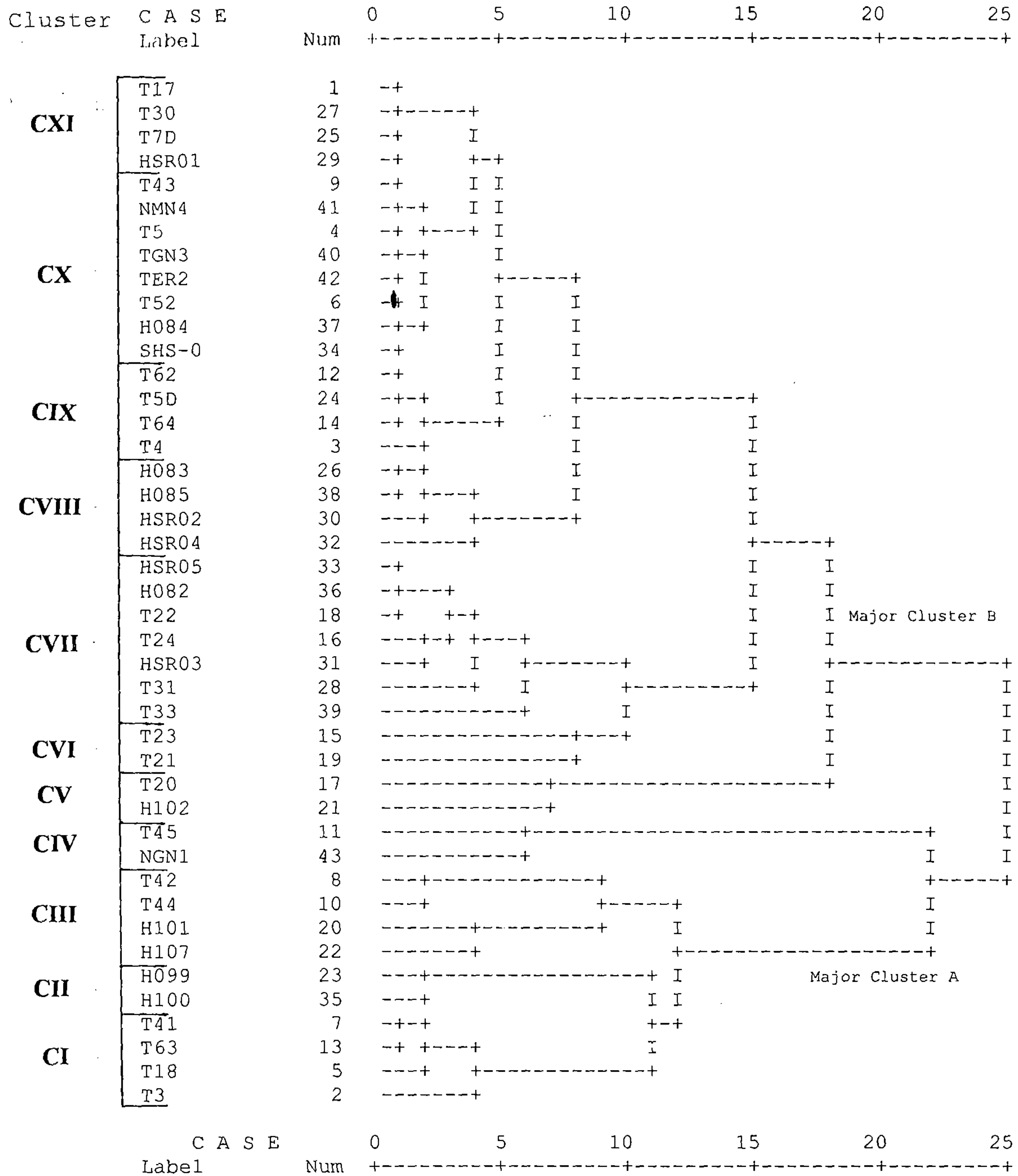
Major cluster A contained 12 genotypes namely, T3, T45, NGN1, H099, H100, T41, T63, T18, T42, H107, H101, T44. Major cluster B was containing 31 genotypes namely, T30, HSR05, H082, T7, HSR01, T17, T24, HSR03, T22, T31, T33, T21, H083, H085, HSR02, HSR04, T43, NMN4, T5, TGN3, TER2, T52, H084, SHS-0, T62, T64, T4, T5D, T20, and H102, and T23.

Cluster CX was having maximum genotypes i.e., 8 followed by CVII (containing 7 genotypes). Cluster CII, CIV, CV and CVI had least number of genotypes (two each). Clusters CI, CIII, CVIII, CIX and CXI comprised four genotypes each.

Hierarchical cluster analysis of seed biochemical and endocarp morphological data:

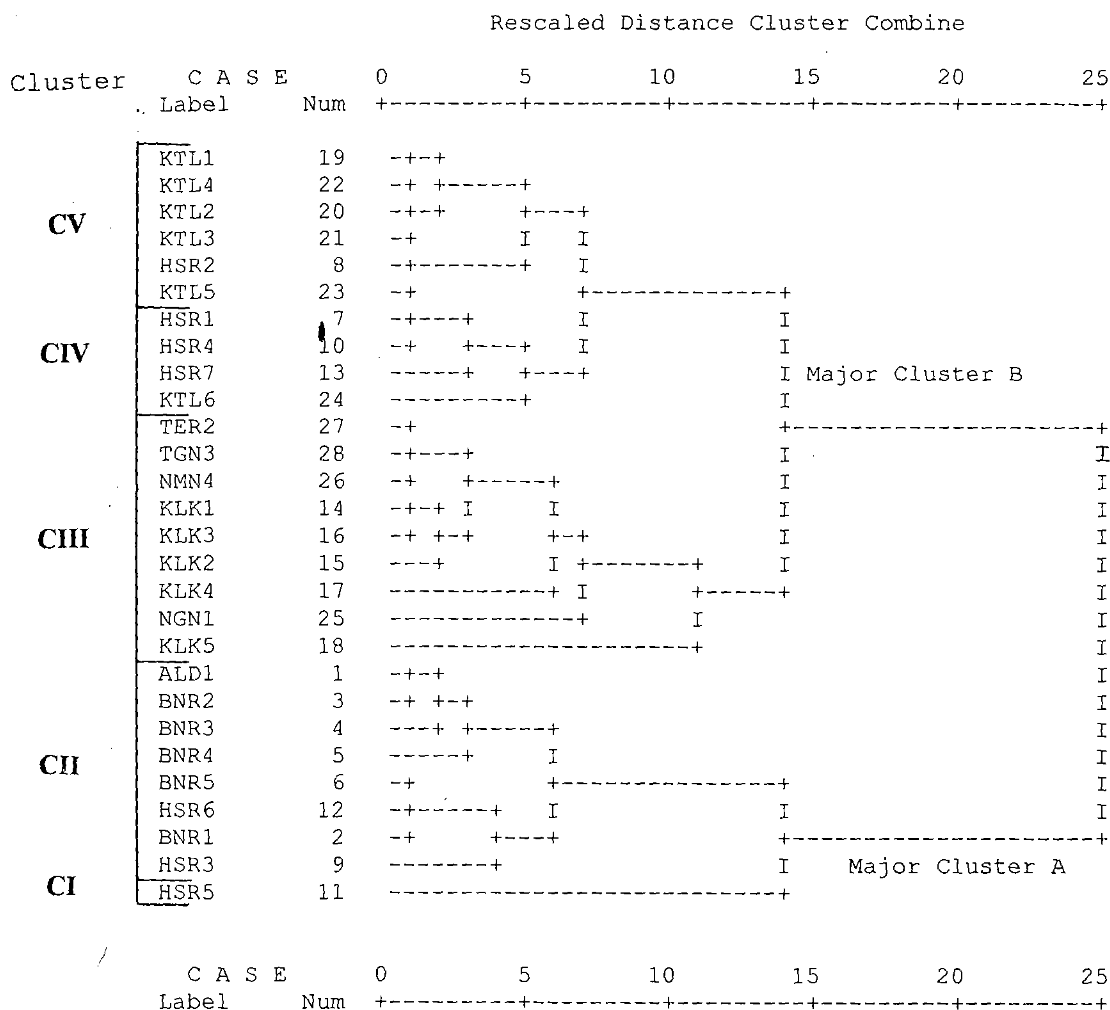
Seed biochemical and endocarp morphological data of 28 genotypes of neem (all 10 endocarp biochemical plus morphological characteristics used) was subjected to hierarchical cluster analysis using squared euclidean distance and UPGMA approach. Two major clusters namely, A and B were discernible from the dendrogram. Cluster A contains nine genotypes containing mainly genotypes from arid (Bikaner) and semi-arid regions (Hisar). One exceptional entry was the genotype from Allahabad which is not having arid type of climate. Cluster B contains 19 genotypes mainly genotypes from semi-arid (Hisar) and other non-semi-arid regions of Haryana namely, Kaithal (Northern plain and central highlands, hot semi-arid eco-region with alluvium-derived soils.), and Kalka (western Himalayas warm sub-humid (including humid) eco-region with brown forest and podzolic soils)) and Pune (Deccan plateau, hot semi-arid eco-region with shallow and medium (including deep) black soils.

Fig. 4.7.2.2. Dendrogram produced from endocarp morphological data of 43 genotypes of *Azadirachta indica* A. Juss. with the help of hierarchical cluster analysis using squared euclidean distance and UPGMA approach (7 endocarp and seed morphological characteristics i.e., without no. of seeds per endocarp)



Dendrogram with z-score standardisation

Table 4.7.2.3. Dendrogram produced from seed biochemical and endocarp morphological data of 28 genotypes of *Azadirachta indica* with the help of hierarchical cluster analysis using squared euclidean distance and UPGMA approach (all ten characteristics were used)



Dendrogram with z-score standardisation

The cluster analysis identified 5 clusters (Fig. 4.7.2.3.) containing between one and nine genotypes. Cluster CIII was having maximum genotypes i.e., 9 followed by CII (containing 8 genotypes). Cluster C I had least number of genotypes (one). Clusters CIV, and CV comprised 4 and 6 genotypes respectively. Major cluster A was further sub-divided into two clusters (CI and CII) and major cluster B was further sub-divided into three clusters (CIII, CIV and CV).

Cluster CI was containing only one genotype namely, HSR5 (Hisar). CII comprised 8 genotypes out of which 5 belonged to Bikaner (Rajasthan) provenance, one was from Hisar, and one belonged to Allahabad. CIII comprised nine genotypes out of which four belonged to Pune and five to Kalka. CIV comprised four genotypes out of which 3 belonged to Hisar and rest to Kaithal. CV comprised 6 genotypes out of which five belonged to Kaithal and one to Hisar.

4.7.3. RAPD analysis

Table 4.7.3.1. depicts the detail of random primers which amplified majority of provenance collections.Number of provenances studied ranged from 2-15 for each primer. This table also enlists about 22 random primers (used singly and / or in combinations of 2-4) which did not give satisfactory amplification and could not be used for further RAPD assays.

Pooled samples of 5 different provenance collections were used for amplification with primer OPC-12. The assay gave 10 amplification products and 40.00 per cent of these markers were polymorphic (**Table** 4.7.3.2.). Fifteen genotypes each were used for amplification with primers OPC-1 and OPC-12. While the former gave six amplification products the latter only gave three products and all these markers were polymorphic (**Table** 4.7.3.3., **Table** 4.7.3.4.).

Twelve genotypes were used for amplification with primer OPC-11. The assay gave four amplification products and all these markers were polymorphic. (**Table** 4.7.3.5.)

Six genotypes each were used for amplification with primers OPD-2 and OPD-3. While the former gave four amplification products the latter only gave seven products. Fifty percent of the markers obtained by OPD-2 were polymorphic and 71.43 per cent of those obtained by OPD-3 were polymorphic. (**Table** 4.7.3.6. **Table** 4.7.3.7.)

Two genotypes were used for amplification with primer OPC-12 + OPD-2. The assay gave seven amplification products and 57.14 per cent of these markers were polymorphic.

Genotype Discriminating Efficiency for various RAPD assays is being depicted in **Table** 4.7.3.8. Discriminating efficiency range from 26.67 per cent to 100.00 per cent. Highest Discriminating Efficiency (100.00 per cent) was achieved with the RAPD assay number 11 wherein primer OPC-12 was used in 5 pooled DNA samples from different provenance collections. Out of 10 pairs of genotypes which were to be discriminated the assay was able to discriminate all of the pairs of pooled genotypes. Lowest Discriminating Efficiency (26.67 per cent) was achieved with the RAPD assay number 7 wherein primer OPD-2 was used in 6 DNA

Table 4.7.3.1. Primers which amplified majority of Provenance collections of *Azadirachta indica*

Primer code	Sequence of primer	Number of genotypes	Bands scored			Genotypes / pooled genotypes used for RAPD analysis (DNA sample number)
			Polymorphic (%age)	Mono-morphic (%age)	Total	
OPC-1	5'-TTCGAGCCAG-3'	15	6 (100)	0 (0)	6	HSR01a (7), HSR01b (8), Adu. (9), H048 (10), H108a (11), H028 (12), HSR01t (13), HSR03 (14), HSR04 (15), H079 (15), H080 (17), H093 (18), H108b (23), GPN-0 (24), HSR02 (25)
OPC-12	5'-TGTCATCCCC-3'	15	3 (100)	0 (0)	3	HSR01a (7), HSR01b (8), Adu. (9), H048 (10), H108a (11), H028 (12), HSR01t (13), HSR03 (14), HSR04 (15), H079 (16), H080 (17), H093 (18), H108b (23), GPN-0 (24), HSR02 (25)
OPD-2	5'-GGACCCAACC-3'	6	2 (50)	2 (50)	4	HSR01a (7), HSR01b (8), Adu. (9), H048 (10), H108a (11), H028 (12)
OPD-3	5'-GTCGCCGTCA-3'	6	5 (71.43)	2 (28.57)	7	HSR01a (7), HSR01b (8), Adu. (9), H048 (10), H108a (11), H028 (12)
OPC-11	5'-AAAGCTGCGG-3'	12	4 (100.0)	0 (0)	4	HSR01a (7), Adu. (9), H048 (10), H108a (11), H028 (12), HSR03 (14), HSR04 (15), H079 (16), H080 (17), H093 (18), H108b (23), GPN-0 (24)
OPC-12 + OPD-2	-	2	4 (57.14)	3 (42.86)	7	HSR01a (7), Adu. (9)
OPC-12	-	5*	4 (40)	6 (60)	10	Kaithal + Kurukshetra (5R), Rohtak (4R), Delhi (3R), Dharwad (2R), Kalka (1R)

*Pooled samples of different provenance collections

samples from different provenance collections. Out of 15 pairs of genotypes which were to be discriminated,the assay was able to discriminate only 4 pairs of pooled genotypes.

Table 4.7.3.9. contains the Similarity (F) and Dissimilarity (1-F) values among pooled Neem genotypes based on OPC-12 primed RAPD polymorphism. Highest similarity value (0.9412) was seen between pooled genotypes 4R (Rohtak) & 3R (Delhi) followed by between 5R (Kaithal + Kurukshetra) & 3R (Delhi) and 5 R & 2R (0.8235). Highest dissimilarity (0.5625) value was observed between 3R (Delhi) & 1R (Kalka), followed by between 3R (Delhi) & 2R (Dharwad) and 2R (Dharwad) & 1R (Kalka) (05556).

Similarity (F) and Dissimilarity (1-F) values among 15 neem genotypes based on OPC-1 primed RAPD polymorphism were calculated. Similarity values range from 0-1. (**Table** 4.7.3.10.). Similarity (F) and Dissimilarity (1-F) values among 15 neem genotypes based on OPC-12 primed RAPD polymorphism was calculated. Similarity value ranged from 0 to 1 (**Table** 4.7.3.11.). Similarity (F) and Dissimilarity (1-F) values among 12 neem genotypes based on OPC-11 primed RAPD polymorphism was calculated. Similarity value ranged from zero to one (**Table** 4.7.3.12.).

Similarity (F) and Dissimilarity (1-F) values among 6 neem genotypes based on OPD-2 primed RAPD polymorphism was calculated. Highest similarity value (1.00) was seen between genotypes 8 (HSR01b Hisar) & 9 (Adu. Aduthurai), 8 (HSR01b Hisar) &10 (H048 Rohtak), 9 (Adu. Aduthurai) & 10 (H048 Rohtak), and 11 (H108a Kalka) & 12 (H028 Narnaul). Highest dissimilarity (0.4286) value was observed between 7 (HSR01a Hisar) & 11 (H108a Kalka) and 7 (HSR01a Hisar) & 12 (H028 Narnaul). (**Table** 4.7.3.13.).

Table 4.7.3.14. contains the Similarity (F) and Dissimilarity (1-F) values among 6 neem genotypes based on OPD-3 primed RAPD polymorphism. Highest similarity value (1.00) was seen between genotypes 9 (Adu. Aduthurai) & 10 (H048 Rohtak), 9 (Adu. Aduthurai) &11 (H108a Kalka), 9 (Adu. Aduthurai) & 12 (H028 Narnaul), 10 (H048 Rohtak) & 11 (H108a Kalka), 10 (H048 Rohtak) & 12 (H028 Narnaul) and 11 (H108a Kalka) & 12 (H028 Narnaul). Highest dissimilarity (0.3333) value was observed between 8 (HSR01b Hisar) & 9 (Adu. Aduthurai), 8 (HSR01b Hisar) & 10 (H048 Rohtak), 8 (HSR01b Hisar) & 11 (H108a Kalka) and 8 (HSR01b Hisar) & 12 (H028 Narnaul).

A total of 15 genotypes were evaluated with the help of primer OPC-1. Total number of alleles detected were 6. ~~All the alleles exhibited null alleles and nine genotypes showed multiple alleles.~~ Allele with highest frequency had a frequency of 66.67 per cent. Number of rare allele was one. A total of 15 genotypes were evaluated with the help of primer OPC-12. Total number of alleles detected were 3. ~~Out of 15 genotypes, 12 exhibited null alleles and three genotypes showed multiple alleles.~~ Allele with highest frequency had a frequency of 80.00 per cent. A total of 12 genotypes were evaluated with the help of primer OPC-11. Total number of alleles detected were 4. ~~Out of 12 genotypes 11 exhibited null alleles and 10~~

Table 4.7.3.2. OPC-12 primed RAPD banding pattern in pooled DNA samples from 5 provenances of *Azadirachta indica*

Band number	Genotypes				
	5R	4R	3R	2R	1R
1	-	-	-	+	-
2	-	+	-	+	+
3	+	+	+	+	+
4	+	+	+	+	+
5	+	+	+	+	+
6	+	+	+	+	+
7	+	+	+	+	+
8	-	+	+	+	+
9	+	+	+	+	-
10	+	+	+	+	+

Table 4.7.3.3. OPC-1 primed RAPD banding pattern in 15 genotypes of *Azadirachta indica*

Band number	Genotypes														
	7	8	9	10	11	12	13	14	15	16	17	18	23	24	25
1	-	-	+	-	+	+	+	+	+	-	-	-	+	+	-
2	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
4	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
6	-	-	+	-	+	+	+	+	+	+	+	-	+	+	-

Table 4.7.3.4. OPC-12 primed RAPD banding pattern in 15 genotypes of *Azadirachta indica*

Band number	Genotypes														
	7	8	9	10	11	12	13	14	15	16	17	18	23	24	25
1	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
2	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
3	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+

Table 4.7.3.5. OPC-11 primed RAPD banding pattern in 12 genotypes of *Azadirachta indica*

Band number	Genotype											
	7	9	10	11	12	14	15	16	17	18	23	24
1	-	+	-	-	-	-	-	-	-	-	-	-
2	-	+	-	-	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+	-	-
4	+	+	+	+	+	+	+	+	+	+	+	-

Table 4.7.3.8. Genotype Discriminating efficiency for various RAPD assays in *Azadirachta indica*

RAPD assay number	Number of genotypes / Pooled samples	Primer(s) used	All possible pairs of genotypes / pooled genotypes which are to be discriminated	Number of pairs of genotypes / pooled genotypes actually discriminated	Number of pairs of genotypes / pooled genotypes not discriminated	Discriminating efficiency (DE) (%)
1	12	OPC-11	66	30	36	45.45
2	6[a]	OPC-1	15	9	6	60.00
3	6[a]	OPC-12	15	8	7	53.33
4	9[b]	OPC-1	36	32	4	88.89
5	9[b]	OPC-12	36	23	13	63.89
7	6[a]	OPD-2	15	4	11	26.67
8	6[a]	OPD-3	15	6	9	40.00
9	15[c]	OPC-1	105	80	25	76.19
10	15[c]	OPC-12	105	63	42	60.00
11	5[*]	OPC-12	10	10	0	100.00

[*]Pooled samples of different provenances; Values in columns 2 with same superscript are same set of genotypes

Table 4.7.3.6. OPD-2 primed RAPD banding pattern in 6 genotypes of *Azadirachta indica*

Band number	Genotype					
	7	8	9	10	12	13
1	-	+	+	+	+	+
2	+	+	+	+	+	+
3	+	+	+	+	+	+
4	+	+	+	+	-	-

Table 4.7.3.7. OPD-3 primed RAPD banding pattern in 6 genotypes of *Azadirachta indica*

	Genotypes					
Band Number	7	8	9	10	11	12
1	+	+	-	-	-	-
2	+	-	+	+	+	+
3	+	+	+	+	+	+
4	+	+	-	-	-	-
5	+	+	+	+	+	+
6	+	-	+	+	+	+
7	+	+	-	-	-	-

Table 4.7.3.9. Similarity (F) and Dissimilarity (1-F) value among different pooled genotypes of *Azadirachta indica* based on OPC-12 primed RAPD polymorphism

Genotypes	Scored bands			F	1-F
	Bands of 1st genotype	Bands of 2nd genotype	Common bands		
5R & 4R	7	9	7	0.875	0.125
5R & 3R	7	8	7	0.8235	0.1765
5R & 2R	7	10	7	0.8235	0.1765
5R & 1R	7	8	6	0.800	0.200
4R & 3 R	9	8	8	0.9412	0.0588
4R & 2 R	9	10	9	0.4737	0.5263
4R & 1R	9	8	8	0.4706	0.5294
3R & 2R	8	10	8	0.4444	0.5556
3R & 1R	8	8	7	0.4375	0.5625
2R &1R	10	8	8	0.4444	0.5556

Table 4.7.3.10. Similarity (F) and Dissimilarity (1-F) value among 15 genotypes of *Azadirachta indica* based on OPC-1 primed RAPD polymorphism

Genotypes	Scored bands			F	1-F
	Bands of 1st genotype	Bands of 2nd genotype	Common bands		
7 & 8	0	0	0	0.0000	1.0000
7 & 9	0	2	0	0.0000	1.0000
7 & 10	0	0	0	0.0000	1.0000
7 & 11	0	2	0	0.0000	1.0000
7 & 12	0	2	0	0.0000	1.0000
7 & 13	0	2	0	0.0000	1.0000
7 & 14	0	2	0	0.0000	1.0000
7 & 15	0	2	0	0.0000	1.0000
7 & 16	0	2	0	0.0000	1.0000
7 & 17	0	1	0	0.0000	1.0000
7 & 18	0	0	0	0.0000	1.0000
7 & 23	0	4	0	0.0000	1.0000
7 & 24	0	5	0	0.0000	1.0000
7 & 25	0	0	0	0.0000	1.0000
8 & 9	0	2	0	0.0000	1.0000
8 & 10	0	0	0	0.0000	1.0000
8 & 11	0	2	0	0.0000	1.0000
8 & 12	0	2	0	0.0000	1.0000
8 & 13	0	2	0	0.0000	1.0000
8 & 14	0	2	0	0.0000	1.0000
8 & 15	0	2	0	0.0000	1.0000
8 & 16	0	2	0	0.0000	1.0000
8 & 17	0	1	0	0.0000	1.0000
8 & 18	0	0	0	0.0000	1.0000
8 & 23	0	4	0	0.0000	1.0000
8 & 24	0	5	0	0.0000	1.0000
8 & 25	0	0	0	0.0000	1.0000
9 & 10	2	0	0	0.0000	1.0000
9 & 11	2	2	2	1.0000	0.0000
9 & 12	2	2	2	1.0000	0.0000
9 & 13	2	2	2	1.0000	0.0000
9 & 14	2	2	2	1.0000	0.0000
9 & 15	2	2	2	1.0000	0.0000
9 & 16	2	2	1	0.5000	0.5000
9 & 17	2	1	1	0.6667	0.3333
9 & 18	2	0	0	0.0000	1.0000
9 & 23	2	4	2	0.6667	0.3333
9 & 24	2	5	2	0.5714	0.4286
9 & 25	2	0	0	0.0000	1.0000
10 & 11	0	2	0	0.0000	1.0000
10 & 12	0	2	0	0.0000	1.0000
10 & 13	0	2	0	0.0000	1.0000
10 & 14	0	2	0	0.0000	1.0000
10 & 15	0	2	0	0.0000	1.0000
10 & 16	0	2	0	0.0000	1.0000
10 & 17	0	1	0	0.0000	1.0000
10 & 18	0	0	0	0.0000	1.0000
10 & 23	0	4	0	0.0000	1.0000
10 & 24	0	5	0	0.0000	1.0000
10 & 25	0	0	0	0.0000	1.0000
11 & 12	2	2	2	1.0000	0.0000
11 & 13	2	2	2	1.0000	0.0000
11 & 14	2	2	2	1.0000	0.0000
11 & 15	2	2	2	1.0000	0.0000
11 & 16	2	2	1	0.5000	0.5000

Table 4.7.3.10. (continued)

Genotypes	Scored bands			F	1-F
	Bands of 1st genotype	Bands of 2nd genotype	Common bands		
11 & 17	2	1	1	0.6667	0.3333
11 & 18	2	0	0	0.0000	1.0000
11 & 23	2	4	2	0.6667	0.3333
11 & 24	2	5	2	0.5714	0.4286
11 & 25	2	0	0	0.0000	1.0000
12 & 13	2	2	2	1.0000	0.0000
12 & 14	2	2	2	1.0000	0.0000
12 & 15	2	2	2	1.0000	0.0000
12 & 16	2	2	1	0.5000	0.5000
12 & 17	2	1	1	0.6667	0.3333
12 & 18	2	0	0	0.0000	1.0000
12 & 23	2	4	2	0.6667	0.3333
12 & 24	2	5	2	0.5714	0.4286
12 & 25	2	0	0	0.0000	1.0000
13 & 14	2	2	2	1.0000	0.0000
13 & 15	2	2	2	1.0000	0.0000
13 & 16	2	2	1	0.5000	0.5000
13 & 17	2	1	1	0.6667	0.3333
13 & 18	2	0	0	0.0000	1.0000
13 & 23	2	4	2	0.6667	0.3333
13 & 24	2	5	2	0.5714	0.4286
13 & 25	2	0	0	0.0000	1.0000
14 & 15	2	2	2	1.0000	0.0000
14 & 16	2	2	1	0.5000	0.5000
14 & 17	2	1	1	0.6667	0.3333
14 & 18	2	0	0	0.0000	1.0000
14 & 23	2	4	2	0.6667	0.3333
14 & 24	2	5	2	0.0000	1.0000
14 & 25	2	0	0	0.0000	1.0000
15 & 16	2	2	1	0.5000	0.5000
15 & 17	2	1	1	0.6667	0.3333
15 & 18	2	0	0	0.0000	1.0000
15 & 23	2	4	2	0.6667	0.3333
15 & 24	2	5	2	0.5714	0.4286
15 & 25	2	0	0	0.0000	1.0000
16 & 17	2	1	1	0.6667	0.3333
16 & 18	2	0	0	0.0000	1.0000
16 & 23	2	4	1	0.3333	0.6667
16 & 25	2	0	0	0.0000	1.0000
16 & 24	2	5	1	0.2857	0.7143
17& 18	1	0	0	0.0000	1.0000
17 & 23	1	4	1	0.4000	0.6000
17 & 24	1	5	1	0.3333	0.6667
17 & 25	1	0	0	0.0000	1.0000
18 & 23	0	4	0	0.0000	1.0000
18 & 24	0	5	0	0.0000	1.0000
18 & 25	0	0	0	0.0000	1.0000
23 & 24	4	5	4	0.8889	0.1111
23 & 25	4	0	0	0.0000	1.0000
24 & 25	5	0	0	0.0000	1.0000

Table 4.7.3.11. Similarity (F) and Dissimilarity (1-F) value among 15 genotypes of *Azadirachta indica* based on OPC-12 primed RAPD polymorphism

Genotypes	Scored bands			F	1-F
	Bands of 1st genotype	Bands of 2nd genotype	Common bands		
7 & 8	1	0	0	0.0000	1.0000
7 & 9	1	0	0	0.0000	1.0000
7 & 10	1	1	1	1.0000	0.0000
7 & 11	1	1	1	1.0000	0.0000
7 & 12	1	1	1	1.0000	0.0000
7 & 13	1	1	1	1.0000	0.0000
7 & 14	1	3	1	0.5000	0.5000
7 & 15	1	3	1	0.5000	0.5000
7 & 16	1	3	1	0.5000	0.5000
7 & 17	1	1	1	1.0000	0.0000
7 & 18	1	1	1	1.0000	0.0000
7 & 23	1	1	1	1.0000	0.0000
7 & 24	1	0	0	0.0000	1.0000
7 & 25	1	1	1	1.0000	0.0000
8 & 9	0	0	0	0.0000	1.0000
8 & 10	0	1	0	0.0000	1.0000
8 & 11	0	1	0	0.0000	1.0000
8 & 12	0	1	0	0.0000	1.0000
8 & 13	0	1	0	0.0000	1.0000
8 & 14	0	3	0	0.0000	1.0000
8 & 15	0	3	0	0.0000	1.0000
8 & 16	0	3	0	0.0000	1.0000
8 & 17	0	1	0	0.0000	1.0000
8 & 18	0	1	0	0.0000	1.0000
8 & 23	0	1	0	0.0000	1.0000
8 & 24	0	0	0	0.0000	1.0000
8 & 25	0	1	0	0.0000	1.0000
9 & 10	0	1	0	0.0000	1.0000
9 & 11	0	1	0	0.0000	1.0000
9 & 12	0	1	0	0.0000	1.0000
9 & 13	0	1	0	0.0000	1.0000
9 & 14	0	3	0	0.0000	1.0000
9 & 15	0	3	0	0.0000	1.0000
9 & 16	0	3	0	0.0000	1.0000
9 & 17	0	1	0	0.0000	1.0000
9 & 18	0	1	0	0.0000	1.0000
9 & 23	0	1	0	0.0000	1.0000
9 & 24	0	0	0	0.0000	1.0000
9 & 25	0	1	0	0.0000	1.0000
10 & 11	1	1	1	1.0000	0.0000
10 & 12	1	1	1	1.0000	0.0000
10 & 13	1	1	1	1.0000	0.0000
10 & 14	1	3	1	0.5000	0.5000
10 & 15	1	3	1	0.5000	0.5000
10 & 16	1	3	1	0.5000	0.5000
10 & 17	1	1	1	1.0000	0.0000
10 & 18	1	1	1	1.0000	0.0000
10 & 23	1	1	1	1.0000	0.0000
10 & 24	1	0	0	0.0000	1.000
10 & 25	1	1	1	1.0000	0.0000
11 & 12	1	1	1	1.0000	0.0000
11 & 13	1	1	1	1.0000	0.0000
11 & 14	1	3	1	0.5000	0.5000
11 & 15	1	3	1	0.5000	0.5000

Table 4.7.3.11. (continued)

