

**Studies on Intraspecific Biodiversity of
Horticultural Traits and *in vitro* Shoot
Morphogenetic Response in Walnut
(*Juglans regia* L.) Germplasm**

Imtiyaz Ahmad Lone

(2007-182-D)



**DIVISION OF POMOLOGY
FACULTY OF POSTGRADUATE STUDIES
SHER-E-KASHMIR UNIVERSITY OF AGRICULTURAL
SCIENCES & TECHNOLOGY OF KASHMIR**

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Horticultural Traits and *in vitro* Shoot
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THESIS

Submitted to

**The Faculty of Postgraduate Studies
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**University of Agricultural Sciences & Technology of Kashmir in
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(POMOLOGY)**

2010





to my beloved Parents

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Pomology,
Shalimar Campus, Srinagar – 191 121
-::o::-

CERTIFICATE – I

This is to certify that the thesis entitled, “**Studies on Intraspecific Biodiversity of Horticultural Traits and *in vitro* Shoot Morphogenetic Response in Walnut (*Juglans regia* L.) Germplasm**” submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy in Agriculture (Pomology)**, to the **Faculty of Postgraduate Studies, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** is a record of bonafide research work carried out by **Mr. Imtiyaz Ahmad Lone (Regd. No. 2007-182-D)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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Title of the Thesis : **Studies on Intraspecific Biodiversity of Horticultural Traits and *in vitro* Shoot Morphogenetic Response in Walnut (*Juglans regia* L.) Germplasm**

ABSTRACT

The present investigation entitled “Studies on intraspecific biodiversity of horticultural traits and *in vitro* shoot morphogenetic response in walnut (*Juglans regia* L.) germplasm” was carried out in order to document the available genetic variability in walnut germplasm and to select elite walnut genotypes possessing superior attributes and quality traits. During the survey, data was recorded on one hundred fifty two (152) walnut trees growing in different areas of Kashmir valley. The study also involved establishment of response of elite walnut selections to different plant growth regulators in shoot morphogenesis.

Remarkable variability was observed in seedling walnut trees for different morphological, nut and kernel characters. Tree canopy/spread varied between (1.20-12.50 m), stem diameter (0.3-3.6 m), leaf length (5.25-24.50 cm), leaf width (2.55-13.50 cm), nut weight (8.5-35.0 g), nut diameter (24.5-50.5 mm), nut length (30.0-60.0 mm), kernel weight (3.50-15.0 g), kernel percentage (31.03-70.0), protein content (14.15-24.25 %) and oil content (50.22-70.0 %). Similarly, variations were also reported for other characters viz., tree vigour, growth habit,

branching habit, leaflet shape, shoot colour, nut shape, shell texture, shell colour, shell seal, shell strength, shell integrity, kernel shrivel and kernel colour.

Woody species have been found to be far more difficult to clone *in vitro* than herbaceous plants. Poor response of the explants from mature woody species to *in vitro* manipulation is usually associated with the problem of browning and explant necrosis. The present studies were conducted on forced explants from three walnut selections (SKUAST 002, SKUAST 008, SKUAST 010). Murashige and Skoog's basal medium supplemented with 0.3 mg/l⁻¹ Benzylamino purine and 0.1 mg/l⁻¹ indole-3-butyric acid gave best response in the establishment of initiating cultures, minimum media browning (80.44 %), minimum explant browning (78.22 %) and minimum mean browning score per explant (9.17 %). The survival (23.45%) and growth of the cultures (21.77%) was also found to be maximum in MS medium supplemented with BAP 0.3 mg l⁻¹ and IBA 0.1 mg l⁻¹.

Key words : Wlanut, *Juglans regia* L., variability, yield component, quality traits, shoot morphogenetic response.

Signature of the Student

Signature of Major Advisor

Dated.....

Dated.....

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Imtiyaz Ahmad Lone

Place : Shalimar, Srinagar

Dated :

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CHAPTER – 1

INTRODUCTION

The Persian walnut (*Juglans regia* L.), known as the English walnut, belongs to the family Juglandaceae. English walnut has its origin in the eastern Europe, Asia minor and points eastward to Himalayan mountains. The native habitat of walnut extends from the Carpathian mountains to Europe across Turkey, Iraq, Afghanistan, South Russia and further eastward into the foot hills of the Himalayas.

The walnut requires a climate which is free from frost in spring and extreme heat in summer. A temperature of even 2 to 3 °C below the freezing point during floral bud swelling, sprouting and/or bloom results in the killing of a large percentage of walnut flowers. Similarly, very hot and continuous spell during summer results in the sunburning of nuts, which subsequently become “blanks”. Such a situation arises if the temperature goes to around 100°F in the shaded area accompanied by low humidity regime for prolonged period. On account of the above climatic requirements of the walnut trees, its cultivation is largely limited to the area between the elevation of 1250 to 2250 m.a.m.s.l. in the Himalayan region. However, it grows successfully in the temperate area of China, Central Asia, Iran, Turkey, Europe and North America.

In India walnuts are usually grown in the mid hill areas of Jammu and Kashmir, Himachal Pradesh, and upper hills of Uttarakhand and Arunachal Pradesh. The soil most suitable for its cultivation should be well-drained and deep silt loamy containing organic matter in abundance. It should not have a fluctuating water level, hard pan and/or sandy sub-soil with alkaline reaction. A soil 2.5 to 3.0 m deep gives best results because the roots can penetrate deep and utilize residual soil moisture during dry spell and also make available sufficient nutrients. Furthermore, availability of sufficient moisture in the leaves can reduce the damage due to sun burning of leaves, shoots and young fruits.

Walnut is grown commercially in about 48 countries with an area of 66, 58, 966 hectares. The world walnut production is about 16, 70, 109 MT. The chief walnut producing countries are China (22%), USA (20%), Iran (12%) and Turkey (10%) (Anonymous, 2007). India accounts for about 2.0 per cent of the world production. In India, Jammu & Kashmir is leading both in area as well as in production with an area of 82.04 thousand ha and production of 146.78 thousand tonnes. However, the productivity level of 1.79 