Genotypes	Scored bands			F	1-F
	Bands of 1st genotype	Bands of 2nd genotype	Common bands		
11 & 16	1	3	1	0.5000	0.5000
11 & 17	1	1	1	1.0000	0.0000
11 & 18	1	1	1	1.0000	0.0000
11 & 23	1	1	1	1.0000	0.0000
11 & 24	1	0	0	0.0000	1.0000
11 & 25	1	1	1	1.0000	0.0000
12 & 13	1	1	1	1.0000	0.0000
12 & 14	1	3	1	0.5000	0.5000
12 & 15	1	3	1	0.5000	0.5000
12 & 16	1	3	1	0.5000	0.5000
12 & 17	1	1	1	1.0000	0.0000
12 & 18	1	1	1	1.0000	0.0000
12 & 23	1	1	1	1.0000	0.0000
12 & 24	1	0	0	0.0000	1.0000
12 & 25	1	1	1	1.0000	0.0000
13 & 14	1	3	1	0.5000	0.5000
13 & 15	1	3	1	0.5000	0.5000
13 & 16	1	3	1	0.5000	0.5000
13 & 17	1	1	1	1.0000	0.0000
13 & 18	1	1	1	1.0000	0.0000
13 & 23	1	1	1	1.0000	0.0000
13 & 24	1	0	0	0.0000	1.0000
13 & 25	1	1	1	1.0000	0.0000
14 & 15	3	3	3	1.0000	0.0000
14 & 16	3	3	3	1.0000	0.0000
14 & 17	3	1	1	0.5000	0.5000
14 & 18	3	1	1	0.5000	0.5000
14 & 23	3	1	1	0.5000	0.5000
14 & 24	3	0	0	0.0000	1.0000
14 & 25	3	1	1	0.5000	0.5000
15 & 16	3	3	3	1.0000	0.0000
15 & 17	3	1	1	0.5000	0.5000
15 & 18	3	1	1	0.5000	0.5000
15 & 23	3	1	1	0.5000	0.5000
15 & 24	3	0	0	0.0000	1.0000
15 & 25	3	1	1	0.5000	0.5000
16 & 17	3	1	1	0.5000	0.5000
16 & 18	3	1	1	0.5000	0.5000
16 & 23	3	1	1	0.5000	0.5000
16 & 24	3	0	0	0.0000	1.0000
16 & 25	3	1	1	0.5000	0.5000
17 & 18	1	1	1	1.0000	0.0000
17 & 23	1	1	1	1.0000	0.0000
17 & 24	1	0	0	0.0000	1.0000
17 & 25	1	1	1	1.0000	0.0000
18 & 23	1	1	1	1.0000	0.0000
18 & 24	1	0	0	0.0000	1.0000
18 & 25	1	1	1	1.0000	0.0000
23 & 24	1	0	0	0.0000	1.0000
23 & 25	1	1	1	1.0000	0.0000
24 & 25	0	1	0	0.0000	1.0000

Table 4.7.3.12. Similarity (F) and Dissimilarity (1-F) value among 12 genotypes of *Azadirachta indica* based on OPC-11 primed RAPD polymorphism

Genotypes	Scored bands			F	1-F
	Bands of 1st genotype	Bands of 2nd genotype	Common bands		
7 & 9	2	4	2	0.6667	0.3333
7 & 10	2	2	2	1.0000	0.0000
7 & 11	2	2	2	1.0000	0.0000
7 & 12	2	2	2	1.0000	0.0000
7 & 14	2	2	2	1.0000	0.0000
7 & 15	2	2	2	1.0000	0.0000
7 & 16	2	2	2	1.0000	0.0000
7 & 17	2	2	2	1.0000	0.0000
7 & 18	2	2	2	1.0000	0.0000
7 & 23	2	1	1	0.6667	0.3333
7 & 24	2	0	0	0.0000	1.0000
9 & 10	4	2	2	0.6667	0.3333
9 & 11	4	2	2	0.6667	0.3333
9 & 12	4	2	2	0.6667	0.3333
9 & 14	4	2	2	0.6667	0.3333
9 & 15	4	2	2	0.6667	0.3333
9 & 16	4	2	2	0.6667	0.3333
9 & 17	4	2	2	0.6667	0.3333
9 & 18	4	2	2	0.6667	0.3333
9 & 23	4	1	1	0.5000	0.5000
9 & 24	4	0	0	0.0000	1.0000
10 & 11	2	2	2	1.0000	0.0000
10 & 12	2	2	2	1.0000	0.0000
10 & 14	2	2	2	1.0000	0.0000
10 & 15	2	2	2	1.0000	0.0000
10 & 16	2	2	2	1.0000	0.0000
10 & 17	2	2	2	1.0000	0.0000
10 & 18	2	2	2	1.0000	0.0000
10 & 23	2	1	1	0.6667	0.3333
10 & 24	2	0	0	0.0000	1.0000
11 & 12	2	2	2	1.0000	0.0000
11 & 14	2	2	2	1.0000	0.0000
11 & 15	2	2	2	1.0000	0.0000
11 & 16	2	2	2	1.0000	0.0000
11 & 17	2	2	2	1.0000	0.0000
11 & 18	2	2	2	1.0000	0.0000
11 & 23	2	1	1	0.6667	0.3333
11 & 24	2	0	0	0.000	1.0000
12 & 14	2	2	2	1.0000	0.0000
12 & 15	2	2	2	1.0000	0.0000
12 & 16	2	2	2	1.0000	0.0000
12 & 17	2	2	2	1.0000	0.0000
12 & 18	2	2	2	1.0000	0.0000
12 & 23	2	1	1	0.6667	0.3333
12 & 24	2	0	0	0.0000	1.0000
14 & 15	2	2	2	1.0000	0.0000
14 & 16	2	2	2	1.0000	0.0000
14 & 17	2	2	2	1.0000	0.0000
14 & 18	2	2	2	1.0000	0.0000
14 & 23	2	1	1	0.6667	0.3333
14 & 24	2	0	0	0.0000	1.0000
15 & 16	2	2	2	1.0000	0.0000
15 & 17	2	2	2	1.0000	0.0000
15 & 18	2	2	2	1.0000	0.0000
15 & 23	2	1	1	0.6667	0.3333
15 & 24	2	0	0	0.0000	1.0000

Table 4.7.3.12. (continued)

Genotypes	Scored bands			F	1-F
	Bands of 1st genotype	Bands of 2nd genotype	Common bands		
16 & 17	2	2	2	1.0000	0.0000
16 & 18	2	2	2	1.0000	0.0000
16 & 23	2	1	1	0.6667	0.3333
16 & 24	2	0	0	0.0000	1.0000
17& 18	2	2	2	1.0000	0.0000
17 & 23	2	1	1	0.6667	0.3333
17 & 24	2	0	0	0.0000	1.0000
18 & 23	1	1	1	1.0000	0.0000
18 & 24	1	0	0	0.0000	1.0000
23 & 24	1	0	0	0.0000	1.0000

Table 4.7.3.13. Similarity (F) and Dissimilarity (1-F) value among 6 Neem genotypes based on OPD-2 primed RAPD polymorphism

Genotypes	Scored bands			F	1-F
	Bands of 1st genotype	Bands of 2nd genotype	Common bands		
7 & 8	3	4	3	0.8571	0.1429
7 & 9	3	4	3	0.8571	0.1429
7 & 10	3	4	3	0.8571	0.1429
7 & 11	3	3	2	0.5714	0.4286
7 & 12	3	3	2	0.5714	0.4286
8 & 9	4	4	4	1.0000	0.0000
8 & 10	4	4	4	1.0000	0.0000
8 & 11	4	3	3	0.8571	0.1429
8 & 12	4	3	3	0.8571	0.1429
9 & 10	4	4	4	1.0000	0.0000
9 & 11	4	3	3	0.8571	0.1429
9 & 12	4	3	3	0.8571	0.1429
10 & 11	4	3	3	0.8571	0.1429
10 & 12	4	3	3	0.8571	0.1429
11 & 12	3	3	3	1.0000	0.0000

Table 4.7.3.14. Similarity (F) and Dissimilarity (1-F) value among 6 genotypes of *Azadirachta indica* based on OPD-3 primed RAPD polymorphism

Genotypes	Scored bands			F	1-F
	Bands of 1st genotype	Bands of 2nd genotype	Common bands		
7 & 8	7	5	5	0.8333	0.1667
7 & 9	7	4	4	0.7273	0.2727
7 & 10	7	4	4	0.7273	0.2727
7 & 11	7	4	4	0.7273	0.2727
7 & 12	7	4	4	0.7273	0.2727
8 & 9	5	4	3	0.6667	0.3333
8 & 10	5	4	3	0.6667	0.3333
8 & 11	5	4	3	0.6667	0.3333
8 & 12	5	4	3	0.6667	0.3333
9 & 10	4	4	4	1.0000	0.0000
9 & 11	4	4	4	1.0000	0.0000
9 & 12	4	4	4	1.0000	0.0000
10 & 11	4	4	4	1.0000	0.0000
10 & 12	4	4	4	1.0000	0.0000
11 & 12	4	4	4	1.0000	0.0000

Table 4.7.3.15. Data on number of alleles, number of genotypes with rare and highest frequency alleles found among some genotypes of Neem in India through RAPD analysis

Primer	Number of genotypes	Total number of alleles	Number of rare allele (one in 10 genotypes)	Frequency of allele with highest occurrence
OPC-1	15	6	1	66.67
OPC-12	15	3	0	80.00
OPC 11	12	4	0	91.67
OPD-2	6	4	0	100.00
OPD-3	6	7	0	100.00

genotypes showed multiple alleles. Allele with highest frequency had a frequency of 91.67 per cent. A total of 6 genotypes were evaluated with the help of primer OPD-2. Total number of alleles detected were 4. ~~Out of 6 genotypes, 3 exhibited null alleles and all the genotypes showed multiple alleles.~~ Allele with highest frequency had a frequency of 100.00 per cent. A total of 6 genotypes were evaluated with the help of primer OPD-3. Total number of alleles detected were 7. ~~Out of 6 genotypes, 5 exhibited null alleles and all the genotypes showed multiple alleles.~~ Allele with highest frequency had a frequency of 100.00 per cent (**Table** 4.7.3.15.).

Average similarity (F) and dissimilarity (1-F) values among different neem genotypes based on RAPD polymorphism is shown in **Table** 4.7.3.16. These values were calculated on the basis of all the primers used. The number of primers used varied from two to six.

Genotype 7 (HSR01a Hisar) was found to have a highest similarity value (0.8571) with genotype 17 (H080 Kurukshetra) followed by genotype 10 (H048 Rohtak) (0.8333) and genotype 9 (Adu. Aduthurai) (0.6486), while this genotype had highest value of dissimilarity (1.00) with genotypes 24 (GPN-0 Hisar) and 25 (HSR-02 Hisar) .

Genotype 8 (HSR01b Hisar) was found to have a highest similarity value (0.7778) with genotype 10 (H048 Rohtak) followed by genotype 9 (Adu. Aduthurai) (0.7368), while this genotype had highest value of dissimilarity (0.3684) with genotypes 11 (H108a Kalka) and 12 (H028 Narnaul). Genotype 9 (Adu. Aduthurai) was found to have a highest similarity value (1.00) with genotype 23 (H108b Kalka) followed by genotypes 11 (H108a Kalka) and 12 (H028 Narnaul) (0.8462), while this genotype had highest value of dissimilarity (1.00) with genotype 25 (HSR-02 Hisar) followed by 18 (H093 Sirmour) (0.7778).

Genotype 10 (H048 Rohtak) was found to have a highest similarity value (1.00) with genotypes 18 (H093 Sirmour) and 25 (HSR-02 Hisar) followed by genotype 11 (H108a Kalka) and 12 (H028 Narnaul) (0.8696) and genotype 9 (Adu. Aduthurai) (0.6486), while this genotype had highest value of dissimilarity (1.00) with genotypes 24 (GPN-0 Hisar) followed by 16 (H079 Nilokheri) (0.6000).

Genotype 11 (H108a Kalka) was found to have a highest similarity value (1.00) with genotype 12 (H028 Narnaul) and 13 (HSR01t Hisar) followed by genotype 10 (H048 Rohtak) (0.8696), while this genotype had highest value of dissimilarity (0.8000) with genotypes 24 (GPN-0 Hisar) followed by 25 (HSR-02 Hisar) (0.5000). Genotype 12 (H028 Narnaul) was found to have a highest similarity value (1.00) with genotype 11 (H108a Kalka) followed by genotype 17 (H080 Kurukshetra) (0.8889), while this genotype had highest value of dissimilarity (0.6000) with genotypes 24 (GPN-0 Hisar) followed by 25 (HSR-02 Hisar) (0.5000).

Genotype 13 (HSR01t Hisar) was found to have a highest similarity value (1.00) with genotype 11 (H108a Kalka) followed by genotypes 9 (Adu. Aduthurai) and 17 (H080 Kurukshetra) (0.8000), while this genotype has highest value of dissimilarity (0.5000) with genotypes 7 (HSR01a Hisar), 16 (H079 Nilokheri), 18 (H093 Sirmour), 24 (GPN-0 Hisar) and 25 (HSR-02 Hisar) followed by 10 (H048 Rohtak) (0.3333). Genotype 14 (HSR03 Hisar) was found to have a highest similarity value (1.00) with genotype 15 (HSR04 Hisar) followed by genotype 16 (H079 Nilokheri) (0.8571), while this genotype has highest value of dissimilarity (0.6667) with genotypes 24 (GPN-0 Hisar) and 25 (HSR-02 Hisar).

Genotype 15 (HSR04 Hisar) was found to have a highest similarity value (1.00) with genotype 14 (HSR03 Hisar) followed by genotype 16 (H079 Nilokheri) (0.8571), while this genotype has highest value of dissimilarity (0.6667) with genotypes 24 (GPN-0 Hisar) and 25 (HSR-02 Hisar). Genotype 16 (H079 Nilokheri) was found to have a highest similarity value (0.8571) with genotypes 14 (HSR03 Hisar) and 15 (HSR04 Hisar) followed by genotype 17 (H080 Kurukshetra) (0.7273) and genotype 9 (Adu. Aduthurai) (0.6486), while this genotype has highest value of dissimilarity (0.8333) with genotypes 24 (GPN-0 Hisar) followed by 25 (HSR-02 Hisar)(0.6667). Genotype 17 (H080 Kurukshetra) was found to have a highest similarity value (0.8889) with genotypes 11 (H108a Kalka) and 12 (H028 Narnaul) followed by genotypes 7 (HSR01a Hisar), 10 (H048 Rohtak) and 18 (H093 Sirmour) (0.8571), while this genotype has highest value of dissimilarity (0.7778) with genotype 24 (GPN-0 Hisar) followed by genotypes 9 (Adu. Aduthurai) and 23 (H108b Kalka)(0.4000).

Genotype 18 (H093 Sirmour) was found to have a highest similarity value (1.00) with genotypes 7 (HSR01a Hisar), 10 (H048 Rohtak) and 25 (HSR-02 Hisar) followed by genotype 17 (H080 Kurukshetra) (0.8571), while this genotype has highest value of dissimilarity (1.00) with genotype 24 (GPN-0 Hisar).

Genotype 23 (H108b Kalka) was found to have a highest similarity value (1.00) with genotype 9 (Adu. Aduthurai) followed by genotype 10 (H048 Rohtak) (0.8333) and genotype 13 (HSR01t Hisar) (0.7500), while this genotype has highest value of dissimilarity (0.8889) with genotype 23 (H108b Kalka). Genotype 24 (GPN-0 Hisar) was found to have a highest similarity value (0.7272) with genotype 23 (H108b Kalka) followed by genotype 13 (HSR01t Hisar) (0.5000), while this genotype has highest value of dissimilarity (1.00) with genotypes 25 (HSR-02 Hisar).

Genotype 25 (HSR-02 Hisar) was found to have a highest similarity value (1.00) with genotypes 7 (HSR01a Hisar),10 (H048 Rohtak), and 18 (H093 Sirmour) followed by genotype 17 (H080 Kurukshetra) (0.6667) and genotype 9 (Adu. Aduthurai) (0.6486), while this genotype has highest value of dissimilarity (1.00) with genotype 24 (GPN-0 Hisar).

Dendrogram of 12 genotypes based on RAPD profile of OPC-1, OPC-11 and OPC-12 primers was constructed using Jaccard similarity coefficient by UPGMA approach of clustering

Table 4.7.3.16. Average similarity (F) and dissimilarity (1-F) values among 15 neem genotypes based on RAPD polymorphism

	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G23	G24	G25
G7	1.0000 (0.0000)	0.8000 (0.2000)	0.6486 (0.3514)	0.8333 (0.1667)	0.7200 (0.2800)	0.7200 (0.2800)	0.5000 (0.5000)	0.6000 (0.4000)	0.6000 (0.4000)	0.6000 (0.4000)	0.8571 (0.1429)	1.0000 (0.0000)	0.6667 (0.3333)	0.0000 (1.0000)	1.0000 (0.0000)
G8	4	1.0000 (0.0000)	0.7368 (0.2632)	0.7778 (0.2222)	0.6316 (0.3684)	0.6316 (0.3684)	NA	NA	NA	NA	NA	NA	NA	NA	NA
G9	6	4	1.0000 (0.0000)	0.5882 (0.4118)	0.8462 (0.1538)	0.8462 (0.1538)	0.8000 (0.2000)	0.6154 (0.3846)	0.6154 (0.3846)	0.4615 (0.5385)	0.6000 (0.4000)	0.2222 (0.7778)	1.0000 (0.0000)	0.3636 (0.6364)	0.0000 (1.0000)
G10	5	4	5	1.0000 (0.0000)	0.8696 (0.1304)	0.8696 (0.1304)	0.6667 (0.3333)	0.6000 (0.4000)	0.6000 (0.4000)	0.4000 (0.6000)	0.8571 (0.1429)	1.0000 (0.0000)	0.4444 (0.5556)	0.0000 (1.0000)	1.0000 (0.0000)
G11	5	4	5	5	1.0000 (0.0000)	1.0000 (0.0000)	1.0000 (0.0000)	0.8333 (0.1667)	0.8333 (0.1667)	0.6667 (0.3333)	0.8889 (0.1111)	0.7500 (0.2500)	0.7273 (0.2727)	0.2000 (0.8000)	0.5000 (0.5000)
G12	5	4	5	5	5	1.0000 (0.0000)	0.7500 (0.2500)	0.8333 (0.1667)	0.8333 (0.1667)	0.6667 (0.3333)	0.8889 (0.1111)	0.7500 (0.2500)	0.7273 (0.2727)	0.4000 (0.6000)	0.5000 (0.5000)
G13	2	2	2	2	2	2	1.0000 (0.0000)	0.7500 (0.2500)	0.7500 (0.2500)	0.5000 (0.5000)	0.8000 (0.2000)	0.5000 (0.5000)	0.7500 (0.2500)	0.5000 (0.5000)	0.5000 (0.5000)
G14	3	2	3	3	3	3	2	1.0000 (0.0000)	1.0000 (0.0000)	0.8571 (0.1429)	0.7273 (0.2727)	0.6000 (0.4000)	0.6154 (0.3846)	0.3333 (0.6667)	0.3333 (0.6667)
G15	3	2	3	3	3	3	2	3	1.0000 (0.0000)	0.8571 (0.1429)	0.7273 (0.0681)	0.6000 (0.4000)	0.6154 (0.3846)	0.3333 (0.6667)	0.3333 (0.6667)
G16	3	2	3	3	3	3	2	3	3	1.0000 (0.0000)	0.7273 (0.2727)	0.6000 (0.4000)	0.4615 (0.5385)	0.1667 (0.8333)	0.3333 (0.6667)
G17	3	2	3	3	3	3	2	3	3	3	1.0000 (0.0000)	0.8571 (0.1429)	0..6000 (0.4000)	0.2222 (0.7778)	0.6667 (0.3333)
G18	3	2	3	3	3	3	2	3	3	3	3	1.0000 (0.0000)	0.1111 (0.8889)	0.0000 (1.0000)	1.0000 (0.0000)
G23	3	2	3	3	3	3	2	3	3	3	3	3	1.0000 (0.0000)	0.7272 (0.2728)	0.3333 (0.6667)
G24	3	2	3	3	3	3	2	3	3	3	3	3	3	1.0000 (0.0000)	0.0000 (1.0000)
G25	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1.0000 (0.0000)

Dissimilarity values are in parentheses; Underlined values are the number of primers / primer pairs used for each pair of genotypes; NA = Not available

Fig. 4.7.3.1. **Dendrogram of 12 genotypes of *Azadirachta indica* based on RAPD profile of OPC-1, OPC-11 and OPC-12 primers using Jaccard similarity coefficient by UPGMA approach of clustering**

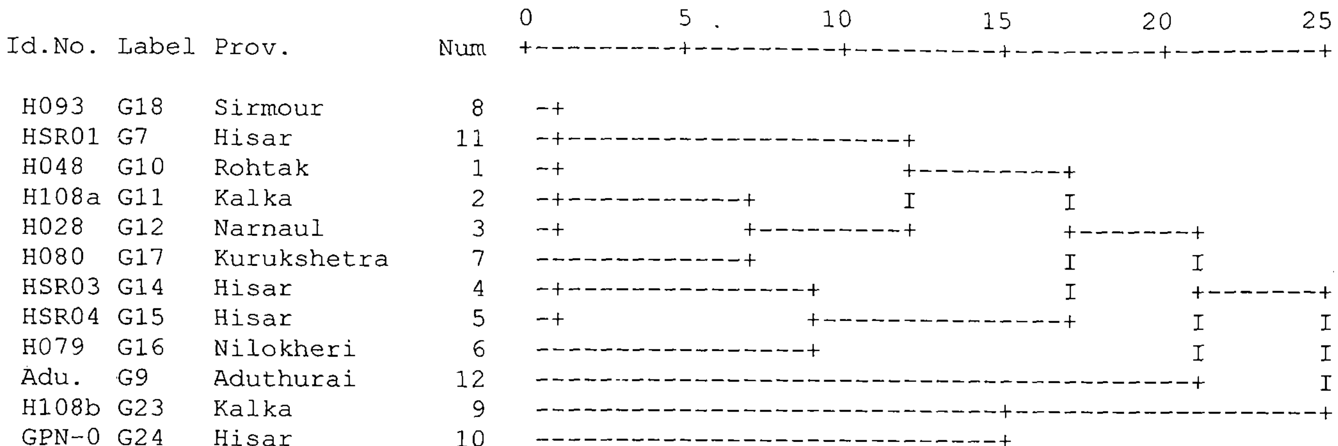
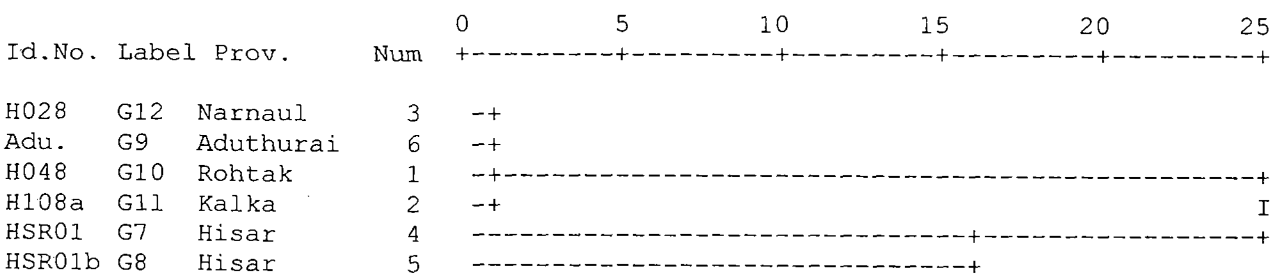


Fig. 4.7.3.2. **Dendrogram of 6 genotypes based on RAPD profile of OPD-2 and OPD-3 primers using Jaccard similarity coefficient by UPGMA approach of clustering**



(Fig. 4.7.3.1.). Genotypes G14 and G15 both from Hisar clustered together due to their very high similarity value. G24 from Hisar was positioned at the periphery of the dendrogram due to its high dissimilarity value with other genotypes. Genotypes G18, G7 and G10 were placed at very distant position compared to G24, G23 ad G9 due to their very high dissimilarity values.

Dendrogram of 6 genotypes based on RAPD profile of OPD-2, and OPD-3 primers was constructed using Jaccard similarity coefficient by UPGMA approach of clustering (Fig. 4.7.3.2.). Genotypes G7 and G8 (progeny of a single tree) from Hisar clustered together due to their very high similarity value. G12 and G10 were positioned at the periphery of the dendrogram due to their high dissimilarity value with other genotypes.

4.7.4. Principal component and Principal factor analyses

Generally, observations are made on a large number of variables, as initially it is not known which variables are more important and useful. In fact one tries to include all possible variables, which are likely to have some connection with the problem. The resulting data matrices become so large that it becomes very difficult to analyze when degree of correlation is very high among the variables.

Principal component analysis helps in identifying most relevant characters and present them in more interpretable and more visualised dimensions through linear combinations of variables that accounts for most of the variation present in original set of variables.

In the present investigation principal components with eigen values greater than one were selected for interpretation (Kaiser, 1958).

Principal component and Principal factor analyses:

Ten morphological and biochemical characteristics of endocarp were considered for principal component and principal factor analyses in 28 genotypes of neem . The first three principal components (PCs) gave eigen values more than one and altogether explained 84.864 per cent of accumulated variability (**Table** 4.7.4.1.). The first PC explained 54.162 per cent of the total variation. The second and third PCs explained 17.557 per cent and 13.146 per cent variation respectively.

Further analysis was carried out using principal factor analysis, as principal component analysis does not assume a definite model, where each observed variable is expressed linearly in terms of a common factor and a unique factor. The common factors account for the correlation among the variables, while each unique factor accounts for the remaining variance (including error) of that variable. Further in PCA, the total variation contained in a set of variables is considered, whereas in factor analysis, interest centres on that part of variance which is shared by the common factors. Also in contrast to principal component analysis, here the variable axes are allowed to interact resulting in distortion of mutual orthogonality.

Table 4.7.4.1. **Total variance explained by different principal components for data of ten seed biochemical and endocarp morphological characteristics of *Azadirachta indica***

Principal Component	Eigen value	% of Variance	Cumulative variance (%)
1	5.416	54.162	54.162
2	1.756	17.557	71.718
3	1.315	13.146	84.864
4	0.763	7.634	92.498
5	0.490	4.904	97.402
6	0.250	2.501	99.903
7	0.008	0.08	99.993
8	0.0007	0.007	100.000
9	~0	~0	100.000
10	~0	~0	100.000

Table 4.7.4.2. **Factor loadings for ten seed biochemical and endocarp morphological characteristics with respect to principal factors in *Azadirachta indica***

Variable	Principal Component			Communalities
	1	2	3	
Unsaturated fatty acids	**-0.971**	0.04	0.200	0.985
Unsaturated fatty acids : saturated fatty acids	**-0.968**	0.02	0.188	0.973
Saturated fatty acids	**0.967**	-0.043	-0.225	0.988
Oleic acid	**-0.903**	0.181	-0.303	0.939
Stearic acid	**0.859**	-0.195	-0.08	0.782
Linoleic acid	**0.718**	-0.228	0.523	0.840
Palmitic acid	**0.604**	0.234	-0.337	0.534
20 endocarp weight	0.264	**0.859**	0.235	0.864
Endocarp length	0.312	**0.700**	0.528	0.867
Oil content	0.014	0.588	**-0.607**	0.714

Bold underline values represent high factor loading for the given character w.r.t. principal factor

PC method was used to extract the principal factors. The advantage of this method is that no distributional assumption is required, whereas other methods like maximum likelihood, assume that the general form of population distribution is known and follow multivariate normal distribution. First the principal factor analysis was carried out without any rotation to derive clear picture of interaction among the characters. Factor loading of different characters (unrotated) are presented in **Table** 4.7.4.2. The results of this table revealed that seven characters (related to fatty acid profile) had very high loading on the first factor. Endocarp length and 20 endocarp weight had high loading on second factor. Oil content of the endocarp had high loading on second factor. This provided a very clear picture regarding the idea of character association with respect to principal factor.

Now among these principal factors, factor 1 and 3 can be regarded as factors for unsaturated fatty acids, ratio of unsaturated and saturated fatty acids and Saturated fatty acids cumulatively. Factors 1, 2 and 3 can be designated as factors for Palmitic acid, Oleic acid, Linoleic acid, 20 endocarp weight and endocarp length. Factors 1 and 2 can be designated as factors for stearic acid. Factors 3 and 2 can be designated as factors for oil content.

Principal factor (PF) scores for all the genotypes were estimated in all the three factors. These scores are presented in **Table** 4.7.4.3. and can be used to propose precise selection indices whose intensity can be decided by variability explained by each of the principal factor.

High principal score for a particular genotype in a particular factor denotes high value for the variable (which the principal factor is representing) in that particular genotype. Perusal of **Table** 4.7.4.3. revealed that genotypes BNR3, BNR2, BNR4, KLK1, KLK2, KLK3, KLK4, KLK5, HSR5, NMN4, and ALD1 had high PF score in factor 1 indicating that they had high unsaturated fatty acids, saturated fatty acids, ratio of unsaturated and saturated fatty acids which this factor is representing. Similarly, PF scores of BNR2, KTL1, KTL4, KLK4, KLK5, HSR1, HSR3, HSR4, HSR5, HSR6, HSR7, NGN1, TER2 and NMN4 were high in factor 2 which point out to the fact that these genotypes had high values for endocarp weight and endocarp length. Likewise, PF scores of BNR1, KTL1, KTL2, KTL3, KTL4, KTL4, KTL5, HSR3, HSR5, HSR6, HSR7, NGN1, and ALD1 were high in factor 3 which point out to the fact that these genotypes had high oil content.

Using the principal factor scores, all the genotypes were plotted for factor 1 and 2, factor 1 and 3 and factor 2 and 3, which cumulatively explained 84.864 per cent variability and accounted for the most important characters (Fig. 4.7.4.1., Fig. 4.7.4.2., Fig. 4.7.4.3.).

These three plots clearly indicated the separation of genotypes with high values of the characteristics towards the axis of factors which represent them. Genotypes BNR3, BNR2, BNR4, and ALD1 which were found to be with lower values of saturated fatty acids stand out towards the negative portion of the factor 1 axis in all the plots. KLK1, KLK2, KLK3, KLK4, KLK5, HSR5, and NMN4, which were found to be with higher yields of saturated fatty acids

Table 4.7.4.3. **Principal factor scores for ten seed biochemical and endocarp morphological characteristics with respect to principal factors in 28 genotypes of *Azadirachta indica***

	Genotype Id. No.	Provenance	F1	F2	F3
1	BNR1	Bikaner	0.07375	0.55823	1.34488
2	BNR2	Bikaner	-1.19207	-1.27442	0.62022
3	BNR3	Bikaner	-2.01462	-0.49527	-0.20212
4	BNR4	Bikaner	-2.43635	0.04561	0.13362
5	BNR5	Bikaner	-0.44438	0.41512	0.38376
6	KTL1	Kaithal	-0.28027	-1.5249	-1.54226
7	KTL2	Kaithal	-0.18694	-0.62912	-0.98676
8	KTL3	Kaithal	-0.59031	-0.57453	-1.00565
9	KTL4	Kaithal	0.00547	-1.36693	-1.49767
10	KTL5	Kaithal	-0.17057	-0.61136	-0.88426
11	KTL6	Kaithal	0.48676	-0.40074	-0.40761
12	KLK1	Kalka	0.88049	0.43518	0.02309
13	KLK2	Kalka	1.33622	-0.34153	-0.65579
14	KLK3	Kalka	0.87066	0.5594	-0.28604
15	KLK4	Kalka	1.3323	-0.7904	0.24496
16	KLK5	Kalka	1.84436	-1.39095	-0.30602
17	HSR1	Hisar	0.05375	0.8931	0.65733
18	HSR2	Hisar	-0.58888	-0.18145	-0.70723
19	HSR3	Hisar	-0.48145	-0.88345	1.8889
20	HSR4	Hisar	-0.22062	1.3743	0.08883
21	HSR5	Hisar	1.3085	-1.49846	2.5819
22	HSR6	Hisar	-0.06336	0.75364	0.86789
23	HSR7	Hisar	-0.02184	1.11064	0.9293
24	NGN1	Pune	0.05884	2.05243	-1.56946
25	TER2	Pune	0.66474	1.21617	-0.1623
26	TGN3	Pune	0.32707	1.33872	-0.39852
27	NMN4	Pune	0.97078	0.95797	-0.11583
28	ALD1	Allahabad	-1.52201	0.25304	0.96282

Table 4.7.4.4. **Total variance explained by different principal components for data of eight endocarp morphological characteristics in *Azadirachta indica***

Principal Component	Eigen value	% of Variance	Cumulative variance (%)
1	4.391	54.886	54.886
2	1.602	20.024	74.910
3	1.223	15.289	90.199
4	0.624	7.802	98.001
5	0.093	1.166	99.167
6	0.056	0.705	99.872
7	0.007	0.081	99.953
8	0.004	0.047	100.000

Fig. 4.7.4.1. **Relationship between first and second principal components showing the distribution of neem trees on the basis PCA analysis of seed biochemical and endocarp morphological characteristics**

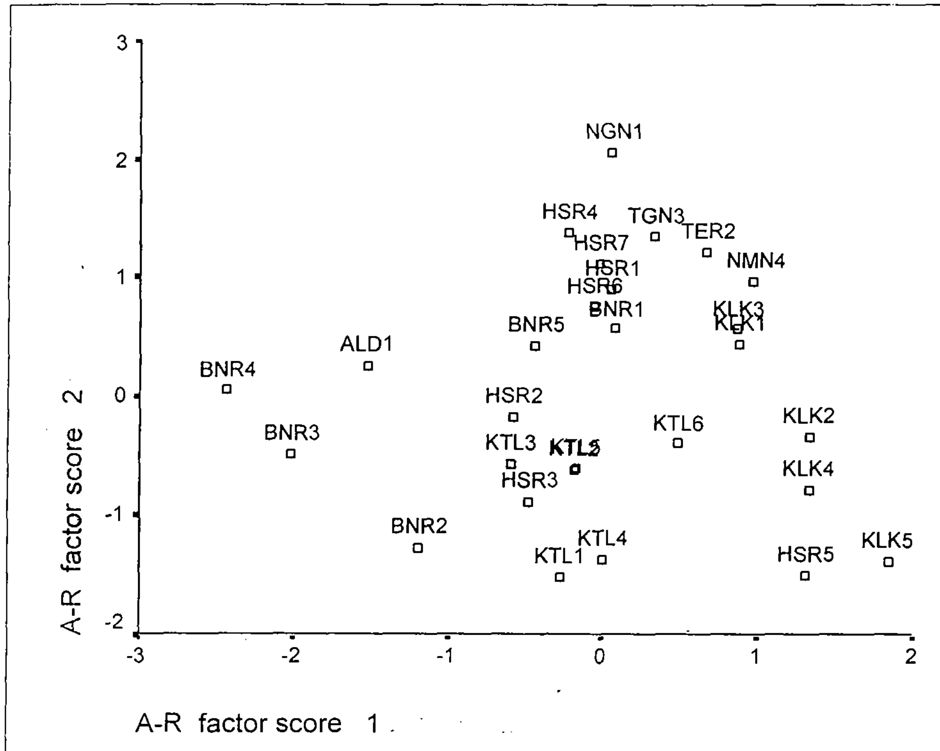


Fig. 4.7.4.2. Relationship between first and third principal components showing the distribution of ~~provenances on th basis of~~ neem trees on the basis PCA analysis of seed biochemical and endocarp morphological characteristics

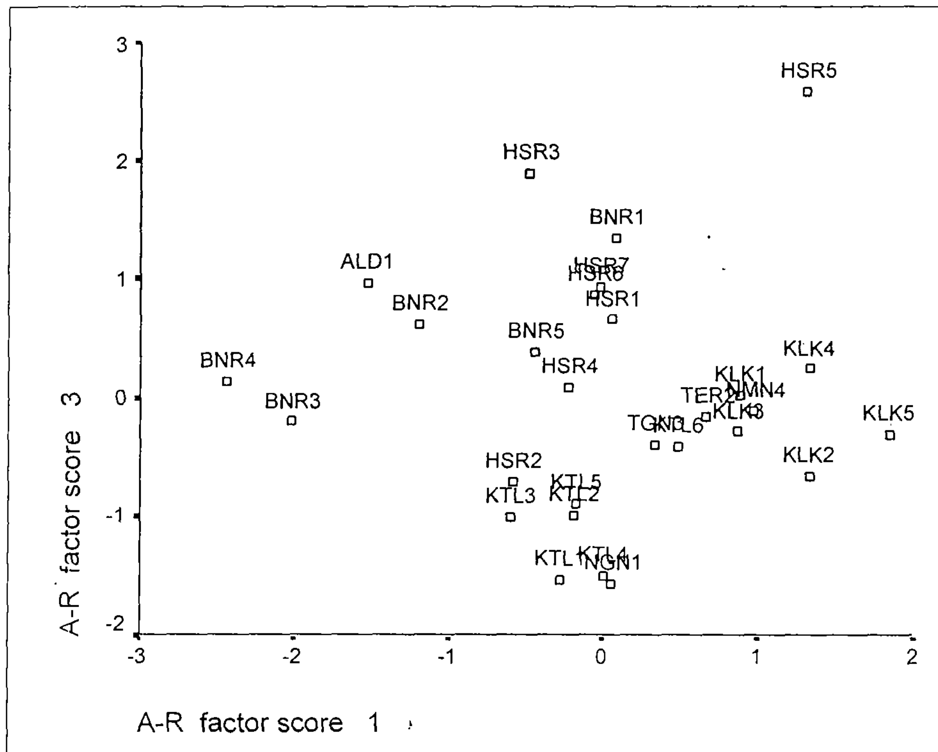
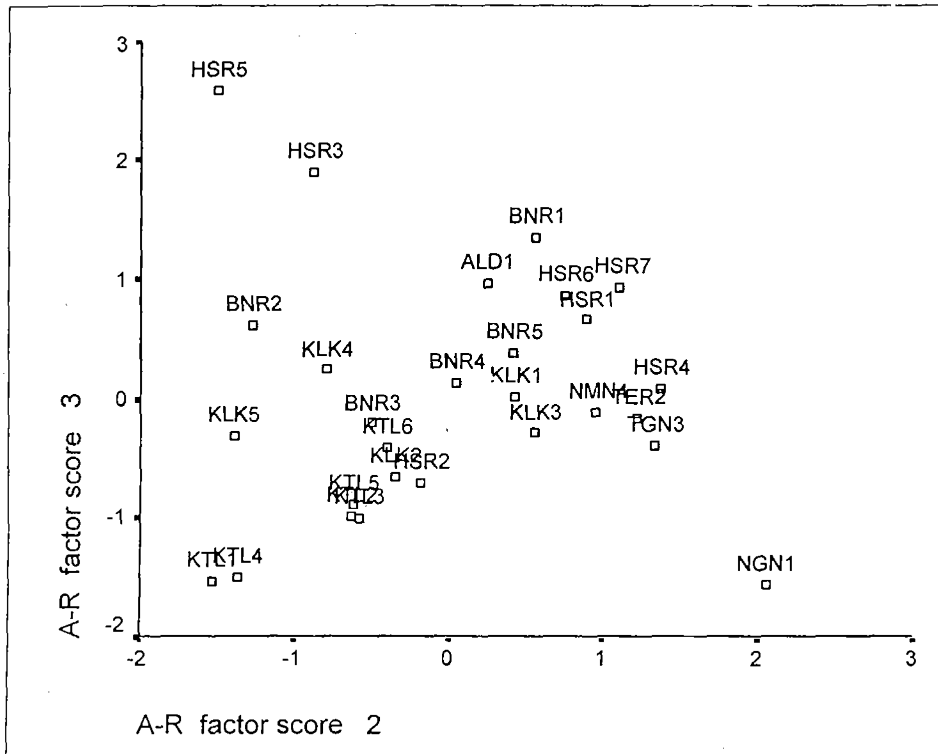


Fig. 4.7.4.3. Relationship between second and third principal components showing the distribution of neem trees on the basis of PCA analysis of seed biochemical and endocarp morphological characteristics



stand out towards the positive portion of the factor 1 axis in all the plots. Genotypes (BNR2, KTL1, KTL4, KLK5, HSR3, HSR5) which were having less 20 endocarp weight and endocarp length, clustered towards the negative side of factor 2 axis (factor accounting for these characters). Whereas, the genotypes (HSR1, HSR4, HSR6, HSR7, NGN1, TER2, TGN3 and NMN4) which were having more endocarp weight and endocarp length clustered towards the positive side of factor 2 axis (factor accounting for these characters). Genotypes KTL1, KTL2, KTL3, KTL4, KTL5, KLK2, KLK5, HSR2, and NGN1 separated more towards the negative side of factor 3 axis which was a factor for oil content indicating that this factor represents these characters efficiently. Genotypes BNR1, BNR2, HSR1, HSR3, HSR5, and HSR7 separated more towards the positive side of factor 3 axis which was a factor for oil content indicating that this factor is an authentic representation for these characters.

When genotypes were plotted on the basis of factor 1 (representing saturated fatty acids) and factor 2 (representing 20 endocarp weight and endocarp length), genotypes BNR3, BNR2, BNR4, and ALD1 were found to be representing low saturated fatty acids and low to moderate levels of 20 endocarp weight and endocarp length. Genotypes KLK2, KLK4, KLK5, and HSR5 were found to be high in saturated fatty acids and moderate levels of 20 endocarp weight and endocarp length.

Similarly, when genotypes were plotted on the basis of factor 1 (representing saturated fatty acids) and factor 3 (representing oil content) genotypes HSR5, KLK4, and NMN4 were found to be high for saturated fatty acids and oil content.

When genotypes were plotted on the basis of factor 2 (representing 20 endocarp weight and endocarp length) and factor 3 (representing oil content) genotypes HSR3, HSR4, HSR7, TER2, TGN3, NMN4 were found to be high for 20 endocarp weight, endocarp length and oil content.

The plot, which used first two factors, were in agreement to some extent with the results obtained by hierarchical cluster analysis. The first three principal factors, explaining about 84.864 per cent of the total variability, were able to show clear differentiation of various clusters. For example, BNR3 and BNR4 clustered together in both hierarchical cluster analysis (HCA) and plots of PC1 and PC3. ALD1 and BNR2 clustered together in both HCA and plots of PC1 and PC3. Three genotypes from Pune namely, TER2, TGN3 and NMN4 clustered together with two genotypes from Kalka KLK1 and KLK3 in both HCA and plots of PC1 and PC3. Four genotypes from Kaithal provenance namely, KTL1, KTL2 KTL3, and KTL4 clustered together with one genotype from Hisar namely, HSR2 in both HCA and plots of PC1 and PC3. (Fig. 4.7.4.3.)

Principal component and Principal factor analyses for morphological data of endocarps:

Eight morphological characteristics of endocarps were considered for principal component and principal factor analyses in 43 genotypes of neem .

Table 4.7.4.5. Factor loadings for eight morphological characteristics with respect to principal factors in *Azadirachta indica*

Characteristic	Components			Communalities
	1	2	3	
Endocarp length	**<u>0.988</u>**	-0.04	-0.105	0.977
Endocarp breath	**<u>0.958</u>**	-0.03	0.124	0.960
Endocarp weight	**<u>0.880</u>**	0.02	-0.421	0.988
Seed weight	**<u>0.833</u>**	0.530	-3.766E-02	0.934
Endocarp coat weight	**<u>0.762</u>**	-0.544	-0.287	0.952
Number of seeds per endocarp	0.202	**<u>0.961</u>**	0.167	0.685
Seed weight : endocarp weight	0.306	-0.280	**<u>0.716</u>**	0.728
Endocarp length : endocarp breadth	0.560	-0.138	**<u>0.629</u>**	0.993

Bold underline values represent high factor loading for the given character w.r.t. principal factor

Table 4.7.4.6. Principal factor scores for eight morphological characteristics with respect to principal factors in 43 genotypes of *Azadirachta indica*

	Tree Id No.	Provenance	F1	F2	F3
2	T3	Bikaner	-0.1195	-0.27972	3.06874
3	T4	Bikaner	0.32658	-0.65163	-0.28817
4	T5	Bikaner	0.11791	-0.22097	0.19282
7	T41	Raipur	0.98239	-0.22125	1.24599
8	T42	Raipur	1.8625	0.83856	-0.3577
9	T43	Raipur	0.17619	0.37928	0.28721
10	T44	Raipur	2.15834	0.62065	0.74153
11	T45	Raipur	0.15786	1.66	2.24657
12	T62	Bhatinda	0.77495	-0.31898	-0.49059
13	T63	Bhatinda	0.84838	-0.60569	1.29066
14	T64	Bhatinda	0.97101	-0.24889	-0.3241
24	T5D	Delhi	0.45438	-0.6859	0.36312
25	T7D	Delhi	-0.75124	-0.0372	0.09806
15	T23	Jodhpur	-1.79538	-1.72888	1.51175
16	T24	Jodhpur	-1.73052	-0.1319	0.35918
17	T20	Jodhpur	-0.08204	-1.21879	-2.20278
18	T22	Jodhpur	-1.50655	-0.35825	0.87624
19	T21	Jodhpur	-0.57911	-1.17925	-0.0189
29	HSR01	Hisar	-0.65875	-0.13901	-0.39425
30	HSR02	Hisar	-0.26935	0.6749	-0.96868
1	T17	Hisar	-0.34214	0.19886	-0.38868
31	HSR03	Hisar	-1.66319	0.17207	-0.2198
32	HSR04	Hisar	-0.5833	1.58247	-1.27738
33	HSR05	Hisar	-1.02919	0.10371	-0.6339
34	SHS-0	Hisar	-0.25874	0.6358	-0.15594
5	T18	Hisar	1.32182	0.18876	0.48355
6	T52	Kaithal	-0.13106	0.25169	0.21695
36	H082	Kaithal	-0.99759	-0.1351	-0.46045
26	H083	Kaithal	-0.07406	1.00704	-0.64866
37	H084	Kaithal	-0.05884	0.19625	-0.14979
38	H085	Kaithal	-0.44576	1.50282	-0.34458
27	T30	Alwar	-0.89172	0.07887	1.6302
28	T31	Alwar	-1.12174	-1.01136	-0.90508
39	T33	Alwar	-1.442	0.15696	-1.37972
40	TGN3	Pune	-0.07107	0.66524	0.0783
41	NMN4	Pune	0.31208	-0.00578	0.3057
42	TER2	Pune	0.08077	1.06187	0.19503
43	NGN1	Pune	0.19932	2.98169	-0.39866
35	H100	Kalka	1.73491	-2.05877	-0.73922
20	H101	Kalka	1.14392	0.25084	-1.31312
21	H102	Kalka	0.08051	-2.08224	-1.09854
22	H107	Kalka	1.78176	-0.05795	-0.83932
23	H099	Kalka	1.11728	-1.83081	0.80641

The first three principal components (PCs) gave eigen values more than one and altogether explained 90.199 per cent of accumulated variability (**Table** 4.7.4.4.). The first PC explained 54.886 per cent of the total variation. The second and third PCs explained 20.024 per cent and 15.289 per cent variation, respectively. Further analysis was carried out using principal factor analysis, as principal component analysis does not assume a definite model, where each observed variable is expressed linearly in terms of a common factor and a unique factor. The common factors account for the correlation among the variables, while each unique factor accounts for the remaining variance (including error) of that variable. Further in PCA, the total variation contained in a set of variables is considered, whereas in factor analysis interest centres on that part of variance which is shared by the common factors. Also in contrast to principal component analysis, here the variable axes are allowed to interact resulting in distortion of mutual orthogonality.

Principal Component method was used to extract the principal factors. The advantage of this method is that no distributional assumption is required, whereas other methods like maximum likelihood assume that the general form of population distribution is known and follow multivariate normal distribution.

First the principal factor analysis was carried out without any rotation to derive clear picture of interaction among the characters. Factor loading of different characters (unrotated) are presented in **Table** 4.7.4.5. The results of this table revealed that five morphological characteristics of endocarps (endocarp weight, seed weight, endocarp coat weight, endocarp length, and endocarp breadth) had very high loading on the first factor. Endocarp length : breadth had high loading on second factor. Seed weight : endocarp weight and number of seeds endocarp^{-1} had high loading on second factor. This provided a very clear picture regarding the idea of character association with respect to principal factor.

Now among these principal factors, factor 1 and 3 can be regarded as factors for endocarp length, endocarp breadth and endocarp weight cumulatively. Factors 1, 2 and 3 can be designated as factors for seed weight : endocarp weight, endocarp length : endocarp breadth, number of seeds endocarp^{-1} and endocarp coat weight. Factors 1 and 2 can be designated as factors for ~~endocarp length~~ seed weight.

Principal factor (PF) scores for all the genotypes were estimated in all the three factors. These scores are presented in **Table** 4.7.4.6. and can be used to propose precise selection indices whose intensity can be decided by variability explained by each of the principal factor.

High principal score for a particular genotype in a particular factor denotes high value for the variable (which the principal factor is representing) in that particular genotype. Perusal of **Table** 4.7.4.6. revealed that genotypes T18, T42, T44, H101, H107, H099, and H100 had high PF score in factor 1 indicating that they had high values for endocarp weight, seed weight, endocarp coat weight, endocarp length, and endocarp breadth which this factor is representing.

Fig. 4.7.4.4. **Relationship between first and second principal components showing the distribution of neem trees on the basis of PCA analysis of endocarp morphological characteristics**

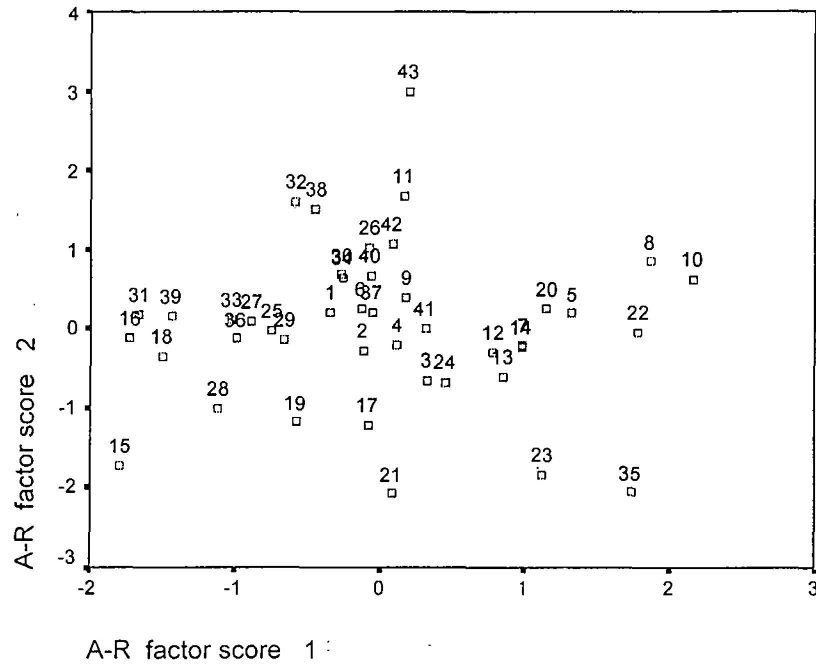


Fig. 4.7.4.5. **Relationship between first and third principal components showing the distribution of neem trees on the basis of PCA analysis of endocarp morphological characteristics**

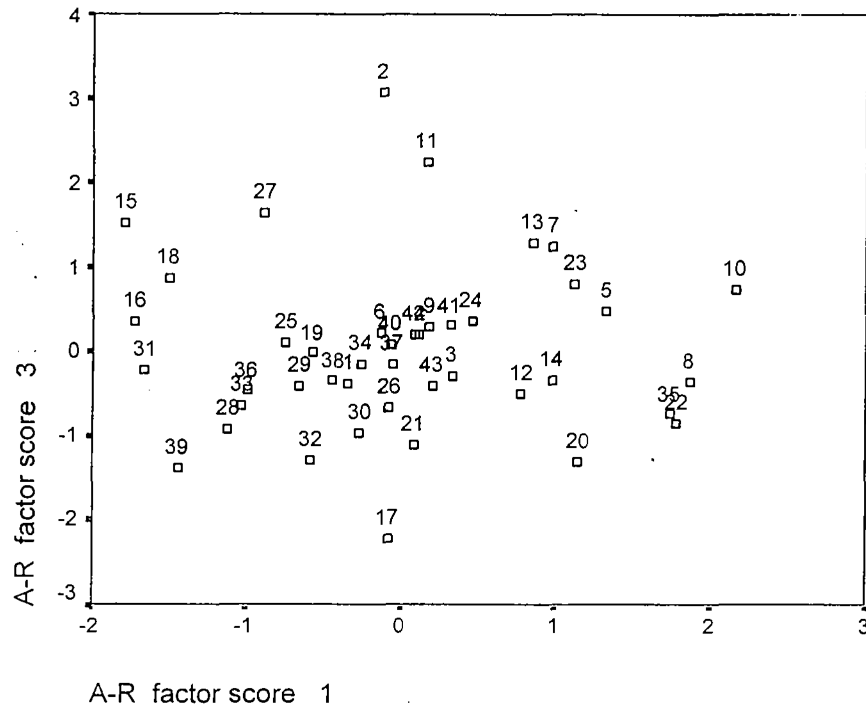


Fig. 4.7.4.6. Relationship between second and third principal components showing the distribution of ~~provenances of~~ neem trees on the basis of PCA analysis of endocarp morphological characteristics

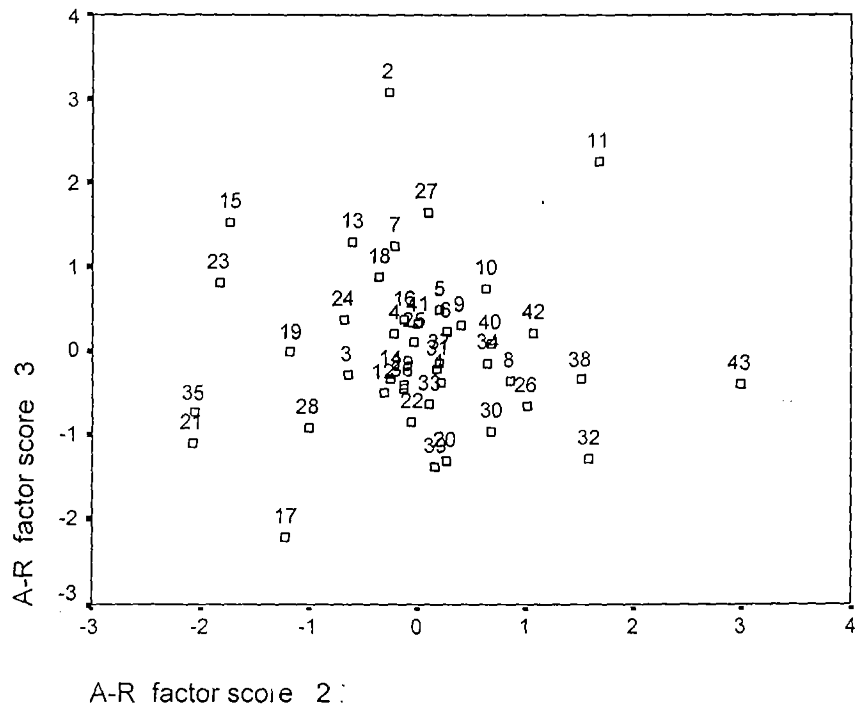


Table 4.7.4.7. Total variance explained by different principal components for data of ten tree architecture characteristics in *Azadirachta indica*

Component	Initial Eigen value			Rotation Sums of Squared		
	Total	% of Variance	Cumulative variance (%)	Total	% of Variance	Cumulative variance (%)
1	4.560	45.602	45.602	3.034	30.337	30.337
2	2.257	22.568	68.170	2.456	24.561	54.897
3	1.211	12.108	80.278	2.367	23.673	78.571
4	1.163	11.630	91.908	1.334	13.337	91.908
5	.489	4.894	96.802			
6	.179	1.786	98.588			
7	0.076	.763	99.351			
8	0.035	.354	99.705			
9	0.019	.195	99.900			
10	0.01	.100	100.000			

Extraction Method: Principal Component Analysis

Table 4.7.4.8. Factor loadings for ten tree architectural characteristics with respect to principal factors in *Azadirachta indica* without rotation

	Component			
	1	2	3	4
Clear bole : dbh	**.814**	.439	.116	.237
Clear bole	.575	-.412	-.146	.550
Clear bole : Height	**.693**	-.327	.359	.453
Clear bole : Unforked height	-.581	.385	.221	**.657**
Crown Spread : dbh	.513	.590	.446	-.228
Crown Spread : Height	-.180	-.396	**.831**	-.237
Height : dbh	.580	**.785**	-.114	-5.678E-02
Unforked height	**.766**	-.480	-.285	-.169
Unforked height : dbh	**.923**	.270	-2.371E-02	-.120
Unforked height : Height	**.824**	-.462	.116	-.116

Bold underline values represent high factor loading for the given character w.r.t. principal factor

Table 4.7.4.9. Factor loadings for ten tree architectural characteristics with respect to principal factors in *Azadirachta indica* with rotation

Characteristic	Component				
	1	2	3	4	Communalities
Clear bole : dbh	**.828**	0.071	.454	-.170	.926
Clear bole	-0.047	.200	**.858**	-.213	.824
Clear bole : Height	.237	.151	**.887**	.236	.921
Clear bole : Unforked height	-0.083	**-.978**	0.026	-0.032	.966
Crown Spread : dbh	**.894**	0.038	-0.062	.239	.862
Crown Spread : Height	-.146	0.003	0.005	**.957**	.937
Height : dbh	**.905**	0.032	-0.057	-.381	.968
Unforked height	0.059	**.833**	.456	-.147	.928
Unforked height : dbh	**.771**	.474	.307	-.162	.940
Unforked height : Height	.212	**.714**	.573	.194	.920

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
Bold underline values represent high factor loading for the given character w.r.t. principal factor

Similarly, PF scores of H083, HSR04, H085, T42 and T45 were high in factor 2 which point out to the fact that these genotypes had high values for endocarp length : breadth. Likewise, PF scores for T41, T45, T63, T23, T30, H099, T22, T44 were high in factor 3 which point out to the fact that these genotypes had high values for Seed weight : endocarp weight and number of seeds endocarp^{-1}.

Using the principal factor scores, all the genotypes were plotted for factor 1 and 2, factor 1 and 3 and factor 2 and 3, which cumulatively explained 90.199 per cent variability and accounted for the most important characters (Fig. 4.7.4.4., Fig. 4.7.4.5., Fig. 4.7.4.6.).

These three plots clearly indicated the separation of genotypes with high values of the characteristics which they represent. Genotypes T18, T42, T44, H101, H107, H099, and H100 which were found to be with higher yields of endocarp weight, seed weight, endocarp coat weight, endocarp length, and endocarp breadth stand out towards the positive portion of the factor 1 axes in all the plots. Whereas, the genotypes (H083, HSR04, H085, T42 and T45) which were having more of Endocarp length : breadth clustered towards the positive side of factor 2 axis (factor accounting for these characters). Genotypes (T41, T45, T63, T23, T30, H099, T22, and T44) separated more towards the positive side of factor 3 axis which was a factor for seed weight : endocarp weight and number of seeds endocarp^{-1} indicating that this factor is an authentic representation for these characters.

When genotypes were plotted on the basis of factor 1 (representing endocarp weight, seed weight, endocarp coat weight, endocarp length, and endocarp breadth) and factor 3(representing Endocarp length : breadth) genotypes T42 and T44 were found to be higher for all the character represented by factor 1 and factor 2. T23 and T31 were found to be lower in all the characteristics represented by two factors.

Similarly, when genotypes were plotted on the basis of factor 1 (representing (endocarp weight, seed weight, endocarp coat weight, endocarp length, and endocarp breadth) and factor 2 (representing seed weight : endocarp weight and number of seeds endocarp^{-1}),genotypes T3, and T45 were found to be high for characteristics represented by factor 1 and number of seeds endocarp^{-1} and seed weight : endocarp weight.

Similarly, when genotypes were plotted on the basis of factor 3 (representing Endocarp length : breadth and seed weight : endocarp weight) and factor 2(representing number of seeds endocarp^{-1}),genotypes NGN1, HSR04, were found high for Endocarp length : breadth. T33 was found to be ~~higher for found~~ high for Endocarp length : breadth and seed weight : endocarp weight.

The plots, which used first three factors, were in partial agreement with the results obtained by hierarchical cluster analysis. The first three principal factors, explaining about 90.199 per cent of the total variability were able to show clear differentiation of various clusters. For

example, T22 and T24 (from Jodhpur provenance) and HSR03 (Hisar) clustered together in both hierarchical cluster analysis (HCA) and plots of PC1 and PC2. H099 and H100 (from Kalka provenance) clustered together in both HCA and plots of PC1 and PC2. Two genotypes from Pune namely, TGN3 and NMN4 clustered together with T5 from Bikaner and T43 from Raipur provenance in both HCA and plots of PC1 and PC3 (Fig. 4.7.4.4. ; Fig. 4.7.6.; **Table** 4.7.2.1.).

The plots, which used first three factors, were in partial agreement with the results obtained by D^2 analysis. Genotypes T4, T41, T62, T64, T63, and T3 clustered in CI (in D^2 analysis) (**Table** 4.7.1.1) also clustered in same cluster in HCA when PC1 and PC2 were plotted. Genotypes HSR03, T22 and T24 clustered in CII (in D^2 analysis) also clustered in same cluster in PCA when PC1 and PC2 were plotted. Genotypes H18 and H101, H101, T42, T44 clustered in CIV (in D^2 analysis) also clustered in same cluster in PCA when PC1 and PC2 were plotted. Genotypes T23 and NGN1 clustered in CIX and CX respectively (in D^2 analysis) were also seed to be plotted in isolation in PCA when PC1 and PC2 were plotted (Fig. 4.7.4.4. and Fig. 4.7.6.).

Principal component and Principal factor analyses for tree architectural data:

Ten tree architectural characteristics were considered for principal component and principal factor analyses in 126 trees of neem .

The first 4 principal components (PCs) gave eigen values more one and altogether explained 91.908 % of accumulated variability **(Table 4.7.4.7.)**. The first PC explained 45.60% of the total variation. The second, third and fourth PCs explained 22.57%, 12.11% and 11.63 variation respectively.

Further analysis was carried out using principal factor analysis. PC method was used to extract the principal factors. First the principal factor analysis was carried out without any rotation to derive clear picture of interaction among the characters. Factor loading of different characters (unrotated) are presented in **Table 4.7.4.8.**. The results of this table revealed that five factors (related to clear bole and unforked height) had very high loading on the first factor. Height : dbh had high loading on second factor. Crown spread : height had high loading on third factor. Clear bole : unforked height had high loading on third factor. While for clear bole, no high loading (0.6) was observed. Therefore, this did not provide a very clear picture regarding the idea of character association with respect to principal factor. Therefore, the next alternative of factor analysis, i.e., with rotation was followed. In this method factor axes are rotated to a simple structure such that each variable loads highly on a single factor and has small to moderate loading on the remaining factors. In the present investigation Varimax rotation method of Kaiser (1958) was used. Factor loading of different characteristics with Varimax rotation are presented in **Table 4.7.4.9.**.

Table 4.7.4.10. **Principal factor scores for ten tree architectural characteristics with respect to principal factors in 116 genotypes of *Azadirachta indica***

S. No.	Genotype Id. No.	Provenance	F1	F2	F3	F4
1	RaJoBa01	Jodhpur	.21787	-.55605	.33858	.85843
2	RaJoBa02	Jodhpur	-.28356	.49335	1.21550	.30226
3	RaJoUn01	Jodhpur	1.17089	-.71628	-1.09690	-.32411
4	RaJoUn02	Jodhpur	1.25609	-.73866	.83153	.77312
5	RaJoUn03	Jodhpur	1.51418	-.71507	-.36969	-1.86888
6	RaAlPc01	Alwar	.58276	-.70687	1.84046	3.83463
7	RaAlVc01	Alwar	-.19275	-.53726	.34817	.28706
8	RaAlAs01	Alwar	-1.00478	-.41212	-.03240	-1.44813
9	HaHiNc01	Nangal Chhoti	-.45907	-.48607	1.55544	-.23700
10	RaChBh02	Bhorugram	4.79658	-1.23080	1.65362	-1.64286
11	HaHiBa01	Bhorugram	-.62590	-.51435	-.09432	-2.15175
12	HaHiGa01	Gaibipur	-.31278	1.24603	1.22465	-.57952
13	HaHiGa02	Gaibipur	.33504	1.93272	-.89164	-.21696
14	HaHiSu01	Surewala	.55312	-.63108	-.22832	-.17998
15	HaFaDh01	Dhani Gopal	.40725	-.60755	.41603	-.09243
16	HaFaJh01	Fatehabad	.38708	-.09303	.62497	-.57890
17	HaFaRd01	Fatehabad	.13249	-.58285	-.74478	.08893
18	HaSiAf01	Sirsa	5.73890	1.85408	-.45767	-2.28109
19	HaHiPi01	Peeranwali	3.22474	-.87816	-1.70395	.96151
20	HaHiUn03	Hisar	.64278	-.63531	.07855	.84439
21	HaHiUn04	Hisar	1.19342	-.69051	-.34981	-.35769
22	HaHiUn05	Hisar	.48822	-.60977	.14132	.21956
23	HaHiUn06	Hisar	.01185	-.57509	-1.34797	-.36691
24	HaHiUn07	Hisar	.36749	.98917	.27455	.12616
25	HaHiUn08	Hisar	.91579	4.32153	-.30884	.16310
26	HaHiUn09	Hisar	.95981	.67433	.98630	3.87255
27	HaHiUn10	Hisar	.80399	-.27983	1.95022	.23105
28	HaHiUn11	Hisar	-.54838	-.52953	-1.82497	-.34594
29	HaHiUn12	Hisar	-.20290	1.42041	.28230	-.31072
30	HaHiUn14	Hisar	-.93594	.66287	.40560	-.56480
31	HaHiUn15	Hisar	-.83060	2.61251	-.41999	.67657
32	HaHiUn16	Hisar	.28309	-.54120	1.82024	-.32479
33	HaHiUn17	Hisar	1.65378	-.72832	-.49929	.53877
34	HaHiNa01	Nalwa	-.03001	-.54426	.59534	.48796
35	HaHiNa02	Nalwa	-1.[illegible]6219	-.43407	-.25837	-1.58400
36	HaBhDu01	Dulehari	-.31120	-.50524	.21817	.75796
37	HaMaMm01	Mahendragarh	-.89690	-.45816	-.39614	.39728
38	HaMaJo01	Jonawas	-.66248	-.47271	-.05126	-.72957
39	HaMaNs01	Nangal Sirohi	-.64025	-.46875	-.68752	.87014
40	HaMaNs02	Nangal Sirohi	.60629	-.64787	-2.37994	1.45619
41	HaMaHu02	Hudina	.90306	-.74981	1.32945	.49691
42	HaMaHu03	Hudina	-.07546	-.53067	.30611	.47629
43	HaMaHu04	Hudina	1.73594	-.05661	1.16501	.25544
44	HaMaAj01	Ajamnagar	.76327	.94885	.73260	.52439
45	HaMaAj02	Ajamnagar	.33256	1.30684	.10222	.62659
46	HaMaRd01	Narnaul	-1.05269	-.40799	-.25810	-.44569
47	HaMaSb02	Saraibahadur	.28985	-.61634	-1.97035	.42052
48	HaReKu01	Kutubpur	-.49093	1.31206	1.40936	-.49353
49	HaReSu01	Suthani	-.11517	-.54620	.14886	-.18255
50	HaReRd01	Bawal	-.30849	-.49385	-.47819	.63011
51	HaGuBa01	Bilaspur	-.38883	-.48353	.47999	-.77866
52	HaGuMa01	Manesar	.16651	3.21795	-.42190	-.98631
53	HaGuMa02	Manesar	-.25539	.76189	-.53730	-.30868

Table 4.7.4.10. Principal factor scores for ten tree architectural characteristics with respect to principal factors in 116 genotypes of *Azadirachta indica* (continued)

S. No.	Genotype Id. No.	Provenance	F1	F2	F3	F4
54	HaGuSi01	Silani	-.14213	-.49768	.28264	-.58032
66	DeNaAn01	Najafgarh	.54713	.49372	.87030	.77444
67	HaGuBh01	Bhondsi	-.87834	-.41731	.40585	-.87133
68	HaGuDh01	Dhunaella	-.88227	-.40161	.36247	-.47706
69	HaGuSh01	Salaheri	-.25603	.15547	-.58567	.88600
70	HaGuSh02	Salaheri	-.57471	.62144	.65738	-.08804
71	HaGuSa01	Salamba	-.33997	.69583	-.22269	-.54674
72	HaFrPa01	Palwal	-.66169	-.39173	2.68600	-.80897
73	HaFrBl01	Palwal	-.35193	-.49332	-.26285	-1.67921
74	DeNrPb01	Pusa	-.16197	-.45086	.31013	-.07786
75	DeNrPb02	Pusa	.06923	-.46525	.78340	-.34332
76	DeNrPd01	Pusa	-.26064	-.47379	-.09805	-.29991
77	DeNrPd02	Pusa	-.53472	-.49396	-1.13703	.12729
78	DeNrPd03	Pusa	-.65256	-.48660	-1.14514	.34773
79	HaSoKu01	Kundli	-.49358	2.53704	-.74639	-.74739
80	HaHiSh01	Sheikhpura	-.56405	2.29104	-1.52521	.40034
81	HaHiGb01	Gujjar Bara	.09202	1.84529	-.81938	1.33987
82	HaHiGb02	Gujjar Bara	1.13698	.25017	-1.10749	1.05770
83	HaHiGb03	Gujjar Bara	.54666	-.64943	.12351	.55372
84	HaHiNa02	Narnaund	-.50369	.62188	.84747	-1.24005
85	HaHiNa03	Narnaund	.34123	-.60415	-.29327	-.56977
86	HaJiGu01	Gulkani	-.40370	-.48234	.92140	.04064
87	HaJiGu02	Gulkani	-.49113	1.23355	-1.14411	.47271
88	HaJiKa01	Kandela	.02775	-.59758	1.66848	.20256
89	HaJiKa02	Kandela	.63899	-.63438	-1.19597	-.31063
90	HaJiAl01	Alewa	-.66507	-.44861	-.29914	1.26664
91	HaJiAl02	Alewa	-.37638	-.53899	-.64638	.85971
92	HaKuKs01	Kurukshetra	-.22994	-.49013	.12361	2.60014
93	HaKuKs02	Kurukshetra	-1.16031	1.13854	-1.50751	.46802
94	HaKaGb01	Kaithal	-1.19882	.58771	.52379	.13291
95	HaKaSc01	Kaithal	-.20512	-.52995	-.65978	1.15547
96	HaKaTb01	Kaithal	-.99811	-.42996	.23946	.16175
97	HaKaTc01	Kaithal	-.09363	-.57970	.58361	-.62618
98	HaKaKe01	Keorke	-.52191	2.12353	-.17718	-1.13471
99	HaAmDh01	Dhin	-.22359	-.54631	-1.42150	-1.72287
100	HaYaJa01	Jagadhari	-.00831	-.55416	-1.20855	.14625
101	HaYaJa02	Jagadhari	.12510	-.10960	.71245	.18864
102	HaYaTa01	Tajewala	-.64274	-.48026	.38866	.40663
103	HpSrBa01	Batamandi	-.71559	-.46951	-.54123	-1.15466
104	HpSrSa01	Satiwala	-1.02715	-.48330	-.72460	-.93381
105	HpSrMn01	Moginand	.36509	.42661	-.42058	.28183
106	HpSrCm01	Chhota Moginand	.46282	-.60581	-1.06374	-2.28152
107	HaAmLa01	Laha	-.78505	-.49223	-1.36632	-.26724
108	HaAmGk01	Garhi Kataha	-.05327	-.57120	-.83419	1.43889
109	HaAmNa01	Naggal	-.39540	2.86356	1.96035	.93076
110	HaKlCa01	Kalka	-.23507	-.55953	.96350	-.67460
111	HaKlNs01	Kalka	-.57938	-.48892	1.08385	-.48001
112	PbPtSa01	Salampur	-.36985	-.54897	-2.08788	1.42109
113	HaKlPi01	Pinjore	-.88037	-.44709	.14855	.38576
114	HaKlPi02	Pinjore	-.81868	-.44972	.61289	-.00837
115	HaKlRe01	Kalka	-.62938	-.48461	-.37198	-.88068
116	HaKlKh01	Kalka	-.30421	-.52830	-.95606	.13988

The results of this **Table 4.7.4.9.** revealed PC method was used to extract the principal factors. Four characteristics (Clear bole : dbh, Crown Spread : dbh, Height : dbh, and Unforked height : dbh) had very high loading on the first factor. Three characteristics (Clear bole : Unforked height, Unforked height, Unforked height : Height) on second; two (Clear bole, Clear bole : Height) on third factor and one (Crown Spread : Height) on fourth factor.

Now among these principal factors, factor 1, 2 and 3 can be regarded as factors for Unforked height : dbh cumulatively. Factor 1 and 3 can be regarded as factors for Clear bole : dbh. . Factor 1, 3 and 4 can be regarded as factors for Clear bole : Height cumulatively. Factors 2 and 3 can be designated as factors for unforked height and unforked height : height. Factors 1 and 2 can be designated as factors clear bole : unforked height. Factors 2, 3 and 4 can be designated as factors for clear bole. Factors 1 and 4 can be designated as factors for height : dbh, crown spread : dbh and crown spread : height. .

Principal factor (PF) scores for all the genotypes were estimated in all the three factors. These scores are presented in **Table 4.7.4.10.** and can be used to propose precise selection indices whose intensity can be decided by variability explained by each of the principal factor.

High principal score for a particular genotype in a particular factor denotes high value for the variable (which the principal factor is representing) in that particular genotype. Perusal of **Table 4.7.4.10.** revealed that 12 trees (RaJoUn01, RaJoUn02, RaJoUn03, RaAlAs01, RaChBh02, HaHiUn04, HaHiNa02, HaHiUn17, HaMaRd01, HaMaHu04, HaHiGb02, HpSrSa01) had high PF score in factor 1 indicating that they had high values for Clear bole : dbh, Crown Spread : dbh, Height : dbh, and Unforked height : dbh which this factor is representing.

PF scores of 17 trees (RaChBh02, HaHiGa01, HaHiGa02, HaSiAf01, HaHiUn08, HaHiUn12, HaHiUn15, HaMaAj02, HaReKu01, HaGuMa01, HaSoKu01, HaHiSh01, HaHiGb01, HaJiGu02, HaKuKs02, HaKaKe01, and HaAmNa01) were high in factor 2 which point out to the fact that these genotypes had high values for Clear bole : Unforked height, Unforked height, .and Unforked height : Height.

Similarly, PF scores of 32 trees (RaJoBa02, RaJoUn01, RaAlPc01, HaHiNc01, RaChBh02, HaHiGa01, HaHiPi01, HaHiUn06, HaHiUn10, HaHiUn11, HaHiUn16, HaMaNs02, HaMaHu02, HaMaHu04, HaMaSb02, HaReKu01, HaFrPa01, DeNrPd02, DeNrPd03, HaHiSh01, HaHiGb02, HaJiGu02, HaJiKa01, HaJiKa02, HaKuKs02, HaAmDh01, , HaYaJa01, HpSrCm01, HaAmLa01, HaAmNa01, HaKlNs01, and PbPtSa01) were high in factor 3 which point out to the fact that these genotypes had high values for Clear bole and Clear bole : Height.

Likewise, PF scores of RaJoUn03, RaAlPc01, RaAlAs01, RaChBh02, HaHiBa01, HaSiAf01, HaHiUn09, HaHiNa02, HaMaNs02, HaFrBl01, HaHiGb01, HaHiGb01, HaJiAl01,

HaKuKs01, HaKaSc01, HaKaKe01, HaAmDh01, HpSrBa01, HpSrCm01, HaAmGk01, PbPtSa01, HaKlKh01 were high in factor 4 which point out to the fact that these genotypes had high scoring for Crown Spread : Height.

Using the principal factor scores, all the genotypes were plotted for factor 1 and 2, 1 and factor 3, 1 and 4, 2 and 3, 2 and 4 and 3 and 4, which cumulatively explained 91.908% variability and accounted for the most important characters (**Fig. 4.7.4.7., Fig. 4.7.4.8., Fig. 4.7.4.9., Fig. 4.7.4.10., Fig. 4.7.4.11., Fig. 4.7.4.12**).

These plots clearly indicated the separation of genotypes with high values of the characteristics towards the axis of factors which represent them. Genotypes which were found to be with lower values of Clear bole : dbh, Crown Spread : dbh, Height : dbh, and Unforked height : dbh generally stand cut towards the negative portion of the factor 1 axis in all the plots, where factor 1 is used.

The trees which were found to be with higher values of Clear bole : dbh, Crown Spread : dbh, Height : dbh, and Unforked height : dbh stand out towards the positive portion of the factor 1 axis in all the plots. Genotypes which were having less Clear bole : Unforked height, Unforked height, and Unforked height : Height of clustered towards the negative side of factor 2 axis (factor accounting for these characters). Whereas, the genotypes which were having more Clear bole : Unforked height, Unforked height, .and Unforked height : Height clustered towards the positive side of factor 2 axis (factor accounting for these characters). Trees separated more towards the negative side of factor 3 axis which was a factor for Clear bole and Clear bole : Height, indicating that this factor represents these characters efficiently. Some Genotypes separated more towards the positive side of factor 3 axis which was a factor for Clear bole and Clear bole : Height,indicating that this factor is an authentic representation for these characters.

Trees separated more towards the negative side of factor 4 axis which was a factor for Crown Spread : Height,indicating that this factor represents these characters efficiently. Some Genotypes separated more towards the positive side of factor 4 axis which was a factor for Crown Spread : Height indicating that this factor is an authentic representation for these characters.

When genotypes were plotted on the basis of factor 1 (representing Clear bole : dbh, Crown Spread : dbh, Height : dbh, and Unforked height : dbh) and factor 2 (representing Clear bole : Unforked height, Unforked height, and Unforked height : Height) trees 25, 52 and 109 were found representing higher Clear bole : dbh, Crown Spread : dbh, Height : dbh, and Unforked height : dbh, while 93, 94, 30 and 31 among others were found representing lower values for these characteristics. Trees 32, 38, 11 and 43 were found representing higher Clear bole : Unforked height, Unforked height, and Unforked height : Height, while tree and 10, 19 were found representing lower values for these characteristics (Fig. 4.7.4.7.).

Fig. 4.7.4.7. **Relationship between first and second principal components showing the distribution of neem trees on the basis of PCA analysis of tree architectural characteristics**

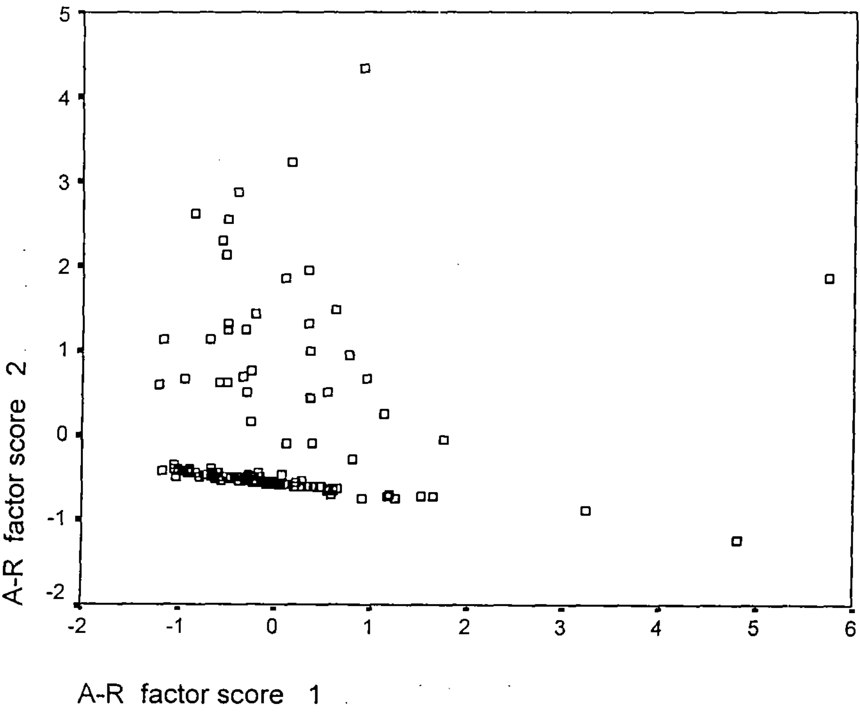


Fig. 4.7.4.8. **Relationship between first and third principal components showing the distribution of neem trees on the basis of PCA analysis of tree architectural characteristics**

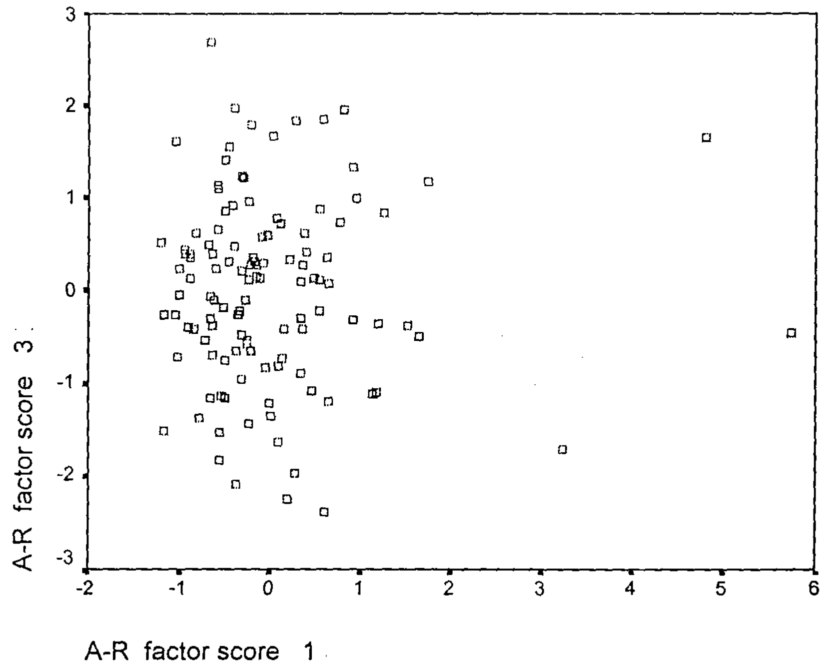


Fig. 4.7.4.9. Relationship between first and fourth principal components showing the distribution of neem trees on the basis of PCA analysis of tree architectural characteristics

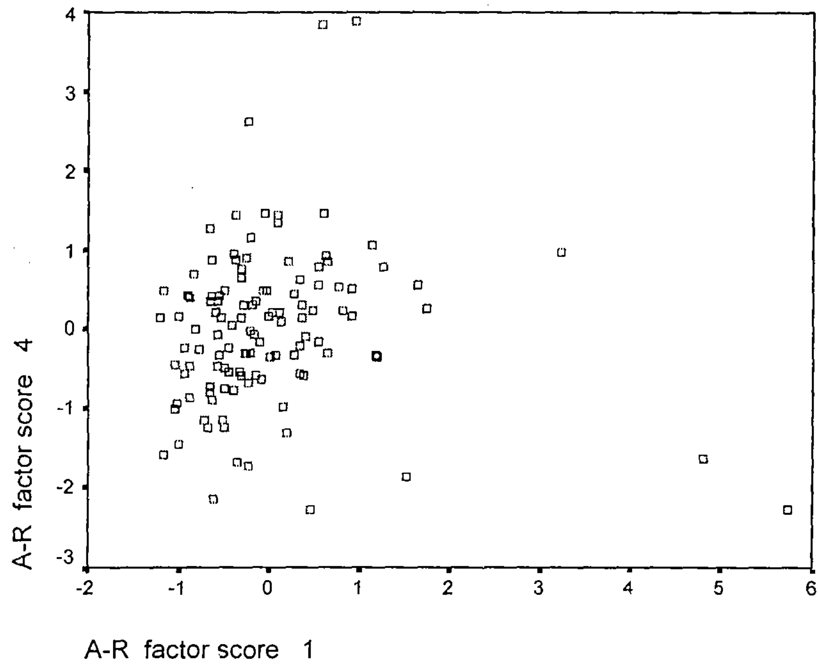


Fig. 4.7.4.10. Relationship between second and third principal components showing the distribution of neem trees on the basis of PCA analysis of tree architectural characteristics

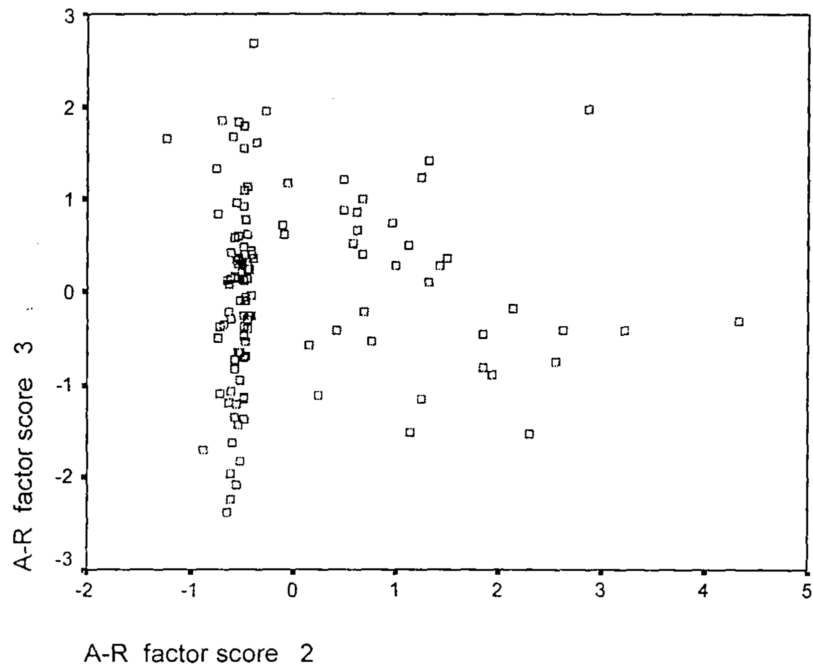


Fig. 4.7.4.11. **Relationship between second and fourth principal components showing the distribution of neem trees on the basis of PCA analysis of tree architectural characteristics**

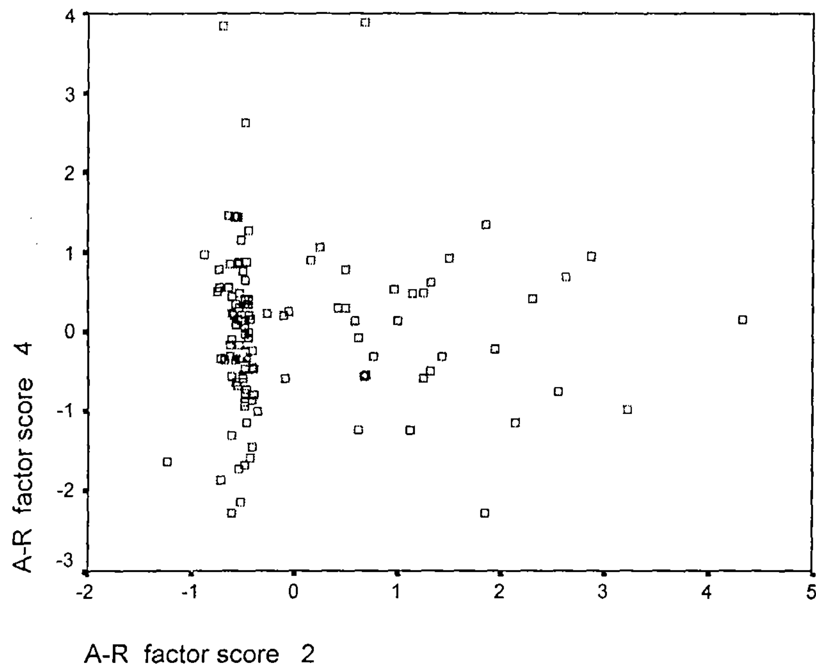
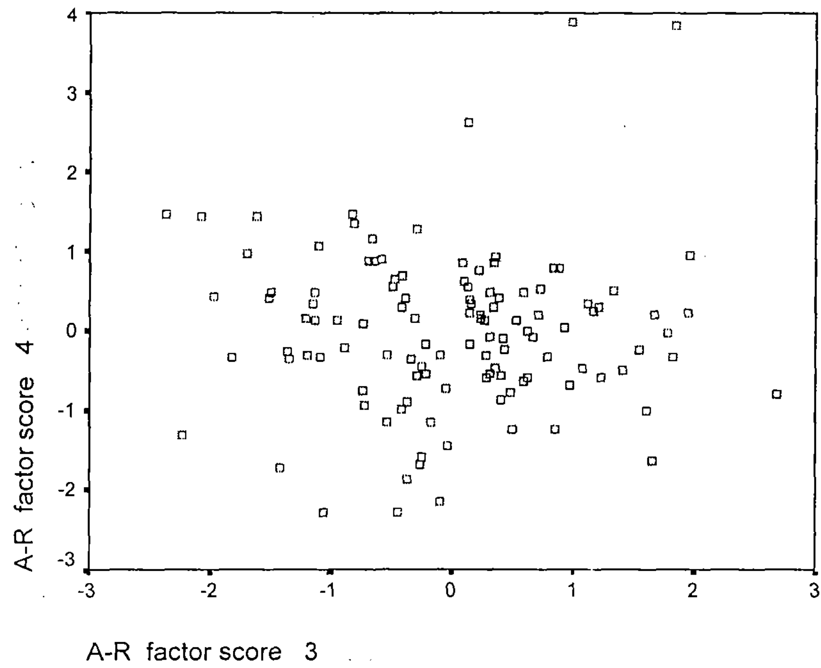


Fig. 4.7.4.12. **Relationship between third and fourth principal components showing the distribution of neem trees on the basis of PCA analysis of tree architectural characteristics**



Similarly, when genotypes were plotted on the basis of factor 1 (representing Clear bole : dbh, Crown Spread : dbh, Height : dbh, and Unforked height : dbh) and factor 3 (representing Clear bole and Clear bole : Height), genotypes 10, 18, and 19 were found to be high for Clear bole : dbh, Crown Spread : dbh, Height : dbh, and Unforked height : dbh and genotypes 61, 93 and 94 among others showed low values for these correlated characteristics. Genotypes 109, 27 and 72 were found to be high for Clear bole and Clear bole : Height and genotypes 12, 55 and 40 showed low values for these two characteristics(Fig. 4.7.4.8.).

Similarly, when genotypes were plotted on the basis of factor 1 (representing Clear bole : dbh, Crown Spread : dbh, Height : dbh, and Unforked height : dbh) and factor 4 (representing Crown Spread : Height), genotypes 10, 18 and 19 were found to be high for Clear bole : dbh, Crown Spread : dbh, Height : dbh, and Unforked height : dbh and genotypes 35 an 93 among others showed low values for these correlated characteristics. Genotypes 6, 26 and 92 were found to be high for Crown Spread : Height and genotypes 11, 106, 5, 10 and 18 showed low values for these two characteristics (Fig. 4.7.4.9.). Similarly, with other plots trees were delineated on the basis of combinations of important correlated variables (Fig. 4.7.10. to Fig. 4.7.4.12).

4.8. Stability analysis

Stability parameters were studied for 20 endocarp weight in some provenance collections of neem (**Table** 4.8.1.1. and 4.8.1.2.). Genotypes KTL6, KTL5, KTL1, and KLK4 showed nearly zero value of $\bar{s}^2_d$ and value of regression coefficient (b) around unity. Therefore, these genotypes are stable for 20 endocarp weight. Rest of the genotypes did not show stability for this characteristic (due to the fact that the value of $\bar{s}^2_d$ is significantly higher than zero). KLK4 showed highest mean amongst the stable varieties followed by KTL6. Genotype KLK1 was having the highest mean but was unstable as indicated by the significant value of $\bar{s}^2$ d. Seven genotypes exhibited above average response (b>1) out of which 4 were stable as indicated by non-significant value of $\bar{s}^2_d$. Four genotypes exhibited below average response (b<1) out of which none was stable as indicated by significant value of $\bar{s}^2_d$. Proportion of predictable genotypes was found to be highest in Kaithal, while lowest proportion of predictable genotypes were found in Hisar provenance.

Stability parameters were studied for oil percent in some provenance collections of neem (**Table** 4.8.2.1. and 4.8.2.2). Genotypes KLK4 and HSR6 showed nearly zero value of $\bar{s}^2_d$ and value of regression coefficient (b) around unity. Therefore, these genotypes are stable for oil percent. Genotypes KLK1, KLK2 and KTL1 showed nearly zero value of $\bar{s}^2_d$ and value of regression coefficient (b) was not near to unity. Therefore, these genotypes are not responsive to environment. Rest of these genotypes did not show stability for this characteristic (due to the fact that the value of $\bar{s}^2_d$ is significantly higher than zero). Amongst the stable varieties,KLK4 showed highest mean amongst followed by HSR6. Five genotypes exhibited above average

Table 4.8.1.1. ANOVA for temporal stability for 20 endocarp weight in 11 provenance collections of *Azadirachta indica*

Source	d.f.	Mean sum of squares
Variety	10	1.119*
Environment	2	1.433*
Variety x Environment	20	0.228*
Total	32	
Pooled Error	60	0.030
Environment x Variety x Environment	22	0.338
Environment (Lin)	1	2.866*
V x E. Lin.	10	0.141*
Pooled Deviation	11	0.286*

Pooled Error MSS for testing Pooled Deviation MSS = 0.01001770

Table 4.8.1.2. Estimates of temporal stability parameters for 20 endocarp weight in 11 provenance collections of *Azadirachta indica*

Genotypes	Provenance	x	b	$\bar{s}^2_d$
KLK4	Kalka	3.577	1.203	0.109
KLK1	Kalka	4.222	0.701	0.326*
KLK2	Kalka	3.720	1.273	1.305*
KLK5	Kalka	3.039	1.584	0.158*
HSR6	Hisar	3.797	0.062	0.185*
HSR1	Hisar	3.469	-0.077	0.450*
HSR3	Hisar	2.148	-0.134	0.135*
HSR2	Hisar	2.874	1.503	0.343*
KTL1	Kaithal	2.459	1.902	0.095
KTL5	Kaithal	2.952	1.435	0.031
KTL6	Kaithal	3.271	1.547	0.010
Pooled mean		3.2299 (±0.3782)	1.0000(±1.0479)	

Table 4.8.2.1. ANOVA for temporal stability for oil per cent in 11 provenance collections of *Azadirachta indica*

Source	d.f.	Mean sum of squares
Variety	10	19.343*
Environment	2	43.079*
Variety x Environment	20	4.543*
Total	32	
Pooled Error	60	0.992
Enviroment x Variety x	22	8.047
Environment (Lin)	1	86.158*
V x E. Lin.	10	4.364*
Pooled Deviation	11	4.294*

Pooled Error MSS for testing Pooled Deviation MSS 0.33060810

Table 4.8.2.2. Estimates of temporal stability parameters for oil per cent in 11 provenance collections of *Azadirachta indica*

Genotypes	Provenance	x	b	$\bar{s}^2_d$
KLK4	Kalka	30.555	1.011	0.785
KLK1	Kalka	26.104	0.108	0.059
KLK2	Kalka	24.372	-0.020	3.064
KLK5	Kalka	27.969	1.617	6.020*
HSR6	Hisar	27.693	1.155	0.114
HSR1	Hisar	26.445	0.720	3.938*
HSR3	Hisar	30.018	0.223	9.953*
HSR2	Hisar	22.243	1.778	10.998*
KTL1	Kaithal	24.054	2.179	8.803*
KTL5	Kaithal	24.688	1.674	11.416*
KTL6	Kaithal	26.702	0.550	0.080
Pooled mean		26.4403(0.1465)	1.000(0.7404)	

Table 4.8.3. ANOVA for temporal stability for endocarp length in 11 provenance collections of *Azadirachta indica*

Source	d.f.	Mean sum of squares
Variety	10	0.037*
Environment	2	0.051
Variety x Environment	20	0.008
Total	32	
Pooled Error	120	0.007

Pooled Error MSS for testing Pooled Deviation MSS 0.00139772

Table 4.8.4. ANOVA for temporal stability for endocarp breadth in 11 provenance collections of *Azadirachta indica*

Source	d.f.	Mean sum of squares
Variety	10	0.016*
Environment	2	0.009
Variety x Environment	20	0.002
Total	32	
Pooled Error	120	0.003

Pooled Error MSS for testing Pooled Deviation MSS 0.00052907

Table 4.8.5. ANOVA for temporal stability for endocarp weight in 11 provenance collections of *Azadirachta indica*

Source	d.f.	Mean sum of squares
Variety	10	0.002
Environment	2	0.004
Variety x Environment	20	0.001
Total	32	
Pooled Error	120	0.002

Pooled Error MSS for testing Pooled Deviation MSS 0.00032476

Table 4.8.6. **ANOVA for temporal stability for seed weight in 11 provenance collections of *Azadirachta indica***

Source	d.f.	Mean sum of squares
Variety	10	0.002
Environment	2	0.003
Variety x Environment	20	0.001
Total	32	
Pooled Error	120	0.004

Pooled Error MSS for testing Pooled Deviation MSS 0.00078924

Table 4.8.7. **ANOVA for temporal stability for endocarp coat in 11 provenance collections of weight *Azadirachta indica***

Source	Sum of squares	Mean sum of squares
Variety	0.008	0.001*
Environment	0.001	0.000
Variety x Environment	0.004	0.000
Total	0.013	
Pooled Error	0.036	0.000

Pooled Error MSS for testing Pooled Deviation MSS 0.00005971

Table 4.8.8. **ANOVA for temporal stability for no. of seeds endocarp^{-1} in 11 provenance collections of *Azadirachta indica***

Source	d.f.	Mean sum of squares
Variety	10	0.006
Environment	2	0.005
Variety x Environment	20	0.005
Total	32	
Pooled Error	120	0.031

Pooled Error MSS for testing Pooled Deviation MSS 0.00624242

Table 4.8.9. **ANOVA for temporal stability for seed weight : endocarp weight in 11 provenance collections of *Azadirachta indica***

Source	d.f.	Mean sum of squares
Variety	10	0.011*
Environment	2	0.051
Variety x Environment	20	0.014
Total	32	
Pooled Error	120	0.013

Pooled Error MSS for testing Pooled Deviation MSS 0.00258138

Table 4.8.10. ANOVA for temporal stability for endocarp length : endocarp breadth in 11 provenance collections of *Azadirachta indica*

Source	d.f.	Mean sum of squares
Variety	10	0.270*
Environment	2	0.005*
Variety x Environment	20	0.018
Total	32	
Pooled Error	120	0.020

Pooled Error MSS for testing Pooled Deviation MSS 0.00405219

response (b>1) out of which 2 were stable as indicated by non-significant value of $\bar{s}^2_d$. Five genotypes exhibited below average response (b<1) out of which two were stable as indicated by non-significant value of $\bar{s}^2_d$. Proportion of predictable genotypes was found to be highest in Kalka, while, lowest proportion of predictable genotypes were found in Hisar provenance.

Analysis of variance for stability was performed in **11** provenance collections of neem for endocarp length (**Table** 4.8.3.). endocarp breadth (**Table** 4.8.4.), endocarp weight (**Table** 4.8.5.), seed weight (**Table** 4.8.6.), endocarp coat weight (**Table** 4.8.7.), number of seeds endocarp^{-1} (**Table** 4.8.8.) seed weight : endocarp weight (**Table** 4.8.9.), endocarp length : endocarp breadth (**Table** 4.8.10.). Genotype and environment interactions for all these eight characteristics were found non-significant which suggested that genotypes have no interaction with environment for the given characteristic.

4.9. Tissue culture studies

The present study was designed to see the efficiency of mature seeds and other vegetative explants (axillary buds, shoot tips and leaves) for shoot regeneration in different media and also establish callus to explore the possibility of *in vitro* production of azadirachtin.

During incubation in dark, segmented seeds started developing callus growth within 18-40 days in all the media but the callusing intensity varied widely from very low to very high. After initial incubation in dark for 30 days, white coloured minute multiple shoots (5-20 per callus) and shoot primordia were observed on some of the media. Highest number of shoots (10-15) was observed in N2 (2mg l^{-1} BA) medium where the cultures were grown on N8 (0.5mg l^{-1} BA + 90g l^{-1}) medium during first subculture. On the other hand, when the cultures were grown only on N2 medium the number of shoots declined drastically (**Table** 4.9.1.). The shoot primordia and multiple shoots could be elongated upon transfer to fluorescent white light.

N1, N4 and N12 were found to provide higher number of shoots per callus (**Table** 4.9.2.). Regenerated shoots were kept on MS medium containing 2mg l^{-1} NAA; 2mg l^{-1} IBA+0.5mg l^{-1} Kin., and 2mg l^{-1} IAA for the purpose of rooting, however no shoot could be rooted. Vegetative explants (axillary buds, shoot tips and leaves) from a mature tree of Hisar provenance were evaluated for callusing in MS medium supplemented with 0.5 mgl^{-1} NAA and 1.0 mgl^{-1} BAP. Callusing could be obtained in 80 per cent of axillary buds and shoot tip explants with in 15-20 days of culture. Leaf explants started callusing within 20-45 days of culture and only 20 per cent of explants showed callusing. Callus cultures could be maintained by frequent sub-culturing in the same medium at an interval of 25-30 days.

Callus cultures produced from axillary buds were analysed for production of azadirachtin after 40 days of incubation. In N6 medium the amount of azadirachtin produced was 0.00027 per cent and in N15 medium a concentration 0.00028 per cent azadirachtin was recorded.

Table 4.9.1. Callus growth and shoot regeneration in mature seed explants of *Azadirachta indica* in different culture media

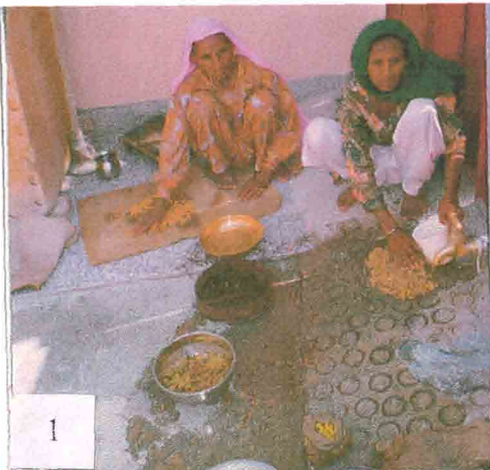
Medium used for		Proportion of explants		Number of shoot callus^{-1}		Number of shoots primordia callus^{-1}		Callus intensity	Callus morphology and comments
First culture	Second subculture	showing callus growth (%)	showing shoot regeneRation (%)	Range	Mean	Range	Mean		
N8	N2	100	30	10-15	11.5(±1.3)	-	-	++	dark brown
N2	N2	100	34	1-3	1.5(±0.7)	1-10	4.1(±2.4)	± to +	creamy to dark brown
N2	N14	100	24	-	-	1-10	5.4(±2.3)	±	dark brown
N2	N13	100	-	-	-	-	-	-	dark brown
N2	N16	100	-	-	-	-	-	±	white to dark brown
N2	N11	100	36	-	-	2-4	-	±	dark brown
N2	N10	100	21	-	-	10-15	12.2(±1.8)	±	dark brown
N4	N2	100	70	1-2	0.9(±1.1)	-	-	++	light brown
N4	N12	100	-	-	-	-	-	+++ to ++++	creamy
N4	N11	100	10	-	-	20-25	22.8(1.9)	++	light to dark brown
N1	N4	100	40	1-4	1.7(±1.1)	-	-	+	light to dark brown
N16	N4	100	-	-	-	-	-	±	light to dark brown
N16	N2	100	-	-	-	-	-	±	dark brown

± = very low; + = low; ++ = moderate; +++ = high; ++++ = very high

Table 4.9.2. Effect of different media on callus growth and shoot regeneration in mature seed explants of *Azadirachta indica*

Medium used for		Proportion of		Mean number of shoots per callus	Callus intensity	Callus morphology
First culture*	Second subculture**	explants showing callus growth (%)	explants showing shoot regeneRation (%)			
N2	N12	100	100	12.2(±1.7)	++	light brown
N2	N4	100	100	16.0(±1.1)	+	light brown
N2	N1	100	100	2.0(±1.2)	++	light brown
N4	N4	100	100	10.4(±1.4)	++	dark brown
N1	N1	100	100	7.3(±1.3)	+	light brown

+ = low; ++ = moderate; *dark incubation at 28±2°C; **transferred to light at 23±2°C after 30 days of incubation in dark at 28±2°C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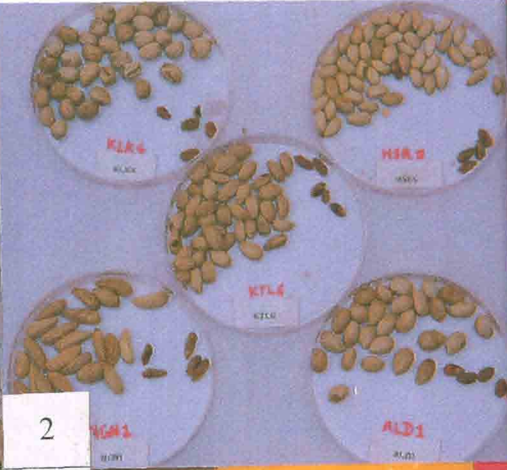
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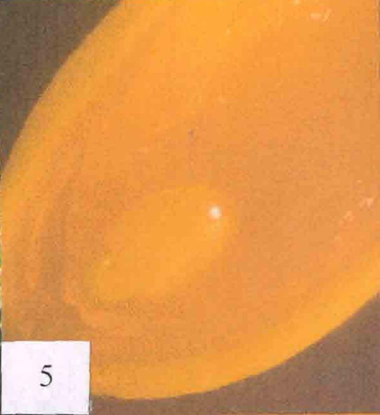
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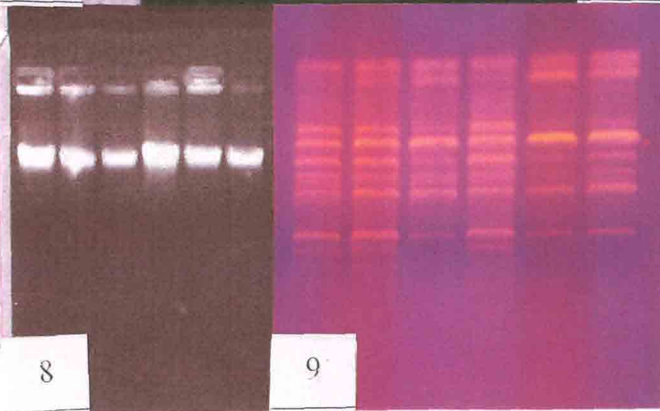
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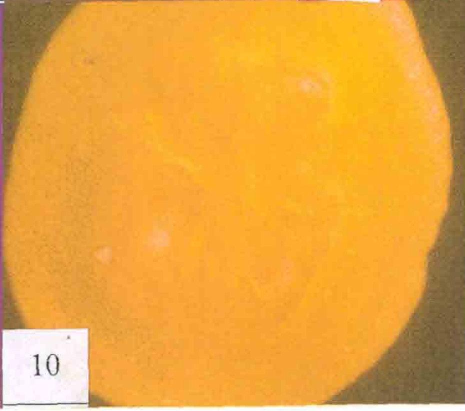
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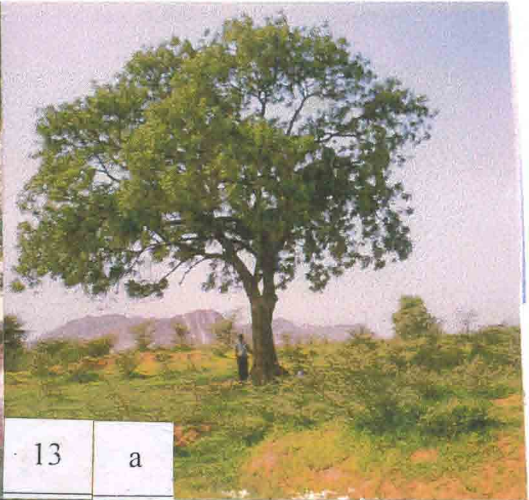

13 a

b

c

d

e

14

Fig. 1. Processing of fruits (drupes) of neem collected from different provenances

Fig 2. Variability with respect to endocarp morphology in different provenance collections of neem

Fig. 3. Method of excision of explants from vegetative shoots of neem tree for the purpose of tissue culture

Fig. 4. Off season flowering (July, 1998) in a tree at Jodhpur, Rajasthan

Fig. 5. Development of embryo in immature seed of neem, set in off season (October, 1998) at Hisar, Haryana

Fig. 6. Off season (October, 1998) concomitant flowering and fruiting in a neem tree at Hisar, Haryana

Fig. 7. Shoot regeneration from callus developed from axillary bud explants in neem

Fig. 8. High molecular weight DNA extracted from young leaves of six neem trees

Fig 9 Typical RAPD profile of different pooled DNA samples of neem trees

Fig. 10. Cross sectional microscopic view of trilocular ovary of neem flower

Fig. 11. Microscopic view of multiple shoot regeneration from axillary bud explants in neem

Fig. 12. Variability with respect to bole forking behaviour in neem trees at Hisar

Fig. 13. Variability with respect to crown shape: (a) mushroom shaped crown of a neem tree from village Nalwa, (District Bhiwani, Haryana; (b) conical shaped crown of a neem tree from Hisar; (c) oblong shaped crown of a neem tree from village Batamandi, (District Sirmour, Himachal Pradesh); (d) cylindrical crown due to plastic behaviour of a young neem tree from Fatehabad, Haryana; (e) round shaped crown of a neem tree from village Dhani Gopal (District Fatehabad, Haryana).

Fig. 14. Off-season (October, 1998) fruit setting in neem at Hisar, Haryana

5. Discussion

Genetic diversity is a fundamental tenet of conservation ethic, and is an important consideration when managing forest stands, ecosystem and landscapes (Libby *et al.*, 1997). Genetic diversity is the most important component of biodiversity and is the foundation of ecosystem stability and forest sustainability because it provides raw material for evolution, survival and adaptation of the species, especially under changed environmental conditions. Genetic diversity needs to be assessed in long term genetic resource collections, in breeding populations, in seed orchards or planting materials producing populations and in production populations (Muona, 1990). For ecologically and socially sustainable forestry, monitoring of genetic diversity in forest trees is also important. Thus genetic diversity includes all levels of variation harboured by plants, from morphological, physiological and biochemical traits. The type and amount of diversity measured in some provenance collections of neem from India using morphological and biochemical classes of measurement is discussed.

Even after attaining industrial importance neem tree has so far not received adequate attention from the point of view of genetic improvement as well as management. A little effort has been made in the past to improve this tree for the economical characteristics like azadirachtin, oil, fatty acids, seed production, etc. Breeding methodology, also has gone practically no further than simple selection on individual plant basis from available germplasm. Further, it is grown across a broad agroclimatic range where there is marked fluctuations in weather conditions, which results in quite unstable and poor yields. It is in this context that there is an urgent need for multidisciplinary research program aiming at genetic improvement. The present study was, therefore, conducted to study the available variability in neem with respect to morphological and biochemical characteristics.

In the present investigation, 221 germplasm accessions of *Azadirachta indica* from nine states of India (Haryana, Rajasthan, Delhi, Himachal Pradesh, Uttar Pradesh, Panjab, Karnataka, Tamil Nadu and Maharashtra) were studied for all or some of the following aspects viz., tree architecture, endocarp morphology, seed viability, provenance evaluation through growth of seedlings in nursery conditions; oil content, fatty acid profile, protein content and azadirachtin content of seeds; temporal stability analysis and divergence analysis. Extensive survey of neem trees was made during July-August, 1998. All the agroclimatic zones of Haryana state and adjoining areas (parts of neighbouring states) were covered under the survey. Considerable variation was recorded both within and between provenances in trees surveyed in Haryana and adjoining areas with respect to quantitative and qualitative tree architectural parameters and their linear associations.

Phenotypic variation can be estimated by ocular comparison of trees or tree measurement for different morphological traits. A large number of morphological, physiological and chemical traits have been shown to be variable either by phenotypic surveys in natural stands or plantations or by progeny tests (Zobel *et al.*, 1960; Dorman, 1976; Khosla *et al.*, 1980 and Bangarwa *et al.*, 1990). Various methods are used for the selection of superior phenotypes (Rudolf, 1956; Ahsan, 1970, Ledig, 1974 and Kotwal, 1983). In the trees under survey, stem form ranges from crooked or forked to nearly straight. Trees with mushroom to cylindrical crown shape are found. Trees are also found to have completely straight stem and low branch angle. Some trees are found to have unusual character of fruits turning brown on the tree itself.

Besides, straightness, main stem : total height and clear bole: total height coupled with low value of crown spread: dbh are desirable for an ideal agroforestry tree (Khosla, 1985; Bangarwa *et al.*, 1990; Singh and Bangarwa, 1996). Association of different morphological characteristics of trees in all the 14 sites combined was studied. Diameter at breast height (dbh) is positively and significantly associated with basal girth, height, crown spread and crown spread : height; and negatively and significantly associated with and height : dbh, clear bole height : height, unforked height: height, unforked height and crown spread : dbh. However, for individual provenances degree of association among various characteristics differed largely. Rank correlation of apical dominance index with branch angle index and fruiting intensity index is found to be negative and significant. The association of crown shape index with apical dominance index and fruiting index is found to be negative and significant. Therefore, observations on qualitative parameters can help in selection of trees for different applications like seed production, agroforestry, etc.

Devender Singh (1994) selected ten neem trees were keeping in view the straightness, higher proportion of main stem and clear bole height. In the present study, highly divergent trees were identified for combination of important tree architectural parameters with the help of principal component and factor analysis. Trees with desirable combinations of clear bole : height, and unforked height : height have been selected based on principal factor analysis. In the present study, of 136 trees studied, 58 candidate plus trees (CPTs) for agroforestry, 45 CPTs for seed production as well as agroforestry and 47 CPTs for seed production were marked. Multiple regression equations (with very high level of goodness of fit) based on the available tree architectural parameters were developed for predicting values of clear bole, unforked height and ratio of unforked height and height. Plus trees have been reported to be selected by various researchers (Kedharnath, 1967; 1982b; Rai, 1986; Emmanuel and Bagchi, 1988) for different species, such as *Tectona grandis, Bombax ceiba, Dalbergia sissoo, Gmelina arborea, Pinus roxburghii, Santalum album.*

Bangarwa (1993) reported in *Dalbergia sissoo*, that straightness varied with different provenances and with the trees within provenances. Stem straightness is a clear indication of high wood quality, easy handling in processing and subsequent use. Height: dbh ratio, crown : dbh ratio, main stem : total height ratio and clear bole : total height ratio also had sample variation in different provenances. Crown : dbh ratio is comparatively higher in arid region populations. In the present investigation, Multiple regression analysis of clear bole height : height, clear bole, unforked height : height and unforked height on all other tree architectural characteristics was performed in 4 provenances individually and by taking all provenance together. In individual provenances and all the 14 sites combined, coefficients of determination (R^2) are very high for all the analyses pointing to very high level of goodness of fit of the regression line. This clearly shows that the parameters taken to study the tree architecture are able to explain the variability for the important traits.

While reviewing variation in Indian tree species, Dogra (1981) emphasized on survey of phenotypic variation for silvicultural characteristics of tree species in their naturally distributed ranges. Kackar *et al.* (1986a) reported variation for morphological characters of *Prosopis cineraria* in natural stands. Jindal *et al.* (1987a) in their studies of natural population of *Tecomella undulata* reported wide variation for various morphological characters. The association between different tree architectural parameters is found to vary from site to site. Khosla *et al.* (1980a) observed strong positive correlation of height with diameter in *Populus ciliata*. Khan (1983) in his study of phenotypic variation in *Pinus wallichiana* in selected areas of Jammu & Kashmir and Himachal Pradesh reported that height in both the states was positively and significantly correlated with diameter, clear bole, and crown width. In the present study also height is positively associated with diameter, crown spread and clear bole

Statistically significant differences (both within and between populations) are observed for most of the endocarp morphological characteristics studied in 43 trees belonging to 12 provenances. Association of endocarp morphological characteristics is studied using simple correlation. Contrasting association behaviour is observed for most of the traits across individual trees. Computed as a ratio of standard deviation of each character to the corresponding entire data mean and expressed as percentage, Coefficients of variation (CV) is useful to compare different characters measured in different units. It is also useful to compare same character in different groups of populations with different sample size, mean and variance.

In the present study, variations in coefficients of variation are observed between regions and within each region for a number of traits ranging from morphological to biochemical. The different levels of regional variability of a particular

character could be due to differences in forces of selection and/or differences in the intensity of a particular selecting force.

A primary goal of many genetic studies is the estimation of variance components associated with individual traits and of covariance terms between traits. Heritability, the proportion of variation in a trait that is contributed by average effects of genes, may be calculated from variance components. The heritability of a trait gives an indication of the ability of a population to respond to selection, and thus, the potential of that population to evolve (Lande, 1982, Mousseau and Roff, 1987). Genetic parameters were studied for endocarp morphological characteristics. Considerable within and between population variation is recorded with respect to all the genetic parameters. For most of the traits heritability varies from low to high. While for number of seeds endocarp^{-1}, the heritability could not be ascertained in most of the provenances. Moderate to high estimates of genotypic coefficient of variation, broad sense heritability and genetic advance obtained for endocarp weight and seed weight, indicates high level of genetic variability and potential of improvement for these traits through selection. Major portion genetic variability is explained by within population genetic variability. High level of correspondence between GCV and PCV indicate that genetic factors explain the phenotypic variability to a very high level in all the provenances and environmental effects were having little or uniform pattern of effect on the phenotype. This is also evidenced by high level of temporal stability of the morphological traits of endocarp in different provenances monitored for three years with the help of model described by Eberhart and Russel (1966) .

These results indicate that the material under study provide ample scope for improvement through selection in these characters. The above findings are in close agreement with those of Salazar (1986) in *Gliricidia sepium*, Shiv Kumar and Banerjee (1986) in *Acacia nilotica*, Kackar *et al.* (1986b) in *Prosopis cineraria*, Bisla and Daulta (1988a) in *Zizyphus mauritiana* and Bagchi and Sharma (1989) in Sandal; Dhillon (1992) in *Dalbergia sissoo*. Broad sense heritability values ranges from 1.82 per cent (number of seeds endocarp^{-1}) to 82.07 (endocarp length). High broad sense heritability estimates for seed length : seed breadth (81.07 per cent) are observed. While for endocarp coat weight, endocarp weight and seed breadth moderate broad sense heritability are observed i.e., 62.99, 68.47 and 69.81 per cent respectively, for seed weight : endocarp weight low estimates (31.99 per cent) are obtained. It indicated that endocarp characters with high estimates are not much influenced by the environment. In general, genotypic correlations are higher in magnitude than the phenotypic ones, which gives an idea that there is inherent association between various characters.

Strong directional, and to some degree stabilizing, selection usually erodes only additive genetic variance while not affecting dominance variance. Consequently, traits

closely associated with fitness should exhibit high levels of dominance variance. Traits that were known to have been subject to intense directional selection (morphological traits for domestic species) had significantly higher dominance estimates than did traits that were assumed not to have been subject to strong selection (morphological traits for wild outbred species) (Crnokrak and Roff, 1995). Strong directional selection (and to a lesser degree, stabilizing selection; Lee and Parsons, 1968; Lacy, 1987) is predicted to erode additive genetic variance and, subsequently, decrease the heritability of a trait (Felsenstein, 1965). As a consequence, the response to selection will be reduced (but the reduction in response may result from other factors: see Falconer (1989) and Lande (1988). Traits for a population at equilibrium are predicted to have low additive genetic variance as it is assumed that selection has moulded them to an optimum (Hegmann and Dingle, 1982) for an alternative explanation). Because they are assumed to be subject to intense selection, traits most closely associated with fitness are predicted to have low heritabilities and subsequently relatively high dominance components (Wright, 1929). A number of studies (Mousseau and Roff, 1987) have shown that life history traits, which are assumed to be closely connected to fitness, have low heritabilities while morphological traits, which are assumed to be more distantly related to fitness have high heritabilities(behavioural and physiological traits are usually intermediate to the above two types of traits). Because selection usually erodes only additive genetic variance (Lynch. 1994), although changes in gene frequency may also cause changes in non-additive variance as well, one would predict that the opposite pattern should be found in terms of dominance variance: life history traits should have relatively high levels of dominance variance while morphological traits should have low levels of dominance variance. In addition to eroding additive variance, selection is also expected to act directly on genetic dominance, resulting in a further relative increase of dominance variance to total genetic variance (Lynch, 1994).

Before deciding on particular breeding design for estimating heritabilities, it is critical to take into consideration the type of traits being measured to obtain accurate estimates (Crnokrak and Roff, 1995). Fisher postulated that differences between heritabilities among traits categories are the result of differences in additive genetic variance, but recent theoretical considerations by Price and Schluter (1991) contest this idea. They proposed that the reason life history traits have low heritabilities is because such traits are composites of many morphological, physiological and behavioural traits. Because life history traits are composites of multiple under lying traits, they are subject to numerous sources of environmental variation which in turn inflates phenotypic variance, thus decreasing the heritability. Low heritability estimates for number of seeds endocarp^{-1} in the current study may be considered as an indication of the fact that this trait is under intense control by morphological, physiological and behavioural characteristics and is

related to some fitness traits or is itself a fitness trait. Infact, inbreeding is a fundamental factor determining evolution of any species. Efforts have been made to estimate inbreeding from the empty seed percentages because of presence of embryonic lethals (Koski, 1971; Kundu, 1999). Empty seed formation in neem observed in the present study is certainly caused by additional factors, such as reduced pollen viability or an environmental stress (drought or cold). The presence of the empty endocarps, as well as fewer seeds per endocarp than expected, indicates that inbreds are selected against. In contrast other endocarp morphological traits like seed weight, endocarp weight, and endocarp length exhibit high heritability estimates possibly pointing to the fact that these characteristics are not related to fitness and survival of neem and are not under intense selection. Likewise, there are pronounced differences in heritability estimates among provenances for most of the provenances, probably suggesting either sampling error or variation in contribution of the respective traits to fitness due to local environmental factors and / or genotypic factors.

The high heritability estimates generally enable the plant breeder to select the desirable plants based on the phenotypic expression for specific improvement. In fact, the information on the estimates of heritability along with those of expected genetic advance (in per cent of mean) are more helpful than that of heritability alone, in predicting the resultant effects of selecting the best individuals (Johnson *et al.*, 1955). The genetic advance. however gives an indication of the expected genetic progress for a particular trait under appropriate selection procedure. It is, therefore necessary that for effective improvement of different traits, all those genetic parameters are considered together. High magnitude of heritability coupled with high genetic advance (per cent of mean) are recorded for the characters under study. Therefore, an inference can be drawn that all traits are quite amenable to selection. Little differences between GCV and PCV values coupled with high heritability estimates for seed weight and endocarp weight indicate that these two characters are highly heritable and that differences between accessions are real.

Correlation among characters are of interest to plant breeders because it helps in the identification of easily measured characters that could be used as indicators of more important (but more complex to score) characters. They are also useful in pointing out the possibility and limitation of simultaneous selection of desirable characters (Amurrio *et al.*, 1993). Endocarp morphology traits are quantitative traits and hence may be controlled by several loci. The more loci involved, the greater the chances of linkages. Such linkages could be broken through the crossing of segregating families. Since crosses are not available yet, other breeding strategies should rather be considered. Examination of other local germplasm as well as diverse germplasm from different provenances would throw more light on the influence of total lead area on seed sizes and

yield. Knowledge of the magnitude and the direction of correlation coefficients between quantitative characters would be beneficial for the interpretation of the patterns of variation. Within the limit of experimental error and environmental effects, high correlation coefficients between characters may show that the characters share some common element of genetic control (i.e., pleiotropy, linkage, etc.) between genes or else from independently controlled characters responding similarly to geographic variation in selection pressures (Thorpe, 1976; Bekele, 1984). The between region correlation coefficient between the characters measures the concordance of their patterns of regional variation, while the within region correlation coefficient between the characters measures both the association arising from genetic factors but is not affected by regional variation (Thorpe, 1976).

This study shows significant positive correlations intra-regionally for some character combinations, it appears that common genetic control is playing a role in bringing about correlations between the various characters. However, it appears that similar response to regional variation is playing a greater role than common genetic control as shown by the many more significant and moderate to high correlation coefficients inter-regionally than intra-regionally. Genotypic correlations are important in selection when one aims at changing two or more characters simultaneously. Statistically significant genotypic and phenotypic correlations are observed between characteristics like endocarp length, endocarp weight, seed weight in some of the populations and all the populations combined suggesting the possibility of enhanced correlated response upon indirect selection for these morphological characteristics.

The direction of correlation coefficients at the genotypic level were generally same as the phenotypic ones. However, the estimates in respect of the former were slightly higher than those of the latter in most of the cases, which is in agreement with the result of earlier works in trees (Dhillon, 1992; Bangarwa, 1993). This envisages a strong inherent association among the various characters but the phenotypic expression of the correlation is lessened under the influence of environment.

The environmental correlations, as worked out from the error variance and co-variance components, include mainly the effect of soil heterogeneity, cultural irregularities and chance error occurring while conducting the experiment (Sikka and Maini, 1962). Such factors cause a change in plant behaviour and may be explained in terms of physiological adjustments. Environmental correlations are generally high in the present study for morphological traits of endocarp indicating less effect of environment in determining of the fate of variation for the given traits.

Seed yield is a quantitative character, which is largely influenced by the environment and hence has a low heritability (Johnson, 1989). As a result, the response to direct selection for seed yield may be unpredictable, unless there is good control of

environmental variation. Plant breeders are seldom interested in one character and therefore, there is the need to examine the relationships among various characters, especially between seed yield and other characters. As the number of independent variables influencing a particular dependent variable is increased a certain amount of interdependence is expected. In such situations, correlations may be insufficient to explain the associations in a manner that will enable breeders to decide on either a direct or an indirect selection strategy (Singh and Singh, 1979).

Path-coefficient analysis (Dewey and Lu, 1959) provides a method of separating direct and indirect effects and measuring the relative importance of the causal factors involved. Several researchers have used this method to assess the importance of the components of yield. Seed yield, being a complex character, is the cumulative and interactive effect of a number of component traits. Direct selection for seed yield per se generally result in low genetic gain because of its low heritability in general dictating plant breeders to realize the importance of component traits. However, because of their complex interactive nature with each other, information on the association of these component traits with yield and also among themselves is of utmost importance. The former can be modified through recombination but the latter may not be easy to overcome and improvement in yield becomes impossible without bringing improvement in component characters. The inclusion of all the component traits in selection scheme is impractical because of obvious reasons and here the knowledge of association of various characters comes to the rescue in formulating an effective and efficient selection scheme.

Separation of the genotypic correlation coefficient into components of direct and indirect effects was done using path coefficient analysis for seed weight and ratio of seed weight and endocarp weight. Seed weight is both directly and indirectly affected by endocarp weight and endocarp coat weight in most of the provenance collections. The negative indirect effect of endocarp coat weight in most of the provenances is not enough to significantly reduce the direct positive relationship between endocarp weight and seed weight. Ratio of seed weight and kernel weight is directly and indirectly affected by all the morphological characteristics of endocarp in all the provenance collections under study. Therefore selection for seed weight is much easier as compared to seed weight : endocarp weight as the former is little influenced by characters like endocarp length and breadth.

Pronounced negative effects of some of the traits are visible on dependent characters in some provenances. Such negative relationships are not universal and hence there is a need to collect and examine diverse germplasm variants with a with a more desirable relationship between seed weight and other seed traits.

Six leaf morphological characteristics of thirteen trees of Hisar provenance were studied. Statistically significant differences are observed for most of the characteristics in

many trees. There is positive association among all the leaf characteristics though only some of the associations were significant. A study of the herbarium at New York Botanical Gardens and Panjab University revealed that the neem trees from arid areas at the foothills of the Himalayas in general have narrow leaflets with sharp dentation in their serrate margin, compared to the trees from a humid climate which have comparatively broader leaflets with a less sharp serrate margin (Puri, 1999). Srivastava *et al.* (1993a) reported variation for leaf morphology (length and breadth) in 15 randomly selected *T. arjuna*. Chauhan (1987), while studying twenty eight clones of *Populus ciliata* collected from their geographical distribution in the western Himalayas, found high range of diversity with regard to leaf area among different provenances. Surendran and Chandrasekharan (1984) studies heritable variation and genetic gain estimates in half sib progenies of *Eucalyptus tereticornis*. Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability estimates and genetic advance as percentage of mean were worked out for eight characters studies in 35 plus trees. The heritability estimates for number of branches, leaf breadth and leaf length : breadth ratio were consistent at different stages of growth.

The adaptation process may be characterized as physiological, genetic or a combination of these two. Whatever the process, there is ultimately a genetic mechanism that drives adaptation (Tigerstedt, 1984). Breeders recognize their need for greater understanding of the gene-controlled physiological processes that produce growth and commercial yield. Physiologically based approach for tree improvement could secure further genetic gains (Donald, 1991). Erickson (1991) suggested that basic research is required on physiological genetics for facing new challenges especially changing environmental conditions. Allen and Meyer (1998) emphasized that results from common garden experiments, suggest that germination differences have a strong genetic basis both among populations and among plants in a population. Seed viability was tested in the seeds collected from 21 trees representing 6 provenance collections (Haryana, Delhi and Rajasthan) after 6 months of storage at 25±2°C. Within and between provenance variation with respect to seed viability in different provenance are observed. Viability of seeds appears to be negatively affected by the oil content of seeds rather than fatty acid profile. Regression equation for linear regression of seed germination on seed oil content gives high coefficient of determination (R^2 = 0.784) pointing to very high level of goodness of fitness of the regression line.

Large seeds in sorghum have been reported to be associated with increased germination percentage, improved stand establishment and increased grain yield (Kwolek *et al.*, 1986; Kabede and Menkir, 1987). Available evidence also indicates that it is possible to improve seed size in sorghum by mass selection (Lothrop *et al.*, 1985).

For several wild species whose seedlings establish in spring, seed populations show relevant variation that can be correlated with habitat conditions. Populations from severe winter sites, where major risk is frost, tend to have a long chilling requirements or to germinate very slowly at low temperatures. Populations from warmer sites, where the major risk is drought, are non-dormant and germinate very rapidly under these same conditions. Seed populations from intermediate sites exhibit variation in dormancy levels, both among and within plants, which spreads germination across a considerable time period (Allen and Meyer, 1998).

The ultimate fate of seeds under natural (wild) conditions depends on their ability to time germination so that survival of seedlings is maximized. Mechanisms that regulate germination timing through seed dormancy are an important aspect of species life history and ecology (Venable, 1989; Meyer *et al.*, 1995, 1997). Many plants have a wide distribution range encompassing a variety of ecological habitats. For such species, contrasting selection pressures associated with different sites can result in highly variable dormancy patterns (Allen and Meyer, 1998). In the present investigation, only germination behaviour is recorded and dormancy pattern could not be ascertained. The wide variation in the viability pattern may be due to physiological reasons (higher oil content, which is found to be associated with low germination) or some sort of dormancy pattern (particularly in long lived seeds).

Neem seed is tropical recalcitrant in nature and the viability of the seed falls off rapidly after two weeks (Dent, 1948). Tropical recalcitrant seeds can be stored for comparatively longer duration by maintaining high seed moisture content and a certain amount of gas exchange. But these seeds are sensitive to low temperatures. In considering seed storage as a mean of conserving germplasm, both the characteristics of tree seed and the technology of storage conditions needs to be understood. Seed moisture content and storage temperature are the major environmental factors affecting the preservation of stored seeds, with seed moisture content usually more critical than temperature (Justice and Bass, 1988). Chaisurisri *et al.* (1986) obtained success in storing neem seeds in a cotton bag at 15°C. Seeds were dried down to a moisture content of 46.18 per cent before storing. Sacande *et al.*(1998) conducted a multifactorial study of conditions influencing longevity of neem seeds. There was hardly any difference in storage behaviour between seed lots, regardless of provenance. Seeds originating from mature yellow fruits had greater longevity than seeds from green or brown fruits.

Provenance evaluation of different provenance collections was done in nursery after a growth period of 3 months. Seeds from 27 trees representing 17 provenance collections were considered. Significant differences among genotypes are found. Morphometric traits such as leaflet ratio, shoot : root ratio, leaf number, and collar diameter have been shown to exhibit clinal variation in neem indicating adaptation to the local environmental

conditions (Kundu, 1999). Seedling length is found to be positively and significantly associated with seed weight. Similar reports are available in number of plants where the seed size influenced the germination percentage positively (Jindal *et al.*, 1999b; Swaminathan and Sivagnam, 1999; Borges *et al.*, 1998) and negatively (Gunasekaran, 1999) both, and affects the seedling growth positively (Basave Gowda and Gowda, 1999; Nizam and Hossain, 1999; Castro, 1999). It is generally believed that larger seeds give rise to seedlings with better performance. On the other hand, the size that a seed reaches is genetically determined by at least two different traits - the genetic variability of the developing embryo, and the genetic variability of the maternal plant, thus the relative contribution of these two traits affect seedling performance by influencing seed size (Castro, 1999). In *Pinus sylvestris* it was observed that the initial growth of the shoot was positively correlated with seed mass. However, after one growing season, seed mass had no effect. Thus, first-year seedling performance seems to be a maternal trait indirectly associated with seed size. This warrants a careful selection of good quality seeds for mass propagation, as it will help in better survival and growth of seedlings in the first year.

Lopez Mata (1987) reported that seed size strongly influenced initial seedling size and the relative size of seedling roots in *Brosimum alicastrum*, which increased in response to habitat dryness and geographic origin. The results suggested that this tree employs an adaptive strategy in response to water deficit during dry seasons in initial phase of seedling establishment. It is noted that collar diameter had positive association with seedling height. These findings confirmed the results of (Dhillon, 1992, Gupta and Patil (1988) in *Leucaena leucocephala,* Shiv Kumar and Banerjee (1986) in *Acacia nilotica,* Salazar (1986 and 1989) in *Gliricidia sepium* and Sindhu *et al.* (1990) in *Santalum album*. Appreciable differences between provenances in respect of seedling height were recorded have been reported Khalil (1985) in *Picea glauca*, Huang (1989) in *Acacia auriculiformis* and Hussein (1989) in *Cordia alloidora*). From the above results, it is suggested that any variation in the mixed population which are provided within identical environment, are governed by genetic factors. Hence, these variations can be exploited in future breeding programmes, thus, eliminating high cost and avoiding the time consuming process which are main drawbacks of direct selection in tree improvement.

Plants can respond to environmental conditions not only by adjusting their own phenotypes but also by altering those of their offspring through changes in the quantity and quality of seed provisioning, and in the structure or biochemistry of the seed coat and fruit tissues. Studies of these cross-generational effects rigorously distinguish environmental from genetic causes of offspring variation and focus on ecologically relevant propagule and seedling traits rather than on propagule mass alone (Mazer and Gorchov, 1996; Sultan, 1996; Lacey *et al.* 1997; Thiede, 1998). Although the mechanisms are not well understood (Lacey *et al.*, 1997). Offspring structure,

development and morphology can be influenced in remarkably specific ways by parent environment. Certain species respond to contrasting growth conditions by changing the structure or thickness of the seed coats or pericarps while maintaining the quantity and quality of the embryo and endosperm tissues that determine initial seedling size (Sultan, 1996; Lacey *et al.*, 1997). The progeny of nutrient-deprive plants can increase root biomass allocation compared with seedling offspring of plants give ample nutrients (Wulf and Bazzaz, 1992). Similarly, the offspring of light-deprived plants can reduce root extension relative to shoot growth compared with offspring of genetically identical plants grown at high light intensity (Sultan, 1996). Such specific plastic changes to seedling growth patterns might allow offspring to maintain critical aspects of function such as root uptake capacity even if the initial seedling biomass is reduced by parental resource deprivation. Seedling offspring or nutrient-deprive *Polygonum* plants produced thinner roots that extended downward more rapidly into the soil, resulting in root systems that were as long as seedlings form nutrient-rich parents in spite of their lower mass (Elmendorf and Sultan, unpublished). Plasticity patterns for ecologically important traits often vary genetically within natural populations, which indicates that the genetic potential for the evolution of adaptive plasticity can exist in many taxa (Sultan, 2000).

The genetic mechanisms that underlie plastic response are as yet poorly known (Schlichting and Pigliucci, 1998 ; Pigliucci and Schmitt, 1999 ; Via *et al.*, 1995), although it has become clear that several different mechanisms might be involved in different aspects of plasticity (Scheiner, 1993a; Wu, 1998). These mechanisms are believed to include environmentally dependent regulatory loci as well as non-epistatic loci at which allelic expression varies with the environment (Via *et al.*, 1995). Because plastic responses involve both environmental perception and the production of the appropriate phenotype (which might entail a suit of anatomical, morphological, physiological and other traits), these responses are likely to be influenced by multiple loci (Wu, 1998).

Population differences in both endogenous production and sensitivity to growth hormones might contribute to different patterns of plastic response. (Emery *et al.*, 1994). Species that consist of highly plastic genotype might be ecological generalists (Sultan, 1995), whereas species whose constituent individuals express limited adaptive plasticity might be restricted to narrower, 'specialist' ecological ranges.

In addition to ecological breadth plasticity, might also contribute to a species' invasiveness. Widespread colonizing species are often characterized by high phenotypic plasticity, which should in theory allow them to inhabit diverse new sites without undergoing local genetic adaptation through natural selection. Work on several colonizing species has confirmed that populations across broad geographic and environmental ranges can show remarkably little genetic or morphological differentiation

but instead consist of genetically similar populations of highly plastic genotypes (Williams, et al. 1995; Hermanutz and Weaver, 1996). Thus plasticity might facilitate the rapid spread of introduced as well as native taxa into new ranges without the evolutionary lag time required to adapt to these unfamiliar habitats through natural selection. Plasticity might also contribute to the ability of species to with-stand sudden environmental changes, such as those caused by human disturbance (Sultan, 2000).

Finally, plasticity can influence patterns of evolutionary diversification. If individual genotypes are sufficiently local plastic to produce phenotypes appropriate to different local environments, natural selection will not occur for genetically distinct, locally specialized ecotypes (Sultan,1995; Sultan, 1987). As neem is able to colonize the difficult sites therefore various aspects of plasticity for various morphological and physiological traits should be studied in this species for benefiting the afforestation programmes and production of superior planting material.

The 43 neem genotypes, evaluated in the present study, exhibit sufficient variability for all the traits. This indicates significance of estimation of other parameters for the material under study. Different workers have emphasized the availability of variability in germplasm since long. Finlay (1971) stressed the importance of continuous infusion of new genetic variability in active plant breeding programmes as it is a major asset for initiating a fruitful crop improvement programme. Such a breeding programme should rested not only on the available gene pool, but also on continuous infusion of newer and superior lines to permit fresh genetic variation (Gustafson, 1974).

Morphological variation in tree, seed, leaf traits are easily recognised in neem populations under study. Seed length, seed breadth, seed weight, kernel weight, seed coat weight, number of kernels seed^{-1}, kernel weight : seed weight and seed length : seed breadth, seedling height, and collar diameter are important and easily measured traits for an early evaluation of seed sources. The considerable amount of variation observed in this study suggests that attractive gain could be obtained through early selection (Lambeth *et al.*, 1983; Zobel and Talbert, 1984; Khasa *et al.*, 1995). Assuming that a significant portion of the phenotypic variation is genetic. It would be possible to make selection for any of the characters within a particular region. It is apparent that between provenance variance is greater than between accessions. In some cases observed within provenance variance is greater than the variance for pooled provenances. Since significant variation is found between provenances and between accessions within provenances, it would be necessary to collect from as many provenances as possible and to adequately sample the variable populations from different localities in a region in order to sample the variation.

In neem, thorough studies on morphological variability of endocarp are yet to come out. No consistent geographic / agroclimatic trend in association among seed

morphological traits indicate that other environmental factors (other than altitude / latitude) and/or non-environmental factors might account for the variation for these particular characters. However, some extent of the observed clinal variation provides a guideline for transfer of seed and plant material. Clinal variations are heritable and offer a good opportunity for plant breeding and selection, especially for designing breeding programs. Information on clinal variation could be useful to predict the performance of hybrids based on their parental populations. Information on clinal variation could be useful to predict the performance of hybrids based on their parental populations. In the present study, conspicuous geographical clines for stearic acid and oleic acid are discovered. The modification of the fatty acid profile might have occurred because of differences in day length, light intensity, humidity and thermo-period, as these phenomena are directly related to the latitudinal gradient (Hill *et al.*, 1998). In the arid regions populations have lower linoleic acid, but higher oleic acid content, while populations in humid regions have higher linoleic acid and lower oleic acid. This could be explained by the lower activity of desaturases in regions with relatively higher atmospheric temperature during the fruit setting season. However, arid and semi-arid regions (Bikaner and Hisar) are found to have higher total unsaturated fatty acids resulting in higher values for ratio of unsaturated and saturated fatty acids compared to humid regions in Haryana.

Kundu (1999) attempted to gain a understanding on how geoclimatic factors prevailing at different sites affect morphological differences between 10 populations collected from India, Bangladesh, Pakistan and Sudan during the seed and seedling stages. Morphological, physiological and allozyme data revealed that there is ample genetic diversity residing in neem at the species, population and individual level. Li Peng *et al.* (1997a) conducted study on genetic structure and patterns of genetic variations among populations in eastern white spruce (*Picea glauca*). Data were collected on seeding heights at various ages, 1-yr. branch number, and 3 yr. bud burst and bud set. Significant differences were found for each trait among provenances and among families within provenances. Regression models showed that patterns provenance variation mainly followed a south-north cline and to a lesser extent a west- east cline.

Positive correlation between collection site variables and plant characteristics would suggest that the variation between accessions is related to agro-ecological variations among the collection sites (Elings, 1991). The correlation between seed azadirachtin and latitude is positive and significant, indicating that environmental factors (related to latitude) might account for the variation for these particular character. Information on the relationships between environmental factors of the collecting sites and morpho-physiological variation of germplasm could enhance the understanding of evolutionary

adaptive patterns, which could assist germplasm collectors and users (Annicchiarico *et al.*, 1995).

Importance of studying mating system parameters for understanding population genetic structure and evolution have been emphasized (Allard, 1975; Rossi *et al.*, 1996). Regulation of the amount of inbreeding plays a major role in adjusting the manner in which genetic variability is organised in a population. Furthermore, mating systems are under genetic control and themselves subject to selection (Brown, 1990). Many tropical species possess mechanisms that ensure or encourage outcrossing. Kundu (1999) detected high level of outcrossing ($t_m = 0.90$) in *A. indica* which indicated that this tree posses a predominantly allogamous mating system. Insect pollination, presence of 'protandry', 'andromonecy' and 'polycarpy' and absence of biparental inbreeding are the probable causes of such high degree of outcrossing (Kundu, 1999).

Inbreeding is a fundamental factor of the population biology of any species. efforts have been made to estimate inbreeding from the empty seed percentages because of presence of embryonic lethals (Koski, 1971; Kundu, 1999). Empty seed formation is certainly caused by additional factors, such as reduced pollen viability or an environmental stress (drought or cold). In the current study, on an average, an endocarp (trilocular) produced only 1-3 seed so that 3-5 ovules are eliminated during seed development. The presence of the empty endocarps, as well as fewer seeds per endocarp than expected, indicates that inbreds are selected against. The present results are presumably due to the presence of embryonic lethals. An analysis of seeds per endocarp showed significant differences indicating genetic differences in tolerance to inbreeding. The 'polycarpic' endocarp structure is also a mechanism to make natural barriers against widespread selfing. The present results support the studies of Singh *et al.* (1996)and Kundu (1999). Singh *et al.* (1996)confirmed neem to be an allogamous species through artificial selfing where fruit set varied from 0 - 0.15 per cent. Based on allozyme data, Kundu (1999) studied mating system of neem and reported that it is a predominantly allogamous species. In contrast, Gupta *et al.* (1996) reported that in neem inbreeding depression was not apparent in neem. In the present study, off-season flowering and fruit setting has been observed in the months of September-October in few trees at Hisar and embryo development is found to be normal. The chances for outcrossing are not there in off season due to the fact that only few trees flower, therefore selfing is evident. Depending on the locality, flowering may range from January to May (Gupta *et al.*, 1995). Sporadic flowering in September-October has been observed quite often, in addition to that in February-March. Shanthi *et al.* (1996b)reported abnormal seedlings in neem in December.

Seed biochemical characteristics (oil, fatty acid, protein content, and azadirachtin) were studied in some provenance collections of neem. Statistically significant differences

(both within and between populations) are observed for seed oil content of five provenances of neem belonging to northern and western India. Maximum average oil content (32.6 per cent) is observed in trees from Hisar provenance. Seed oil content in most of the provenances is not consistently and significantly correlated with morphological parameters of seeds. Age of the tree appeared to have no significant effect on the oil yield. Kadir *et al.*(1998) studied the oil content of seeds of neem trees in Peninsular Malaysia. The sample from Jalan Sungai Batu Pahat, Perlis, northern Peninsular Malaysia contained the highest percentage of neem oil (63.8 per cent). These estimates are higher as based on seeds and not endocarp. In the present study the estimates have been based whole of the endocarp.

Sridharan and Venugopal (1998) worked on the effect of environmental conditions on the yield of oil in neem. Seeds from different locations had significant variations in their oil content. Climatic factors at the different locations were correlated with oil contents of seeds, with a significant positive correlation of sunshine hours during September-March with seed oil content. However, in the present investigation no relationship is found between environmental conditions and oil yield.

Fatty acid profile of seeds from six provenance collections of neem belonging to northern and western India were studied. Considerable variation is observed for 2 saturated (palmitic acid and stearic acid) and 2 unsaturated (oleic acid and linoleic acid) fatty acids. Oleic acid is the major fatty acid. Ratio of unsaturated to saturated fatty acids exhibited a wide range. Palmitate ranges from 13.8 to 19.9 per cent, stearate ranges from 10.5 to 17.7 percent. The two unsaturated fatty acids namely oleate and linoleate acids ranged from 44.8 to 67.8 per cent and 3.35 to 19.64 percent respectively.

Kaushik and Vir (2000) reported fatty acid analysis for neem seed samples from different provenances of the Rajasthan state in India. Significant variability in individual fatty acids was observed. The palmitic acid ranged from 16 to 34 per cent, stearic acid from 6 to 24 per cent, oleic acid from 25 to 58 per cent and linoleic acid from 6 to 17 per cent. Authors suggested that this variability can be exploited for selection of trees and for studying the genetic variability in neem. These selections can also be utilized for genetic improvement of the tree. Ali *et al.* (1996) conducted studies on the fatty acids and glyceride compositions of neem seed oil. The fatty acid and glyceride compositions of seed oil were studied. It was observed that neem seed grown under the soil and climatic conditions of Bangladesh contains 40 per cent of acrid bitter greenish yellow to brown oil with a strong disagreeable garlic-like odour. The content of triglycerides varied from 89.0 to 89.9 per cent, diglycerides from 2.7 to 3.2 per cent and monoglycerides from 2.6 to 3.0 per cent depending on the soil texture in the areas where the plants were grown. The fatty acid composition of oil was analysed by GLC. Oleic, palmitic, stearic, linoleic were 55.8, 16.5, 2.5, 20.5 per cent , respectively, of the total fatty acids.

No uniform/significant association is observed among different fatty acids in most of the accessions except that between the two unsaturated fatty acids under study. Fatty acid profile (stearic acid and oleic acid) of seeds shows a strong association with geographical position and agroclimate of the sampling sites. Correlation studies among different fatty acids show significant as well as non-significant associations. Palmitic acid is associated with stearic acid positively, in two provenances (with arid and semi-arid climate) and negatively in three provenances (with mesic climate). However, a reverse trend is observed in case of association of palmitic acid with linoleic acid, wherein, positive correlation is obtained in mesic agro-climate and negative in case of arid and semi-arid agro-climate. The two unsaturated fatty acids are invariably negatively (though not significantly in every case) associated with each other.

No consistent correlation among fatty acids could be advantageous for manipulating fatty acid profile or selecting desirable ecotypes from the existing variability. The study demonstrates that neem genotypes available from different agro-ecological regions of India have considerable potential to offer high oil content and various combinations of fatty acids (particularly higher content of unsaturated fatty acids) which can be exploited as such (by clonal multiplication to exploit genetic variation) or put to further genetic improvement through traditional/molecular breeding.

Among different fatty acids, three fatty acids namely, oleic, linoleic and linolenic are essential fatty acids and have to be provided through diet. It has been further suggested that linolenic acid and erucic acid are to considerably removed if not eliminated completely, in view of their nutritional quality in case of crucifers, and sunflower, etc. (Appleqvist, 1972; Downey *et al.*, 1975). Neem oil is found to completely devoid of erucic acid and linolenic acid in the samples studied so far, but rich in linoleic acid (3.35-19.64 per cent) and oleic acid (44.84-67.81 per cent).

Ratio of unsaturated fatty acids to saturated fatty acids exhibited considerable variation ranging from 1.62 (Kalka) to 2.96 (Bikaner). Due to possibility of development of genotypes rich in unsaturated fatty acids, neem oil can be exploited to formulate low cholesterol causing cooking oils. Thus it offers great scope to further manipulate the content of unsaturated fatty acids by studying samples from diverse agro-ecological regions and /or genetic manipulations involving tissue culture techniques. Efforts can be directed towards development/selection of genotypes with higher content of essential unsaturated fatty acids and lower content of saturated fatty acids.

A reduction in palmitate and stearate would enhance the nutritional quality by lowering total saturated fatty esters, and reduction of linolenate would improve the oxidative stability of the oil (Dutton *et al.*, 1951). Although there are major genes for reduced palmitate and linolenate, the traits can be considered quantitatively inherited due

to environmental effects and the influence of modifying genes (Graef *et al.*, 1988; Fehr *et al.*, 1992). Stearate content also is considered a quantitative trait because no major genes have been reported for reduction of the fatty ester.

Multiple regression analysis of linoleic acid on all other seed characteristics (namely, palmitic acid, stearic acid, oleic acid, unsaturated fatty acids, saturated fatty acids, ratio of unsaturated and saturated fatty acids, 20 endocarp weight, and endocarp length,) in neem was done. High value of coefficient of determination points to very high level of goodness of fitness of the regression line. On the contrary the multiple regression analysis for oil content yielded very poor goodness of fit of regression line, suggesting greater effect of environment than the genes.

Statistically significant differences (both within and between populations) were observed for seed protein content in individual neem trees. Average protein value for the seed samples of 10 provenances is 12.91 per cent with a range of 10.06 to 16.63 per cent. Correlation coefficient of protein with oil content showed a significant value ($P = 0.5$) of 0.686. This association may be exploited for selection of genotypes with both high oil and protein contents.

Statistically significant differences between individual trees are observed for seed azadirachtin content. Average azadirachtin value for the seed samples of 7 seed samples from 5 provenance collections from Haryana is 0.3987 per cent with a range of 0.281 per cent to 0.583 per cent and a coefficient of variation of 31.85. Seeni Rengasamy and Parmar (1995) studied azadirachtin A- content in neem seeds, in ecotypes from 11 agroecological regions of India. Higher content was reported in coastal, arid and semi-arid regions. In Haryana, azadirachtin seems to be associated with latitude. However, in order to ascertain it further and to exploit this information further it is necessary to survey more area in the state.

In natural populations, variance components are of considerable interest for evolutionary studies (Lande, 1982) and, increasingly, for conservation purposes (Storfer, 1996). In natural populations, however, information on relationships may be unreliable or unavailable. Molecular marker information [restriction fragment length polymorphism (RFLP), minisatellites, microsatellites, RAPDs etc.] from the population of interest provides a means of circumventing this problem, by allowing estimation of relationship on a pair-wise basis (Thompson, 1975; Ritland, 1996a) without the need for pedigree reconstruction. These estimates of relationships may be combined with phenotypic information gathered from the same individuals, allowing inferences to be made about variance components (Ritland, 1996b).

An understanding of the extent and distribution of genetic variation within neem populations is essential for devising sampling strategies which efficiently capture genetic diversity for selection trials and subsequent distribution of material to farmers that fulfils

the dual aims of high genetic variation (which may provide an adaptive capacity to varying conditions) and reasonable performance.

Protocol for fingerprinting the neem genotypes using RAPD (Random Amplified polymorphic DNA) technique has been standardised and preliminary investigations with 15 genotypes and pooled DNA samples of 5 populations indicate within and between population genetic variability. A total of 20 random sequence commercially available deca-nucleotide primers of 60 percent or more of G + C content were used for the amplification of DNA for RAPD analysis. Of these 15 primers failed to amplify DNA of any of the neem genotypes, while 5 primers could amplify most of the individual 15 DNA samples and all of the 5 pooled DNA samples. Such non amplifying primers have also been reported by other workers. Zhenshen *et al.* (1996) used 150 primers for studying polymorphism in Chinese common wild and cultivated rice, 41 of which did not amplify any genotypes.

Total number of DNA fragments amplified by a specific primer / primer pair in individual DNA samples varied from minimum of zero to maximum of seven. However, polymorphic bands produced by different primers / primer pair ranged from 3 to 6. Total number of DNA fragments amplified by a OPC-12 in pooled DNA samples varied from minimum of six to maximum of ten. However, polymorphic bands produced were four only. Polymorphism was determined by calculating pairwise dissimilarity value (1-F) among different neem genotypes. The formula for genotype discriminating efficiency (GDE) of individual primers was formulated. GDE ranges from 26.67 to 88.89 percent for individual genotypes. Dendrogram of 12 genotypes based on RAPD profile of OPC-1, OPC-11 and OPC-12 primers was constructed using Jaccard coefficient by UPGMA approach of clustering. Highly divergent genotypes could be delineated by this exercise.

Rafalski *et al.* (1991) postulated that in RAPD reaction, the composition of amplified products is determined by a competition between potential priming site in the template rather than by the total number of priming site available. It is for this reason, variations in amplification and polymorphism is observed for different primers. The comparison of dissimilarity (1-F) values (Nei and Li, 1979) obtained with specific oligonucleotide primers differed in their usefulness in detecting DNA polymorphism within neem species. This suggested that it could be possible to identify a set of primers that bind at more sites of neem genome. These hyper-variable RAPD primers would generally facilitate the application of RAPDs in breeding and may be particularly useful for DNA fingerprinting of new genotypes.

The population of bands obtained when pooled leaf samples is subjected to RAPD analysis is not always a summation of all the bands present in all of the individuals, presumably because of competition effects for the primer within the reaction tube.

However, use of the sampling method must lead to a more representative population of bands for an accession than use of DNA from a single plant.

With regard to the suitability of numerical techniques for the interpretation of RAPD data, agglomerative clustering method of UPGMA was used. It is a popular method in which dendrograms reveal information based on similarity/dissimilarity between accessions and hence it is possible to identify readily closely-related accessions.

However, homology of co-migrating RAPD products has been previously demonstrated for different species of *Glycine* and *Allium* (Wilkie *et al.*, 1993) Indirect (but very significant) supporting evidence of allelism is derived from the conformity of taxonomic classifications based on RAPD data to those which may be widely accepted and based on more conventional approaches involving morphology, cytology and enzyme electrophoresis. We,therefore,feel that the assumption in our calculations that co-migrating bands are allelic, is not unrealistic. However, to confirm allelism conclusively the inheritance of band/s in the progenies of appropriate cross/es must be studied.

Fingerprinting reliability through RAPD has been questioned since comigrating bands from different individuals do not necessarily represent homologous amplification products, but the presence of comigrating bands only reduces the absolute similarities, not the relative similarities nor the relationships among clusters (Adams and Rieseberg, 1998). Hence fragment size and frequency can be considered a reliable predictor of homology of closely related individuals (Nicese *et al.*, 1998), provided that they can differentiate between at least two populations.

Although neem tree is an ancient domesticated tree, very little information is known at the molecular level about the different provenances used. In an attempt to shed light on provenance identification and biodiversity, reproducible RAPD markers developed in this study are useful for genotypic identification. Presence of high molecular weight bands confirms that polysaccharides, if present, do not interfere with polymerisation (Pandey *et al.*, 1996). The absence of spurious bands suggests that the non-specific priming of RNA is effectively absent.

Several reasons have led us to use bulk, in addition to individual DNA to characterise the neem -tree provenances: (i) A genotype is considered as a group of individuals that has been selected for expression of specific traits in a background of otherwise randomly distributed genetic variation. Then a bulk sample of 10 individuals may be expected to represent the markers linked to traits (Yang and Quiros, 1993). Bulk DNA samples of several genotypes for a single provenance may reveal markers that distinguish between them in a significant number of cultivar members. It has been described that 20 bands and 10 individuals are needed to detect a difference between two populations with a type-1 error of 5 per cent, a power of 90 per cent and distance less than 0.1 per cent (Gherardi *et al.*, 1998). The high level of polymorphism probably reflects the outcrossing nature of

neem in accordance with results described by Kundu (1999) on the basis of isozyme and DNA polymorphism studies (Singh *et al.*, 1999). Isozyme studies exhibited distinct genetic differentiation in different populations. On the basis of isozyme studies, Kundu (1999) suggested strong natural selection in the populations or genetic erosion due to restricted population size.

Sometimes a polymorphism characterised one genotype by its presence and absence. The results obtained comprised a unique fingerprint for some of genotypes studied, allowing unequivocal identification of each genotype, even in case of progenies of same tree and closely growing trees in a provenance. This study indicates that the use of RAPD technique to detect genetic variation at the level of DNA among neem genotypes is sensitive and powerful. This could be useful for determining the best choice of parents in order to generate mapping population for tagging genes for useful traits.

Menkir *et al.* (1997) concluded that genetic distance determined by RAPD markers may help in selecting the most diverse cultivar or accession for introgression. In general, tropical woody plants maintain high level of within-population genetic variation (Loveless, 1992). Variation in genetic diversity within species is usually related with geographic range, mode of reproduction, mating systems, seed dispersal and fecundity (Brown and Moran, 1979; Loveless, 1992; Hamrick *et al.*, 1992). High genetic diversity detected in this study is presumably due to all these prevalent background factors as all the populations and individual genotypes studied are widely distributed. The high gene diversity and the degree of genetic differentiation in this study are also manifested by the special phenomenon of 'protandry' (pollen mature before stigma become receptive), and 'polycarpy' (endocarp having more than one carpel) in this species. The results of the present study on genetic diversity concur other studies on neem (Kundu, 1999), with those of other tropical species such as *Tectona grandis* (Kertadikara and Prat, 1995) and *Acacia albida* (Joly *et al.,* 1992).

Determinants of genetics structuring in plant population include mating system (Loveless and Hamrick, 1984) natural (and possibility artificial) selection, evolutionary history, life- history characteristics and mechanism of gene flow (Schaal, 1980; Ellstrand *et al.*, 1989; Hamrick and Godt, 1989; Hamrick *et al.*, 1992). Together, these factors can lead to complex genetic structuring within populations which is often difficult to resolve. The use of biochemical and molecular markers can enhance understanding of such complexities. Random amplified polymorphic fragments are normally inherited in a biparental dominant Mendelian manner (Carlson *et al.*, 1991). Primers varied in ability to detect polymorphism and in reproducibility. Five primers which consistently revealed informative patterns over several independent PCR runs, on DNA extracted from fresh leaf and over a range of DNA concentrations, were chosen for analysis of the entire set of genotypes.

The various statistics used to estimate and partition genetic variation at a detailed level within plant population cannot be applied easily to RAPD data obtained from outcrossing species because of dominance. Codominant products are occasionally revealed (at one locus in the present study), but these are rare (Williams *et al.,* 1990). Despite this, approximate estimators have been devised recently for various population-genetic parameters using RAPDs(Lynch and Milligan, 1994), although these rely on an assumption of Hardy Weinberg equilibrium. As individual half-sib families of neem do not approach this equilibrium, further approximations regarding the inter-relationship of RAPD product frequency and underlying genetic variation are required and a relatively large sample of RAPDs must be scored to provide an effective description of population structure.

In a group of randomly mating, outcrossing individuals the theoretical expectation for genetic partitioning within and among half- sib families is 75 percent and 25 percent, respectively (Hartl, 1987). Although the number of populations used in the present study is relatively low, which limits the ability to estimate, the populations partitioning among the populations was achieved.

Cluster analysis shows that differentiation appears to be clinal and indicates a relationship between geographical distance and genetic similarity based on RAPDs. Although greater resolution must partly reflect the greater number of markers available via RAPDs, additional factors associated with RAPD dominance and relative phenotypic neutrality may also be involved. In particular, there is some evidence of non-neutrality at some isozyme loci (Karl and Avise, 1992). Whatever the source of greater RAPD discrimination, an increased understanding of detailed population structure is provided which is potentially relevant for the design of collection strategies to capture genetic variation optimally. Within the population studies, RAPDs indicate a significant degree of geographically related differentiation among sub-populations, which suggests that stratified sampling may be necessary to capture effectively most genetic variation. The degree of sub-population discrimination available with RAPDs is greater than the associated with isozymes, so the RAPDs may provide a more useful tool than isozymes in the design of collection strategies which retain more of the variation contained within populations (Dawson *et al.*, 1995).

Many methods are available for the clonal propagation of trees. Among the various *in vitro* methods, somatic embryogenesis is the method of choice over other routes like direct or indirect organogenesis and bud proliferation, because of certain advantages viz., probable single-cell origin of the regenerants, a very high multiplication rate, and possibility of adaptation to artificial or synthetic seeds. Currently some published reports are available on somatic embryogenesis in neem i.e., Shrikhande (1993), Su *et al.* (1997) and Murthy and Saxena (1998). Other reports on *in vitro* regeneration in neem pertain to

shoot proliferation from nodal explants (Yaseen, 1994), and decapitated seedlings (Rangaswamy and Promila, 1972), shoot and root formation from anther derived callus (Gautam *et al.*, 1993) and transformation attempts (Naina *et al.*, 1989). The present study was designed to see the efficiency of mature seeds for shoot regeneration / somatic embryogenesis in different media and also establish callus and suspension cultures to explore the possibility of *in vitro* production of azadirachtin. During incubation in dark, segmented seeds start developing callus growth within 18-40 days in all the media but the callusing intensity varies widely from very low to very high. After initial incubation in dark for 60 days, white coloured minute multiple shoots (5-20 per callus) and shoot primordia are observed on some of the media. Highest number of shoots (10-15) are observed in N2 (2mg l^{-1}BA) medium where the cultures are grown on N8 (4.0mg l^{-1} BA + sucrose 90g l^{-1}) medium during first subculture. On the other hand, the cultures are grown only on N2 medium the number of shoots declined drastically. The shoot primordia and multiple shoots could be elongated upon transfer to fluorescent white light. Four media viz., N1, N4, N12 and N15 are found to provide higher number of shoots per callus. Murthy and Saxena (1998) used a dark incubation period of only one week while we have extended dark incubation to 60 days. Due to this fact these authors first got deformed seedlings and then embryogenesis, on the other hand we obtained regeneration directly from seeds or seed callus. This might be the reason of comparatively higher number of multiple shoots (upto 50) in our study than those obtained by Murthy and Saxena (1998). Zypman *et al.* (1997) Tissue culture methods and cloning of the neem tree for bioinsecticide production. Light yellow friable callus and shoots were initiated from hypocotyl explants of neem on MS medium supplemented with 0.5 mg IAA and 1mg BA l^{-1}. Shoots were regenerated from nodal buds and calli.

Regenerated shoots were kept on MS media containing 2mg l^{-1} NAA; 2mg l^{-1} IBA+0.5mg l^{-1} Kin., and 2mg l^{-1} IAA for the purpose of rooting, however no shoot could be rooted. Vegetative explants (axillary buds, shoot tips and leaves) from a mature tree of Hisar provenance were evaluated for callusing in MS medium supplemented with 0.5 mgl^{-1} NAA and 1.0 mgl^{-1} BAP. Callusing could be obtained in 80 per cent of axillary buds and shoot tip explants within 15-20 days of culture. Leaf explants started callusing within 20-45 days of culture and only 20 per cent of explants showed callusing. Callus cultures could be maintained by frequent sub-culturing in the same medium at an interval of 25-30 days. Jaiswal and Narayan (1984) reported regeneration of plantlets from stem tissue of neem. George and Kulkarni (1997) reported that explants of leaflets, rachis, inflorescence axis and cotyledons from locally growing trees in Maharashtra callused with high frequency in an *in vitro* culture medium of MS + 5 per cent (V/V) coconut water + 2 µM BAP in 2-3.5 weeks.

Joshi and Thengane (1996) reported *in vitro* propagation of neem by shoot proliferation. Two to three shoots per explant were observed after 20 days. An increase in multiplication frequency of shoots per explant (45-50) and average shoot length (4.7 cm) was observed after 14-16 weeks. Eeswara *et al.* (1997b)developed a standard procedure for the micropropagation of the neem. Micropropagated shoots were initiated from leaf explants, taken from potted plants (6- or 18-months old) grown from seed. However, in the present investigation no shoot could be regenerated from leaf tissues. Venkateswarlu *et al.*(1998) worked on micro-propagation of plus neem tree and evaluation of field transferred plants. Successful shoot bud induction and multiplication could be achieved.

Yasseen (1994) studied shoot proliferation and plant formation from neem. Tawfik (1997) reported micropropagation and plant regeneration of neem tree. Four types of explants (shoot-tips, stem nodes, petiole and leaf segments) were used. Shoot tips and stem explants were cultured on woody plant medium (WPM) supplemented with kinetin and benzyladenine (BA) each at 0, 0.5, and 1 mg l^{-1}.

Freshly explanted tissues of woody plants, particularly angiosperms, often secrete brown/black pigments, composed mainly of oxidized polyphenols and tannins, into the medium in response to wounding. Phenolics released into the medium inhibit shoot growth and can kill the plant material; also the explants taken from older specimens tend to produce more phenolics (Chalupa, 1987). Phenolic compounds that are largely located in vacuoles, upon injury are mixed with the contents of plastids (which contain polyphenoloxidases) and other organelles. Polyphenols are oxidized to form quinones, which are highly reactive compounds that polymerize rapidly and form covalent bonds with proteins (Loomis and Battaile, 1966). Oxidized polyphenolic compounds inhibit enzyme activity (Hu and Wang, 1983) and may result in the decline or lethal browning of the explants. Explant browning has been successfully controlled in a number of explants like shoot tip, leaf, nodal segment, cotyledon, etc. by using a variety of pretreatments; presoaking in ascorbic acid, L-cysteine-HCl, citric acid, meracaptoethanol (Anderson, 1975; Monaco *et al.*, 1977; Welander, 1988); presoaking in sterile distilled water (Cresswell and Nitsch, 1975; ~~Vieitez, 1980b,~~ Durand-Creswell *et al.*, 1982; ~~Vieitez *et al.*, 1983)~~; growing the cultures in dark (~~Ziv and Halevy, 1983;~~ Barghchi and Alderson, 1985,); incorporating L-cysteine HCl, polyvinylpyrollidone, activated charcoal and ascorbic acid in the medium (Somers *et al.*, 1982; Srivastava and Steinhaner, 1982; Dabin and Bonharmont, 1983). In the present investigation, a solution containing 0.5 g l^{-1} of ascorbic acid, 0.5 g l^{-1} of citric acid and 0.1 g l^{-1} PVP was used to control the browning of the explants completely.

Callus cultures produced from axillary buds were analysed for production of azadirachtin after 40 days of incubation. In N6 medium (MS medium + 0.8 mg l^{-1} α-naphthalene acetic acid + 2.0 mg l^{-1} 6-benzyl aminopurine) the amount of azadirachtin produced is 0.00027 per cent and in N15 medium (MS medium + 2.0 mg l^{-1} kinetin) a concentration 0.00028 per cent azadirachtin is recorded. Veeresham *et al.* (1998) were

able to produce of azadirachtin from callus cultures of neem. Callus cultures, initiated from leaves and flowers of neem (collected from India), were established for the production of azadirachtin. Twelve-week-old flower callus produced 2.46 per cent azadirachtin. Wewetzer (1999) studied effect of different nutrient media and two carbohydrate sources on the azadirachtin content of callus cultures of neem derived from leaf, root, shoot and bark. Level of differentiation was higher on medium supplemented with maltose but overall, average azadirachtin contents were higher when sucrose was used as carbohydrate source. The yield of azadirachtin in the present investigation is quite low, however only two media and one explants were studied. The production could have been higher in case of trial with more variables.

Classification studies have special significance in germplasm studies because of two specific reasons. First, it is difficult to evaluate large number of lines through breeding plans obviously due to practical limitations and second because many of the accessions may be genetically more or less similar due to their common ancestor. Various approaches like geographical diversity (Dhawan and Singh, 1961; ~~Mali *et al.*, 1962;~~ Singh and Joshi, 1966), coefficient of radical likeness (Pearson, 1926), Hutchinson's polygraph (Hutchinson, 1936), discriminate function (Fisher, 1936), metroglyph and index score analysis (Anderson, 1957) had been suggested for classification and selection considering many variables simultaneously. But these failed to provide foolproof measure of genetic diversity and its quantitative assessment. D^2 statistic of Mahalanobis (1930, 1936), a measure of group distance based on multiple characters permits precise quantitative comparison among all pairs of population along with their classification before affecting actual crosses. It is based on second degree statistics and is self-weighing on the basis of genetic variability of characters involved. Also D^2 value between any pair of populations amount to a measure of genetic divergence (Rao, 1952). Therefore, D^2 analysis was carried out in the present study to gain an insight in the genetic divergence in the material under study.

D^2 values for all possible (903) combinations among 43 genotypes (using all 8 endocarp morphological characteristics used) were calculated and these genotypes are grouped into ten clusters. Cluster CI consisted of maximum number of genotypes i.e., 12. This envisaged that the genotypes grouped in this cluster are more or less genetically similar to each other and apparent wide diversity is mainly due to the remaining 31 genotypes distributed over rested of the nine cluster. Clusters namely, CII, CIII, and CIV consisted of 10, 7, and 5 genotypes respectively. CV, CVI, and CVII had 2 genotypes each, whereas and CVIII and CIX and CX are represented by one genotype each. The genotypes of CVIII, CIX and CX are extraordinary for one or the other character that made them so divergent from others. CVIII represented low seed weight : endocarp

weight. CIX represented low seed weight. CX represented low high endocarp length and high endocarp length : endocarp breadth.

The study further reveals from clustering pattern that genotypes from different geographic regions are grouped together in a cluster and vice-versa suggesting that geographical diversity does not necessarily represent genetic diversity. This is in line with the results obtained earlier using D^2 analysis by Mehndiratta *et al.* (1971), Tiwari and Singhania (1989), Ezeaku *et al.* (1999) who reported that geographic diversity although important, was not the only factor responsible in determining the genetic diversity.

The main objective of forming cluster and to find out the intra and inter-cluster distances is to provide relevant information for selection of diverse parents for hybridization programme without making actual crosses (Bhatt, 1970). The lesser magnitude of intra-cluster distances than those of inter-cluster distances indicated that the genotypes grouped in a common cluster diverged very little from one another as compared to the genotypes of different cluster. Large inter-cluster distances are recorded especially in single genotype clusters in relation to other clusters signifying them to be extra-ordinary for one or more characters and made them divergent form others.

The cluster means reflect appreciable variation for all the characters among different clusters. These differences were more pronounced for seed weight, endocarp weight and seed length. The clusters VIII, IX and X, though represented by single genotypes each are to have accumulated a few unusual attributes like very high value for endocarp length in CX. Bagchi (1992) conducted genetic divergence studies with the help of Mahalanobis's generalized D^2 statistic among 42 provenances of *Acacia nilotica* in search of bigger seeds and to find contribution of individual characteristics towards divergence. Cluster analysis resulted in two groups of which one was the bigger group having 34 provenances and the other smaller with 8 provenances. Second group contained the provenances belonging to Rajasthan only, whereas the first group included samples from all the other states. First group was best having higher group mean values for all the three characteristics. The higher intra-cluster distance in first group indicates higher variability within the group and it is quite likely that extreme individuals from this group may help in selection. Hybridisation between widely divergent groups may also help producing greater genetic diversity which may be utilized for further selection.

After clustering, widely divergent groups may be utilized for hybridisation, if possible to increase the genetic diversity for furtherance in selection or, a superior group may directly be utilized for short-term plantation purposes for an improved seedling vigour and consequently increased growth.

It may be noted that seed sources from different locations were clustered together to form the clusters. This signifies that all seed sources from a single zone may not

necessarily form a single cluster and the pattern of genetic nearness is not dependent on the geographical nearness. This generally happens when seed sources are of different genetic makeup and are distantly related. Similar results were obtained in agricultural crops when selections from the same cross did not form a single cluster due to wider relations between the two parents (Murthy and Arunachalam, 1966; Murthy and Pavate 1962; ~~Singh and Gupta, 1968).~~

For more objective interpretation of the results obtained from D^2 analysis, the actual D^2 values among the genotypes rather than inter-cluster D^2 statistic is totally arbitrary. Rana and Murty (1971), Sidhu and Singh (1975) and Singh and Gupta (1979) reported the classification according to D^2 analysis as subjective, firstly because of the cluster formation method, secondly that sometimes genetically related genotypes may be grouped into different clusters and vice-versa and thirdly that the number and composition of clusters varies greatly under the influence of environment. Therefore, they suggested that in the absence of more precise method, it becomes necessary to use more than one method to offset these limitations to a certain extent.

Peeters and Martinelli (1989) discussed several advantages of multivariate algorithm hierarchical cluster analysis. Firstly, it allows mixing of both quantitative and qualitative data which allows utilizing all the information available on the sample. Secondly, in this method each entry is treated as an individual entity of equal weight contrary to other multivariate techniques that are based on variation of group of entries. Thirdly, it defines the degree of relatedness among the samples and can predict the degree of segregation of given samples thus making it a powerful tool to precisely classify the population.

Endocarp morphological data of 43 genotypes of neem (all 8 endocarp morphological characteristics used) were subjected to hierarchical cluster analysis with the help of squared euclidean distance and UPGMA approach. Two major clusters were visible. First cluster contained only one genotype (NGN1) from Pune while second cluster (containing rest of the genotypes) was further divided into 2 sub-clusters.

The association among the different genotypes is presented in the form of dendrogram prepared using rescaled distances. The genotypes which are lying nearer to each other in the dendrogram are more similar to one another than those lying apart. The resemblance coefficient between the two genotypes is the value at which their branches join. The dendrogram also showed the relative magnitude of resemblance among the different clusters. The grouping of genotypes originating from different eco-geographic regions into one cluster could be attributed to ancestral affinity and due to operation of similar force of natural and artificial selection resulting in perpetuation and stabilization of similar genotypes (Murthy and Arunachalam, 1966).

Seed biochemical and endocarp morphological data of 28 genotypes of neem (all 10 endocarp biochemical plus morphological characteristics used) was subjected to

hierarchical cluster analysis of using squared euclidean distance and UPGMA approach. Two major cluster namely, A and B were discernible from the dendrogram. Cluster A contains 9 genotypes containing mainly genotypes from arid (Bikaner) and semi-arid regions (Hisar). One exceptional entry is the genotype from Allahabad which is not having arid type of climate. Cluster B contains 19 genotypes mainly genotypes from semi-arid (Hisar) and other non-semi-arid regions of Haryana namely, Kaithal (Northern plain and central highlands, hot semi-arid eco-region with alluvium-derived soils.), and Kalka (western Himalayas warm sub-humid (including humid) eco-region with brown forest and podzolic soils) and Pune (Deccan plateau, hot semi-arid eco-region with shallow and medium (including deep) black soils. The hybridization among diverse parents is likely to produce heterotic hybrids.

A phenetic relationship is referred to as grouping of taxa by overall existing similarity. It measures the currently existing similarity among taxa and thus ignores the direction of characters state changes during evolution. Phylogenetic relationship on the other hand, groups plant on the basis of shared derived characters, and therefore, the resulting clusters estimate ancestor-descent relationships (Sneath and Sokal, 1973). In the current study, cluster analysis of biochemical and morphological traits classified distinct groups in some cases according to agroclimate associated with their respective regions. The provenance, Bikaner is found to have close affinity with Hisar provenance.

No complete correspondence is observed between the geographical and divergence pattern. Kang and Lee (1996), Lin *et al.* (1998) and Ayana and Bekele (1999) used cluster analysis to classify different germplasm collections of sorghums and partitioned them into different clusters. In most of the studies no definite association between geographical and genetic diversity were reported, however, Ayana and Bekele (1999) found that a greater proportion of accessions of similar adaptation zones and accessions from regions of origin with similar agro-climatic conditions were grouped together. This implies that geographic diversity is not the only factor determining genetic divergence and is one among the several factors determining the genetic divergence. Therefore, parental selection for hybridization should be based on the criteria of genetic diversity.

Usually the number of variables included to classify the germplasm becomes perceivably large when the above mentioned idea is considered and makes it difficult to estimate the coefficients of functions mathematically. Under such circumstances, principal component analysis comes handy to partition the variability into mutually orthogonal components retaining the same amount of variability contained in the original set of variables.

Owing to lack of knowledge regarding relative importance and usefulness of variables, the investigator tries to include all the possible variables, making the data matrix perceivably large, complicated, unmanageable and beyond comprehension.

Therefore, the investigator requires a technique here for systematic reduction and summarisation of data.

Principal component analysis, basically a data reduction technique, was initially floated by Pearson (1901) and later developed by Hotelling (1933), offers solution to this complex problem by transforming the original set of variables into a smaller set of linear combinations that account for most of the variability of the original set. The objectives of principal component analysis is to identify the minimum number of components, which can explain maximum variation out of the total variance (Anderson, 1972; Morrison, 1978 and Dillon and Goldstein, 1984).

The first principle component absorbs and accounts for maximum proportion of total variability in the set of all variables and remaining components account for progressively lesser and lesser amount of variation. Same trend is observed in the present study. For seed biochemical and morphological traits combined, the first principal component accounted for maximum variability i.e. 54.16 per cent which reduced gradually to 0.007 per cent in 8th and nearly zero in 9th principle component. Kang and Lee (1996); and Ezeaku *et al.* (1999) conducted principles component analysis in sorghum and transferred many correlated variables into a few independent principal components explaining much of the variability of the original set. The first three principals components, having eigen values greater than one and altogether explaining more than 80 per cent the total variation, were retained for further studies (Kaiser, 1958 and Jeffers, 1967).

In the light of critical comments of Sneath and Sokal (1973), further analysis was carried out using principal factor analysis. In principal component analysis, the total variation contained in set of variables is considered whereas, in principal factor analysis interest centres on that part of variance only, which is shared by the common factors leaving aside the unique factor (including error) of the variable. Moreover, in contract to principal component analysis, here the component axes are allowed to interact resulting in distortion of mutual orthogonality. Thus the magnitude of change in values and results of principal factor analysis as compared to principal component analysis can be predominantly ascribed to influence of environment and interaction among the principal axes.

Ten morphological and biochemical characteristics of endocarp were considered for principal component and principal factor analyses in 28 genotypes of neem. The first three principal components (PCs) gave eigen values more than one and altogether explains 84.864 per cent of accumulated variability. Seven characters (related to fatty acid profile) have very high loading on the first factor. Endocarp length and 20 endocarp weight have high loading on second factor. Oil content of the endocarp have high loading on second factor. This provides a very clear picture regarding the idea of character association with respect to principal factor.

The clear cut grouping of similar type of variables by getting loaded on common principal factor elaborates the successful transformation of eight interrelated variables into three independent principal factors explaining about 84.86 per cent of the variability of the original set.

Principal factor (PF) scores for all the genotypes are estimated in all the three factors. These scores can be used to propose precise selection indices whose intensity can be decided by variability explained by each of the principal factor. Using the principal factor scores, all the genotypes are plotted for factor 1 and 2; factor 1 and 3; and factor 2 and 3. These three plots clearly indicate the separation of genotypes with high values of the characteristics towards the axis of factors which represent them. Genotypes with contrasting fatty acid profiles and morphological traits and combinations thereof could be delineated with different plots.

Eight morphological characteristics of endocarp were considered for principal component and principal factor analyses in 43 genotypes of neem. The first three principal components (PCs) give eigen values more than one and altogether explained 90.2 per cent of accumulated variability. Five morphological characteristics of endocarp (endocarp weight, seed weight, endocarp coat weight, endocarp length, and endocarp breadth) have very high loading on the first factor. Endocarp length : breadth and Seed weight : endocarp weight had high loading on third factor and number of seeds endocarp^{-1} have high loading on second factor. Principal factor (PF) scores for all the genotypes were estimated in all the three factors. Using the principal factor scores, all the genotypes are plotted for factor 1 and 2; factor 1 and 3; and factor 2 and 3. These three plots clearly indicated the separation of genotypes with high values of the characteristics which they represent. Genotypes with contrasting endocarp morphology and combinations of traits could be delineated with different plots.

Principal component analysis has been used in neem to differentiate populations on the basis of variation in rainfall and leaf area. It also indicated that moisture availability plays an important role in differentiation of neem plantations (Kundu, 1999). Lopez Mata (1987) studied genecological differentiation in nine provenances of *Brosimum alicastrum*. Principal component analysis and linear correlation indicated the existence of ecoclinal variation in seed size (weight and diameter). Li Peng *et al.* (1997a) conducted study on genetic structure and patterns of genetic variations among populations in eastern white spruce (*Picea glauca*). Data were collected on seeding heights at various ages, 1-yr. branch number, and 3 yr. bud burst and bud set. Two Principal components composed 87 per cent of the total variation for all traits. Regression models explained between 19 and 65 per cent (an average of 47 per cent) of provenance variation and showed that patterns of provenance variation mainly followed a south-north cline and to a lesser extent a west- east cline.

Using multivariate analyses (D^2 analysis; hierarchical cluster analysis; and principal component and principal factor analysis) it has been possible to delineate highly divergent samples with respect to endocarp morphological and seed biochemical characteristics. Clustering pattern is found to have partial dependence on geographical location of the germplasm accessions. Some similarity is observed in clustering pattern obtained by different multivariate techniques.

The breeder's aim is to develop cultivars that are stable across a range of environments. This is particularly important for forest tree species especially for marginal environments. However, if cultivars are being selected for a large group of environments then stability and yield across all environments are of major importance and yield in a specific environment is of marginal importance (Piepho, 1996).

The interaction of a genotype with the environment is a universal phenomenon, irrespective of the plant material. Inference about genetic makeup are based on phenotypic data and phenotype is the result of both genetic and non-genetic (environmental) components. The effect of non-genetic components is not independent of genetic component as the genotypes respond differently to varied environments. This interplay of genetic and non-genetic forces is known a genotype-environment interaction (Comstock and Moll, 1963).

In natural populations many random effects (e.g. maternal effects,) as well as fixed effects (e.g., sex, or year of birth) may have considerable influence on quantitative traits (Thomas, *et al.*, 2000). Frankel (1958) proposed two possible ways in which a plant breeder can develop varieties exhibiting a low G X E interaction: (i) the sub-division of heterogeneous area for which the varieties are being bred, into smaller regions or in other words, stratification of environments such that each of the strata is more homogeneous with its own characteristic varieties, (ii) the introduction of varieties which exhibit a high degree of stability in performance over a wide range of environments, i.e., varieties having wide and general adaptability. The first strategy may not be very efficient but the second strategy, which has been receiving increasing attention in recent years, interests the breeder a great deal.

In the recent past, the methods have been developed which could be used to provide reliable estimates of these interactions. The analytical approaches are, statistical (Yates and Cochran, 1938; Finlay and Wilkinson, 1963, Eberhart and Russel, 1966) and other is genetical (Mather and Jones, 1958; Jinks and Stevens, 1959; Bucio Alanis, 1966; Bucio Alanis and Hill, 1966; Perkins and Jinks 1968a, b and Breese, 1969) are now available by which the results from experiments conducted in different environments can be interpreted.

Environmental variations seemed to be of importance in determining performance and therefore evaluation based on several years and locations is a right strategy to be pursued

in the breeding programme. Data from multiple years are more reliable for stability analysis (Yue *et al.*, 1997). Since each environment consists of a combination of various factors which influence performance, it is difficult to specify all the differences between environments in relation to these factors. As pointed out by Hill (1975), the use of independent physical environmental factors instead of the performance based environmental index is not devoid of problems since random sampling of the environment with respect to these factors would be difficult.

Hildebrand (1990) suggested the selection of materials that maintain productivity in poor environments or that are superior in favourable environments rather than those with regression coefficients equal to 1. Genotypes with b equal to 1 are less productive in poor environments than those with low regressions and also yield less in favourable environments as compared to those with high regressions. Low regressions indicate adaptation to poor environments and could be utilized to cater for low input agriculture.

Analysis of variance for stability was performed in 11 provenance collections of neem for eight endocarp morphological characteristics. Genotype and environment interactions for all these eight characteristics were found non-significant which suggested that genotypes have no interaction with environment for the given characteristic. Stability parameters were obtained using the statistical analysis described by Eberhart and Russel (1966). Twenty seed weight and seed oil content exhibit significant genotype-environment interaction and differences are observed with respect to temporal stability. For these two characteristics stable genotypes could be identified.

The mean squares due to genotypes and years are significant when treated against pooled error for both the traits, indicating the presence of variation among genotypes and years for both the characters. The genotype x environment interaction is significant for both the characters (20 endocarp weight and oil content of seeds) when tested against pooled deviation. It indicates that the genotypes interacted considerably with the environment in the expression of both the characters. Highly significant mean squares due to environment (linear) indicated considerable differences among environments and their predominant effect on both the characters.

The genotype x environment interaction (linear) is significant for 20 endocarp weight as well as oil content when tested against pooled error and pooled deviation indicating that genotype x environment interactions are linear as well as non-linear. The genotypes differed significantly for their stability for both the characters as evident by different values for mean sum of squares for genotype x environment (linear) and pooled deviations for two characters.

Based on the regression coefficient b, the genotypes tested could be categorized as those having more than average stability, average stability and below average stability for both traits in the range of environments they encountered. HSR2 and KLK5 appeared to

be sensitive to changes in environments due to their b values of >1. Therefore, these materials can be considered as sensitive and having below average stability for 20 endocarp weight. Among the 11 genotypes, KLK4 showed more than average mean performance, regression coefficient of slightly more than 1 and non-significant deviation from regression, indicating its stable performance for oil content. HSR2, KLK5, and KTL5 appeared to be sensitive to changes in environments due to their b values of >1. Therefore, these genotypes can be considered as sensitive and having below average stability for oil content. Zhou Weijun *et al.* (1997) reported most of the cultivars cotton cultivars tested at 7 locations in China to be stable for oil content.

Plant architecture can vary in response to the environment, as has been shown for the number and length of sylleptic versus proleptic branches in Populus trees (Wu and Stettler, 1998). In herbaceous plants, shading can alter the plant's architecture as a result of effects on meristem initiation and fate as well as organ size and structure (Huber *et al.*, 1999). Developmental plasticity might be limited to early stages of the life cycle or might vary in timing among different genotypes, populations or species (Pigliucci and Schlichting, 1995; Bell and Sultan, 1999; Gedroc *et al.*, 1996). Young neem trees growing in vicinity of other trees with competition for light the crown spread is quite less and straightness is quite high. This indicate highly plastic architecture of neem in young stages.

Temporal variation in the environment is generally thought to be less efficient in maintaining genetic polymorphism than spatial variation. However, if there is delayed germination or diapause, in some situations the conditions for genetic polymorphism are greatly broadened in a temporally variable environment (Hedrick, 1995). Levene (1953) first gave the conditions for a polymorphism when there is spatial variation in the environment. Conditions for maintenance of polymorphism when there is temporal variation in selection appear to be much more restrictive than those for maintaining variation from spatial variation in fitness (Hedrick *et al.*, 1976).

Some organisms may be able to avoid an environment for which they are not adapted when there is temporal variation in the environment because they can exist in a life stage that does not encounter the effects of the environment. For example, some plants have extensive seed pools and the seeds that do not germinate do not experience many of the environmental effects encountered by the seeds that do germinate. Recently, Ellner and Hairston (1994) have extended these ecological models to show that the conditions for maintenance of genetic variation can be greater in fluctuating environments in which there is the opportunity for genotypes to escape unfavourable environments via the storage mechanism. Off season flowering observed in neem coupled with high longevity of seeds at ambient temperature observed in some genotypes in various provenances could be a mechanism to escape unfavourable environments or to produce the offsprings in

an entirely different environment leading to more probability of survival of diverse individuals and thus maintaining the diversity due to temporal variation.

It is generally believed that most of the quantitative and physiological traits are liable to environmental modification and may respond to different types of stress. Thus, same population may respond differently in different environments. The clinal variation observed in the study of fatty acids (especially stearic and oleic) probably indicate genetic and / or physiological adaptation of these traits in response to temperature and other environmental factors during fruit setting period. Furthermore, the western populations in Haryana and Rajasthan are distinct from eastern populations of Haryana in having lower amount of linoleic and stearic acid. Cluster analysis of 28 genotypes based on fatty acids, oil content and seed morphological characteristics reveals two distinct groups of populations according to rainfall associated with their respective origins, indicating possible adaptation to the local climatic conditions. Kundu (1999), on the basis of cluster analysis of physiological and growth traits, reported three distinct groups of populations in neem according to the rainfall, indicating adaptation to local climate in neem.

Genetic polymorphisms are regulators of the breeding system of populations in many cases. For example, pre- and post-zygotic incompatibility, and different sexes are the foremost genetic ~~mechanism that~~ mechanisms that prevent self-fertilization (Stern and Roche, 1974). The probable presence of an embryonic lethal may be an instance of post-zygotic incompatibility in neem. A small possibility for seed formation from self-fertilized zygote in neem has been reported by Singh *et al.* (1996). Protandry may be an example of pre-zygotic incompatibility (Gupta *et al.*, 1996b) in this species. Self-compatibility in plant populations is generally known to be an adaptive strategy (Stern and Roche, 1974). The variation recorded in seed development in the 'trilocular endocarp' (having six ovules per endocarp) appears to be an additional adaptive feature; a phenomenon that explains increased probability to produce outbred seeds. The observed polycarpy in this study seems to be an effective mechanism for avoiding inbreeding that ultimately directs a predominantly outcrossing mating system in this species. the andromonoecious habit is an advanced reproductive feature in neem, which is probably an evolved form of the dioecious habit. Similar observations were made by Kundu (1999c).

As plasticity studies have extended beyond simple effects on plant growth, it has been found that key life-history traits such as sex expression and breeding system, reproductive allocation and phenology can vary in responses to the environment. For example, the proportions of staminate and hermaphroditic flowers in an andromonoecious *Solanum* were shown to depend on plant resource status, confirming a long-standing ecological hypothesis (Diggle, 1994). Normally self-incompatible plant

can switch to self-fertilization in response to floral age and lack of prior fruit development (Vogler *et al.,* 1998). This plasticity for self-compatibility results in a 'delayed selfing' strategy that insures reproduction if out crossing fails. This case makes it clear that a plastic switch can occur in response to a plant's internal environment as well as to resource availability or other external cues (Sultan, 2000).

Neem trees growing in areas with a warm winter bloom first, followed by areas where the winter is comparatively colder. Depending on the locality, flowering may range from January to May (Gupta *et al.*, 1995). Sporadic flowering in September-October has been observed quite often, in addition to that in February-March. Shanthi *et al.* (1996b) reported abnormal seedlings in December from these trees and suggested that these trees may be used as germplasm. In the Murshidabad area of West Bengal (India), the tree flowered throughout the year (Guhabakshi, 1984). Neem normally flowers in April [in Tamil Nadu] and the fruits are ready for harvesting by July, although the fruiting season sometimes extends to August. The seeds of neem have a short period of viability (only a few months after harvest) so late flowering forms would be useful for solving seed procurement problems for later planting. Such a genotype was identified in Tirupur by Shanthi *et al.* (1996b) As well as flowering and fruiting at the normal times this tree flowered in September and produced fruits in December. Seed germination and seedling characteristics were compared from the 2 periods; germination was 83 per cent in the early flowering type and 67 per cent in the later flowering type. Seedling growth was similar, but there were more abnormal seedlings (12 vs. 2 per cent) in the later flowering type, probably due to selfing. Late flowering types would be useful for breeding studies. In the present study flowering and fruiting has been observed in Hisar provenance in October, 1998. As no other tree are flowering at that time, selfing is evident. Fruit development is normal with well developed embryos. The off season flowering may have genetic control or may be due to plastic response of the trees to the environment or combination of both. However this variation in phenology is very important in fixing traits by selfing and other application for producing variability.

Plastic reproductive timing and allocation have been documented in several herbaceous species (Geber, 1990; Pigliucci, 1997). These changes are likely to directly affect plant fitness and therefore population persistence and response to natural selection for example (Galloway, 1995). A selection experiment confirmed that these contrasting reproductive patterns reflect different fitness priorities in the two types of environment: in poor sites, plants have shorter life spans and maximizing early flower production is advantageous; in favourable sites, where plants live longer, greater allocation to vegetative growth followed by later flowering maximizes fitness (Galloway, 1995). Season and duration of reproductive phenoperiods vary according to location and climate. In bi-modal climates there are sometimes two flowering and fruiting seasons.

Variations with respect to phenology, flowering time encountered in the present study points to either highly plastic nature of neem or genetic variation. Nevertheless, as only few individual behave erratically for the phenology, some genetic regulation of plasticity is expected.

Fundamental processes in speciation and evolution are adaptation, natural selection and competition. There are three close relatives of Indian neem, which were previously regarded as varieties of neem. These are *A. siamensis* (VAL.) (Thai neem); A. excelsa (Jack) Jacobs (Sentang or Marrango), and *Melia azedarach* L. (Persian lilac or Chinaberry). Boonsermsuk and Jitjamnong (1989) reported the differences in peroxidase isozyme patterns and pollen morphology of *A. siamensis* and *A. indica*. Natural hybrids between *A. siamensis* and *A. indica* have been recorded in Thailand (Lauridsen *et al.*, 1991). These hybrids are intermediate in their characteristics. Kundu (1999), on the basis of isozyme studies, demonstrated that evolution within *A. indica* has taken place by allelic differentiation. Dendrograms based on morphological and biochemical and molecular data generated in the present study suggest a tendency of speciation in neem as evident by highly divergent genotypes. Further studies involving a number of populations covering a wide geographical range would provide clearer evidence for the degree of speciation in this species. The RAPD analysis of 15 genotypes and 5 populations in the present study shows clear allelic differentiation among the individuals. Limited genotypes are used in the present investigation. Nevertheless, the present data on RAPD for neem populations provides substantial evidence of the species in different ecological races or ecotypes.

Breeders have successfully improved crops despite not knowing the genes affecting quantitative traits. The numbers of genes controlling quantitative traits in different crops are yet unknown, although rough estimates include 69 loci for oil and 173 loci for protein content in the maize kernel (Dudley and Lambert, 1992). Experiments in many plant species have indicated that few quantitative trait loci have large effects, whereas many loci have smaller effects (Kearsey and Farquhar, 1998). Most of important traits in crops are quantitative and are controlled jointly by many loci. In maize as a model species, it has been found through computer simulation that gene information is most useful in selection when few loci (e.g., 10) control the trait. With many loci (≥50), the least squares estimates of gene effects become imprecise. It is estimated gene information consequently improves selection efficiency among hybrids by only 10 per cent or less, and actually becomes detrimental to selection, as more loci become known. Increasing the population size and trait heritability to improve the estimates of gene effects also improves phenotypic selection, leaving little room for improvement of selection efficiency via gene information (Bernardo, 2001). Little or no information on molecular aspects is available in neem on various traits, therefore a larger cross-sectional collection

of neem germplasm and understanding the interaction of different traits at phenotypic level in varying environments may be sufficient in leading future improvement in this tree for the desirable traits.

This study is an indication that variability for tree architecture, phenology, endocarp morphological traits, seed biochemical characteristics, seedling growth characteristics, and seed viability is present in nature. For effective exploitation of this variability, it is desirable to undertake an elaborate survey over a number of years, incorporating still greater cross-sections of agro-ecological regions and microclimates in the Indian sub-continent to identify consistently high yielding ecotypes and/or potentially productive habitats within each agro-ecological region. This exercise would help in locations of suitable ecotypes and also help in proper planning and ascertaining the economic viability of commercial plantations of neem for the purpose of commercial production.

Due to long life cycle, some reliable markers for high yielding genotypes may be useful in breeding programs of neem. Morphological markers, limited in number, often do not reliably portray genetic relationships because of environmental interactions and the largely unknown genetic control of the traits. Moreover, in the present study no consistent/significant correlation of oil yield and other important biochemical characteristics with seed morphology could be found. This warrants development of some reliable biochemical markers (viz., isozymes, polypeptide pattern, etc.) and molecular markers (based on DNA sequences) for predicting the production potential of future plantations with respect to oil, fatty acids, azadirachtin, etc.

In summary, our results show that there is a wide range of variation residing in the studied materials at within and between provenance levels. Therefore, future germplasm collection should concern all levels of variation. Morphological, physiological and molecular marker data have revealed ample genetic diversity in neem at population and individual levels. The clinal variation observed in some morphological, physiological and biochemical characteristics in neem suggests that for future germplasm sampling and conservation, more attention should be paid to populations along gradients of growing period, rainfall and temperature. The enormous variation documented in the present investigation would serve future improvement and conservation tasks.

Biological evolution is usually considered a series of processes involving descent of organisms with modification marked by successive adaptations to environmental conditions, governed by competition and natural selection acting on variation. The fundamental components of evolution are variation, speciation and phylogeny (Radford, 1986). The genetic system and its components determine the capability of a population to undergo evolutionary changes. The foregoing accounts indicate that there is large genetic diversity in neem at population and individual levels.

6. Summary and Conclusions

Two hundred and twenty one germplasm accessions of neem from nine states of India (Haryana, Rajasthan, Delhi, Himachal Pradesh, Uttar Pradesh, Panjab, Karnataka, Tamil Nadu and Maharashtra) were studied for all or some of the following aspects *viz.* tree architecture, endocarp morphology, seed viability, provenance evaluation through growth of seedlings in nursery conditions; oil content, fatty acid profile, protein content and azadirachtin content of seeds; temporal stability of endocarp morphological characteristics and seed oil content; and divergence analysis. In addition, thirteen local trees were taken up for studying leaf morphology and tissue culture experiments.

The investigations on architecture of neem trees comprised survey of 14 sites of Haryana and adjoining states. The tree architectural parameters *viz.* diameter at breast height (dbh), basal girth, total height, clear bole height, unforked height, crown spread, total height : dbh, clear bole height : total height, unforked height : total height, crown spread : dbh, crown spread : total height; and qualitative parameters (stem straightness index, crown shape index, branch angle index, apical dominance index, fruiting intensity index, tree health, colour of leaves, and leafiness) were recorded for individual trees of each site selected at random The data were statistically analysed in order to find out the extent of phenotypic variation in natural populations and to estimate association among quantitative and qualitative characteristics.

Considerable variation was recorded both within and between provenances in trees in Haryana and adjoining areas with respect to tree architectural parameters and their linear associations. Stem form ranged from crooked or forked to nearly straight. Out of 136 trees, six trees with conical crown shape were found scoring four with respect to crown shape index. Two trees were found to show cylindrical crown shape scoring five with respect to crown shape index. Twenty three trees were found to have completely straight stem scoring five with respect to stem straight. index, while 46 trees were found to have scored four with respect to stem straight. index. Sixteen trees were found to have unusual character of fruits turning brown on the tree itself. Sixteen trees were observed to have low branch angle.

Association of different architectural characteristics of trees in all the 14 sites combined was studied. Diameter at breast height was positively and significantly associated with basal girth, height, crown spread and crown spread : height; and negatively and significantly associated with and height : dbh, clear bole height : height, unforked height: height, unforked height and crown spread : dbh, however within provenances degree of association amongst various characteristics differed largely. Based on data of 126 trees, the rank correlation of apical dominance index with branch angle index and fruiting intensity index was found to be negative and significant. The

association of crown shape index with apical dominance index and fruiting index was found to be negative and significant.

Out of 136 trees covered under survey, 58 candidate plus trees (CPTs) for agroforestry, 45 CPTs for seed production as well as agroforestry and 47 CPTs for seed production were marked. Multiple regression equations (with very high level of goodness of fit of the regression line) based on the available tree architectural parameters could be developed for predicting values of clear bole, unforked height and ratio of unforked height and height both in individual sites and all the sites taken together. Highly divergent trees could be identified for combination of important tree architectural parameters with the help of principal component and factor analysis.

Ten tree architectural characteristics were considered for principal component and principal factor analyses in 126 trees of Neem. Principal component method was used to extract the principal factors. Four characteristics (clear bole : dbh, crown Spread : dbh, height : dbh, and unforked height : dbh) had very high loading on the first factor. Three characteristics (clear bole : unforked height, unforked height, unforked height : height) on third; two (clear bole, clear bole : height) on third factor and one (crown Spread : Height) on fourth factor.

Principal factor scores for all the genotypes were estimated in all the three principal factors. Using the principal factor scores, all the genotypes were plotted for by taking two principal. These plots clearly indicated the separation of genotypes with high values of the characteristics which they represent. Genotypes with contrasting tree architectural characteristics and their combinations could be delineated with different plots.

Statistically significant differences (both within and between populations) were observed for most of the endocarp morphological characteristics studied in 43 trees belonging to 12 provenances. Association of endocarp morphological characteristics was studied using simple correlation. Contrasting association behaviour was observed for most of the traits across individual trees. Genetic parameters were studied for endocarp morphological characteristics and considerable within and between population variation was recorded. For most of the traits heritability varied from low to high. While for number of seeds endocarp^{-1} the heritability could not be ascertained in most of the provenances. Moderate to high estimates of genotypic coefficient of variation, broad sense heritability and genetic advance were obtained for endocarp weight and seed weight, indicating high level of genetic variability and potential of improvement for these traits through selection. Major portion of genetic variability was explained by within population genetic variability.

Statistically significant genotypic and phenotypic correlations were observed between characteristics like endocarp length, endocarp weight, seed weight in some of the populations and for most of the characteristics when all the populations were combined

suggesting the possibility of enhanced correlated response upon indirect selection for these morphological characteristics.

Separation of the genotypic correlation coefficient into components of direct and indirect effects was done using path coefficient analysis for seed weight and ratio of seed weight and endocarp weight. Seed weight was both directly and indirectly affected by endocarp weight and endocarp coat weight in most of the provenance collections. Ratio of seed weight and kernel weight was directly and indirectly affected by all the morphological characteristics of endocarp in all the provenance collections under study.

Six leaf morphological characteristics of thirteen trees of Hisar provenance were studied. Statistically significant differences were observed for most of the characteristics in many trees. There was positive association amongst all the leaf characteristics though only some of the associations were significant.

Seed viability was tested in the seeds collected from 21 trees representing six provenance collections from Haryana, Delhi and Rajasthan after six months of storage at 25±2°C. Within and between provenance variation with respect to seed viability was observed. Viability of seeds appeared to be affected by the oil content of seeds rather than fatty acid profile. Regression equation for linear regression of seed germination on seed oil content gives high coefficient of determination ($R^2 = 0.784$) pointing to very high level of goodness of fit of the regression line.

Provenance evaluation of different provenance collections was done in nursery after a growth period of 3 months. Seeds from 27 trees representing 17 provenance collections were considered. Significant correlation was found between seedling height and collar diameter. Seedling length was found to be positively and significantly associated with seed weight.

Statistically significant differences (both within and between populations) were observed for seed oil content of 27 neem trees from five provenances of neem belonging to northern and western India. Maximum average oil content was observed in trees from Hisar provenance. Seed oil content in most of the provenances was not consistently and significantly correlated with morphological parameters of seeds. Age of the tree appeared to have no significant effect on the oil yield.

Fatty acid profiles of seeds of 28 neem trees from six provenance collections belonging to northern and western India were studied. Considerable variation was observed for two saturated (palmitic acid and stearic acid) and two unsaturated (oleic acid and linoleic acid) fatty acid. Oleic acid was found to be the major fatty acid. Ratio of unsaturated to saturated fatty acids exhibited a wide range. No uniform/significant association was observed amongst different fatty acids in most of the accessions except that between the two unsaturated fatty acids under study. Fatty acid profiles (stearic acid

and oleic acid) of seeds showed a strong association with geographical position and agroclimate of the sampling sites. The study demonstrates that neem genotypes available from different agro-ecological regions of India have considerable potential to offer high oil content and various combinations of fatty acids (particularly higher content of unsaturated fatty acids) which can be exploited as such (by clonal multiplication to exploit genetic variation) or put to further genetic improvement through traditional/molecular breeding.

Multiple regression analysis of linoleic acid on all other seed characteristics (namely, palmitic acid, stearic acid, oleic acid, unsaturated fatty acids, saturated fatty acids, ratio of unsaturated and saturated fatty acids, 20 endocarp weight, and endocarp length,) in *Azadirachta indica* was done. Coefficient of determination (R^2) was very high (0.99), pointing towards very high level of goodness of fit of the regression line.

Statistically significant differences were observed with respect to seed protein content in 10 neem trees. Average protein content was 12.91 per cent with a range of 10.06 to 16.63 per cent. Correlation coefficient of protein with oil content showed a significant value. Protein content of seeds was found to be positively associated with longitude. Statistically significant differences between individual trees were observed for seed azadirachtin content. Average azadirachtin content for the seed samples of seven seed samples from five provenance collections from Haryana is 0.3987 per cent with a range of 0.28 per cent to 0.58 per cent and a coefficient of variation of 31.85.

D^2 values for all possible (903) combinations among 43 genotypes (using all eight endocarp morphological characteristics) were calculated and these genotypes were grouped into ten clusters. Cluster CI consisted of maximum number of genotypes i.e., 12. Clusters namely, CII, CIII, and CIV consisted of 10, 7, and 5 genotypes, respectively. The next clusters, *viz.* CV, CVI, CVII and CVIII had 2 genotypes each, whereas CIX and CX were represented by one genotype each. The genotypes of CIX and CX were extraordinary for one or the other character that made them so divergent from others.

Endocarp morphological data of 43 genotypes of neem (all 8 endocarp morphological characteristics used) were subjected to hierarchical cluster analysis with the help of squared euclidean distance and UPGMA approach. Two major clusters were visible. First cluster contained only one genotype (NGN1) from Pune while second cluster was further divided into 2 sub-clusters.

Seed biochemical and endocarp morphological data of 28 genotypes of neem were subjected to hierarchical cluster analysis of using squared euclidean distance and UPGMA approach. Two major clusters were discernible from the dendrogram. First Cluster contained 9 genotypes containing mainly genotypes from arid (Bikaner) and semi-arid regions (Hisar). One exceptional entry was the genotype from Allahabad which is not having arid type of climate. Second cluster contained 19 genotypes mainly

genotypes from semi-arid (Hisar) and other non-semi-arid regions of Haryana namely, Kaithal; and Kalka (Shivalik foothills) and Pune (Maharashtra).

Ten morphological and biochemical characteristics of endocarp were considered for principal component and principal factor analyses in 28 genotypes of neem. The first three principal components (PCs) gave eigen values more than one and altogether explained 84.864 per cent of accumulated variability. Seven characters (related to fatty acid profile) had very high loading on the first factor. Endocarp length and 20 endocarp weight had high loading on second factor. Oil content of the endocarp had high loading on third factor. This provided a very clear picture regarding the idea of character association with respect to different principal factors.

Principal factor scores for all the genotypes were estimated in all the three factors. Using the principal factor scores, all the genotypes were plotted using all the pairs of factors. Three factor plots clearly indicated the separation of genotypes with high values of the characteristics towards the axis of factors which represent these characteristics. Genotypes with contrasting fatty acid profile could be delineated with different plots and some highly divergent genotypes could be identified.

Eight morphological characteristics of endocarp were considered for principal component and principal factor analyses in 43 genotypes of neem. The first three principal components gave eigen values more than one and explained 90.2 per cent of accumulated variability. Five morphological characteristics of endocarp (endocarp weight, seed weight, endocarp coat weight, endocarp length, and endocarp breadth) had very high loading on the first factor. Endocarp length : breadth had high loading on second factor. Seed weight : endocarp weight and number of seeds endocarp^{-1} had high loading on second factor. Principal factor scores for all the genotypes were estimated in all the three principal factors. Using the principal factor scores, all the genotypes were plotted for factor pairs and genotypes with contrasting endocarp morphology could be delineated with different plots.

Using multivariate analyses (D^2 analysis; hierarchical cluster analysis; and principal component and principal factor analysis) it was possible to delineate highly divergent samples with respect to endocarp morphological seed biochemical characteristics. Clustering pattern found to have partial dependence on geographical location of the germplasm accessions. Some similarity was observed in clustering pattern obtained by different multivariate techniques.

Analysis of variance for assessing temporal stability was performed in 11 trees of neem for eight endocarp morphological characteristics. Genotype and environment (year) interactions for all these eight characteristics were found non-significant which suggested that genotypes have no interaction with environment for the given characteristic. However, twenty endocarp weight and seed oil content exhibited significant genotype-

environment interaction and differences were observed with respect to temporal stability. For these two characteristics stable and unstable genotypes could be identified.

Tissue culture studies were designed to see the efficiency of mature seeds and other vegetative explants (axillary buds, shoot tips and leaves) for *in vitro* shoot regeneration and also to explore the possibility of *in vitro* production of azadirachtin in callus cultures. During incubation in dark, segmented seeds developed callus growth and shoot primordia. The shoot primordia and multiple shoots could be elongated upon transfer to fluorescent white light. Regenerated shoots could not be rooted. Callus was induced in vegetative explants (axillary buds, shoot tips and leaves) from a mature tree of Hisar provenance. Callus cultures could be maintained by frequent sub-culturing. Callus cultures produced from axillary buds were analysed for production of azadirachtin after 40 days of incubation. In N6 (MS medium + 0.8 mg l^{-1} NAA + 2.0 mg l^{-1} BAP) medium the amount of azadirachtin produced was 0.00027 per cent and in N15 (MS medium + 2.0 mg l^{-1} kinetin) medium a concentration 0.00028 per cent azadirachtin was recorded.

Protocol for fingerprinting the neem genotypes using RAPD (Random Amplified Polymorphic DNA) technique was standardised and preliminary investigations with 15 genotypes and pooled DNA samples of 5 populations indicated genetic variability within and between populations. A total of 20 random sequence commercially available deca-nucleotide primers of 60 percent or more of G + C content were used for the amplification of DNA for RAPD analysis. Of these, 15 primers did not amplify DNA of any of the neem genotypes, while five primers could amplify most of the individual 15 DNA samples and all of the five pooled DNA samples. Total number of DNA fragments amplified by a specific primer / primer pair in individual DNA samples varied from minimum zero to maximum seven. However, polymorphic bands produced by different primers / primer pair ranged from three to six. Total number of DNA fragments amplified by OPC-12 in pooled DNA samples varied from minimum of six to maximum of ten. However, polymorphic bands produced were four only. Polymorphism was determined by calculating pairwise dissimilarity value (1-F) among different neem genotypes. The formula for genotype discriminating efficiency of individual primers was formulated and genotype discriminating efficiency ranged from 26.67 to 88.89 per cent for individual genotypes. Dendrogram of 12 genotypes based on RAPD profile of OPC-1, OPC-11 and OPC-12 primers was constructed using UPGMA method and pair wise Jaccard similarity coefficient between genotypes. Highly divergent genotypes were found by this exercise.

Morphological, physiological and molecular marker data revealed ample genetic diversity in neem at population and individual levels. Clinal variation observed in seed biochemical characteristics like stearic acid and oleic acid suggests that for germplasm conservation attention should be paid to populations according to latitudinal and longitudinal gradients.

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Appendix 1. List of provenance collections of *Azadirachta indica* collected for the present study

State	Locations covered	Number of trees selected/surveyed
Haryana	Hisar[a, b], Barwala, Sirsa, Fatehabad, Uklana, Hansi, Agroha, Namaund, Jhumpa, Siwani, Bhiwani, Tosham, Charkhi Dadri, Mahendergarh, Namaul, Rewari, Bawal, Gurgaon, Sohna, Nuh, Palwal, Ballabhgarh, Faridabad, Jajhar, Bahadurgarh, Rohtak, Meham, Namaund, Jind, Narwana, Asandh, Sonepat, Kaithal[a, b], Karnal, Nilokheri, Kurukshetra, Pehowa, Ambala, Jagadhari, Kalesar, Narayangarh, Kalka[a, b], & Pinjore.	134
Delhi	IARI Campus[b].	9
Rajasthan	Bikaner**[a b], Jodhpur[b], Alwar[b], & Bhorugram.	16
Himachal Pradesh	Nahan, Kala Amb.	4
Maharashtra	Pune**[a]	4
Tamil Nadu	Chitoor, Uthupathy, Irrukangundi, Vallapaddy, Sattur, Andipatti, Thiruvanna malai, Adhu..urai, Mettupalayam, Karur, Gankayam, Palladam, Sathy, Erode, Gobi, Kurumandur, Pallathur, Namakkal, Velankanni, Gudiatham, E. K. L. Puram, Anthiyur, S. N. Palayam, Cuddalore, Kanyakumari, Pondy, Paramgudi, Paratharami, Thiruvarur, Sengottai, Krishnagiri, Aruppukottai, Kumulur, Madras, Tirunelveli, Trichy, Thiruvallur, FC & RI, Ramnad, Sathankulam.	41*
Karnataka	Bijapur.	3*
Panjab	Rajpura, Patiala, Malerkotla, Central zone*, & Kandi zone*.	2 + 2*
Uttar Pradesh	Dehradun**[a], Jwalapur, Kanpur, & Mirzapur	4 + 2*
	Total	221

*Seeds received from Neem Network participants from Tamil-Nadu, Karnataka, & Panjab

** not collected in year 1998; [a] collected in the year 1995; [b] collected in the year 1997

Appendix 2. Method adopted for nomenclature

In the Accession number of trees :
first two letters indicate the state;
next two letters indicate the district;
next two letters indicate village/location;
last two numerals indicate the serial number of the tree collected from a particular site.

e.g. States: Ha = Haryana; Ra = Rajasthan; De = Delhi; Hp = Himachal Pradesh; Pb = Panjab;

Districts : Hi = Hisar; Jo = Jodhpur; Al = Alwar; Ch = Churu; Fa = Fatehabad; Si = Sirsa; Ma = Mahendrgarh; Bh = Bhiwani; Re = Rewari; Gu = Gurgaon; Jh = Jhajjar; Ro = Rohtak; Fr = Faridabad; Nr = New Rajendra Nagar; Na = Najafgarh; Pu = Pusa; So = Sonepat; Ji = Jind; Kr = Karnal; Ka = Kaithal; Ku = Kurukshetra; Am = Ambala; Ya = Yamunanagar; Kl = Kalka; Pt = Patiala; Sr = Sirmour; Pw = Parwanoo;

Appendix 3. Plant material used in the present study

Explant	Provenance	Explants source
axillary buds, stem segments, leaves, shoot tips, mature seeds	Hisar	Young tree

Appendix 4. Description of the explants used in tissue culture studies

Explant / inoculum	Size	Description
Axillary buds	10-30 mm	Excised from the shoots with a portion of stem on both ends, contains 1-2 axillary buds
Shoot tips	15-40 mm	Contain 2-4 axillary buds in addition to the shoot tip (growing tip)
Leaves	-	Contain the leaf lamina and the rachis portion, either the leaf was segmented into 2-4 parts or intact leaf explants were inoculated after injuring the lamina and rachis with a blade
Stem segments	5-15 mm	Stem + callus developing on the lower internodal portion of axillary bud was used for further culturing

Appendix 5. **Description of sampling sites considered for studies on tree architectural parameters of *Azadirachta indica***

Site	N	Provenance(s)
Site 1	9	Delhi
Site 2	12	Gurgaon + Faridabad
Site 3	8	Rohtak + Jhajjar
Site 4	17	Alwar + Rewari
Site 5	18	Hisar
Site 6	12	Jind + Narnaund
Site 7	8	Kaithal + Kurukshetra
Site 8	8	Ambala + Yamunanagar + Patiala
Site 9	4	Sirmour
Site 10	7	Kalka + Pinjore
Site 11	6	Jodhpur
Site 12	6	Bhiwani+Bhorugram
Site 13	9	Hisar+Sirsa
Site 14	11	Sirmour + Kalka + Pinjore
Total	135	

N = number of accession

Appendix 6. **Description of sampling sites and germplasm accessions considered for studies on endocarp morphological characteristics of *Azadirachta indica***

Site	N	Germplasm accessions included	Y	Provenance
1	2	T17, T18	1997	Hisar-I
2	3	T3, T4, T5	1997	Bikaner
3	5	T41, T42, T43, T44, T45	1997	Raipur
4	3	T62, T63, T64	1997	Bhatinda
5	5	T23, T24, T20, T22, T21	1998	Jodhpur
6	5	H099, H100, H101, H102, H 107,	1998	Kalka
7	2	T5D, T7D	1998	Delhi
8	3	T30, T31, T33	1998	Alwar
9	6	HSR01, HSR02, HSR03, HSR04, HSR05, SHS-0	1998	Hisar-II
10	4	NGN1, TER2, TGN3, NMN4,	1995	Pune
11	3	H082, H084, H085	1998	Kaithal
12	4	DRN1, DRN2, DRN3, DRN4	1995	Dehradun
Total	43			

N = number of accession ; Y = year of collection

Appendix 6a. **Description of sampling sites and germplasm accessions considered for studies on endocarp morphological characteristics of *Azadirachta indica* collected in more than one year**

Provenance	Year of collection		
	1995	1997	1995
Kalka	H099	81	KLK4
Kalka	H100	82	KLK1
Kalka	H101	83	KLK2
Kalka	H102	80	KLK5
Kalka	H107	NC	KLK3
Hisar	HSR01	12	HSR6
Hisar	HSR02	11	HSR1
Hisar	HSR03	13	HSR3
Hisar	HSR04	14	HSR2
Hisar	HSR05	10	NC
Hisar	SHS-0	15	HSR4
Jodhpur	T20	20	NC
Jodhpur	T21	21	NC
Jodhpur	T24	24	NC
Jodhpur	T23	23	NC
Jodhpur	T22	22	NC
Alwar	T30	30	NC
Alwar	T31	31	NC
Alwar	T33	33	NC
Delhi	T5D	T5	NC
Delhi	T7D	T7	NC
Delhi	NC	T1	T1
Delhi	T6D	T6	NC
Kaithal	H082	50	KTL1
Kaithal	H083	51	KTL5
Kaithal	H084	53	KTL6
Kaithal	H085	54	NC
Kaithal	NC	52	KTL2
Bikaner	NC	1	BNR1
Bikaner	NC	2	BNR2

NC = not collected

Appendix 7. Detail of DNA samples from individual plants of *Azadirachta indica* used for RAPD analysis

Sr. No.	DNA sample Identification Number	Accession Number*	Provenance	Source plant
1	G 7	HSR01a	Hisar	Single plant in the nursery
2	G 8	HSR01b	Hisar	Single plant in the nursery
3	G 9	Adu.	Aduthurai	Single tallest plant in the nursery
4	G 10	H048	Rohtak	Single plant in the nursery
5	G 11	H108a	Kalka	Single plant in the nursery
6	G 12	H028	Narnaul	Single plant in the nursery
7	G 13	HSR01t	Hisar	Single tree
8	G 14	HSR03	Hisar	Single tree
9	G 15	HSR04	Hisar	Single tree
10	G 16	H079	Nilokheri	Single plant in the nursery
11	G 17	H080	Kurukshetra	Single plant in the nursery
12	G 18	H093	Sirmour	Single plant in the nursery
13	G 23	H108b	Kalka	Single plant in the nursery
14	G 24	GPN-0	Hisar	Single tree
15	G 25	HSR02	Hisar	Single tree

*according to the year 1998 collection

Sr. No. = serial number during RAPD analysis and dendrogram construction

Appendix 8. Detail of pooled DNA samples of different provenance collections of *Azadirachta indica* used for RAPD analysis

S. No.	DNA sample Identification Number	Accession Number* of parent trees (number of seedlings pooled)	Provenance	Description of source of leaves fro DNA isolation
16	1R	H105(4), H108(2), H106(3), H107(1), H101(1), H099(1)	Kalka	12 seedlings pooled
17	2R	UAS Dharwad-2 (2), UAS Dharwad-3 (1)	Dharwad	3 seedlings pooled
18	3R	T7 (1), H065 (1)	Delhi	2 seedlings pooled
19	4R	H048 (2), H046, H049 (2)	Rohtak	5 seedlings pooled
20	5R	H080 (1), H084 (1)	Kaithal + Kurukshetra	3 seedlings pooled

*according to the year 1998 collection

S. No. = serial number during RAPD analysis

Appendix 9. Reagents and stock solutions for DNA isolation and electrophoresis

1. 50X TAE (Tris-acetate buffer)

Stock	Volume
Tris Base	242g
Glacial acetic acid	57.1ml
0.5M EDTA pH 8.0	100ml

Add water to make volume 1000ml

Mix well and store at 4°C

2. 6X Gel buffer

BPB	0.25%
Sucrose	4g

Add water to make volume 10ml; Mix well and store at 4°C

3. Wash-I (76% ethanol, 0.2M Sodium acetate)

Stock	Amount to be added for making 100ml
Ethanol	76ml
3M $CH_3COONa.3H_2O$	6.67ml
Water	17.33ml

4. 3M Sodium acetate solution

$CH_3COONa.3H_2O$	408.1g
Water	400ml

Adjust pH to 4.8 with glacial acetic acid

Add water to make volume 1000ml and autoclave

5. 0.5M EDTA pH 8.0

Dissolve 186.12g EDTA disodium salt in 600ml of water. Adjust pH to 8.0 with 6N NaOH. Make volume to 1000ml with water

6. CTAB buffer

Stock	Amount to be added for making 100ml buffer
Water	46ml
1M Tris pH 8.0	20ml
5M NaCl	28 ml
0.5M EDTA pH 8.0	4 ml
β-Mercaptoethanol	2.0ml
CTAB	2.0g

Add CTAB to warm (60-65°C)

Add β-Mercaptoethanol just prior to use

7. Wash-II (76% ethanol, 10mM Ammonium acetate)

Stock	Amount to be added for making 100ml
Ethanol	76ml
7.5M Ammonium acetate	0.133ml
Water	23.867ml

8. Tris EDTA (TE) buffer (10mM Tris HCl pH 8.0, 1mM EDTA pH 8.0)

Stock	Amount to be added for making 100ml
1M Tris HCl pH 8.0	10ml
0.25M EDTA	4ml

Add water to make volume 1000ml and autoclave

9. 1M Tris pH 8.0

Dissolve 121.1g Tris base in 600 ml water. Adjust pH to 8.0 with concentrated HCl. Make up the volume to 1000 ml.

Appendix 10. Temperature cycles used in RAPD analysis

Step	Function	Temperature (^{0}C)	Duration (minutes)
I	Initial denaturing step	94	3
II	Denaturing	94	1
	Annealing	40	1
	Extension	72	3
Repeat step II 40 times (40 cycles)			
III	Final Extension step	72	15

After final extension the samples were held at $4^{O}C$ until sample retrieval. The samples were stored in sub-zero temperature before electrophoretic separation.

Appendix 11. Detail of random primers used for RAPD analysis

Primer code	Sequence of primer	GC content (%)
OPC-1	5'-TTCGAGCCAG-3'	60
OPC-12	5'-TGTCATCCCC-3'	60
OPD-2	5'-GGACCCAACC-3'	70
OPD-3	5'-GTCGCCGTCA-3'	70
OPC-11	5'-AAAGCTGCGG-3'	60

Other primers used but failed to produce amplification products were OPC-2, OPC-8, OPC-5, OPC-3, OPC-6, OPC-4, OPC-10, OPC-09, OPC-13, OPC-14, OPC-7, OPC-11, OPC-15, OPC-1 + OPC-12, OPC-12 + OPD-2, OPC-1 + OPD-2, OPC-1 + OPD-3, OPC-12 + OPD-2, OPC-8 + OPC-5, OPC-3 + OPC-6, OPC-7 + OPC-11, OPB-7 + OPH-14 + OPI-5, OPB-7 + OPD-3 + OPC-9 + OPD-2, OPC-2 + OPC-6 + OPC-14 + OPC-10

Appendix 12. Composition of Murashige and Skoog (1962) medium

Constituent	Quantity (mg l^{-1})
NH_4NO_3	1650
KNO_3	1900
$MgSO_4.7H2O$	370
$CaCl_2.2H2O$	440
KH_2PO_4	170
$FeSO_4.7H2O$	27.8
Na_2EDTA	37.3
$MnSO_4.H2O$	22.3
$ZnSO_4.7H2O$	8.6
H_3BO_3	6.2
KI	0.83
$Na_2MoO_4.2H2O$	0.25
$CuSO_4.5H2O$	0.025
CoCl.6H2O	0.025
Myo-inositol	100
Nicotinic acid	0.5
Pyridoxine HCl	0.5
Thiamine HCl	0.1
Glycine	2.0
Sucrose	30000

Appendix 13. Composition of media based on MS medium

Medium Code	Growth regulators (mgl^{-1})		Glycine (mgl^{-1})	Sucrose (gl^{-1})
	Auxin	Cytokinin		
N1	-	Kin = 2.0	2.0	30
N2	-	BA = 2.0	2.0	30
N4	NAA = 0.4	BA = 1.0	2.0	30
N6	NAA = 0.4	BA = 1.0	2.0	90
N8	-	BA = 4.0	2.0	90
N10	NAA = 1.0	Kin = 0.5	4.0	30
N11	NAA = 1.0	Kin = 0.5	8.0	30
N12	NAA = 0.4	BA = 1.0	2.0	30
N13	2,4-D = 2.0	-	2.0	30
N14	IAA = 1.0	Kin = 0.5	2.0	30
N15	NAA = 0.8	BA = 2.0	2.0	30
N16	2,4-D = 1.0	Kin = 0.5	2.0	30

Appendix 14. Definition of Concepts

Provenance / origin: Provenance, source and origin: In forestry provenance refers to the population of trees growing at a particular place. It denotes the geographical area from which seeds or other propagules are obtained (Callaham, 1964; Jones and Burley, 1973). Provenance means simply the source of population sample representing a defined are (Matyas, 1997).

Adaptation: Adaptation is a central phenomenon of evolutionary biology. All organisms are adapted to both their physical and biological environment. Jepsen et al. (1949) defined adaptation as correlation in a way useful to the organism, between structure, function and environment. Thus, biological adaptation can be considered to be biological axiom.

Adaptation is a change of the genetic program of a plant or animal, which increases its 'fitness in the prevailing environment. Again fitness is the average success of genotype in producing surviving offspring compared with a reference genotype (Usually with an average of the population, or one of th parent genotype). Natural selection is the mechanism that increases the fitness and enhances the adaptation of the individual or the population to the existing environmental conditions.

The concept of fitness and adaptedness seem to gain more importance in cultivated ecosystems, because of concerns of high input and projected climate instability (Matyas, 1997).

'Origin' represents a geographic place with in the natural distribution of a species form where seeds or other propagation materials are collected. The word 'provenance' has been used in an inconsistent way, although it generally refers to the 'source' (Tigerstedt, personal communication). The basic difference between origin and source is that natural selection may have changed the genetic composition and thus adaptedness of the population.

However, in this study population and provenance are considered as synonymous. Here, the term 'provenance' in a broad sense includes all seed sources of neem anywhere in the world.

Provenance research defines the genetic and environmental components of phenotypic variation associated with geographic source. Assuring sources of seeds to give well adapted, productive trees in directing breeding of inter-racial and inter-specific hybrids towards adaptation to the particular localities. The scope of provenance research includes a) studies of inherent adaptive variation related to "ecological variability within species", and b) studies of the inherent non-adaptiveness differences that might result from isolation or other factors.

Cline: The cline concept was proposed by Huxley (1938). It considers a phenotypic trend of genetic origin, within a species, across the environmental gradients. The environmental gradients may be geographical or ecological in nature. A cline is the change in gene frequency of an allele or (genotype) over space. A cline, by definition, is based on a single characteristic that shows continuous variation. Recognition of clines for a trait such as drought or cold tolerance in tree improvement is very important.

Ecotype: An ecotype is a group of plants of similar genotype that occupy a specific ecological niche. Turesson (1922) suggested the concept of ecotype. According to him, it is a genotypical response of a species to a particular habitat. The key characteristic of ecotypes is that they represent the adaptation of whole genotypes or gene complexes to specific environments. The ecotype and cline concepts have widespread utility in forestry.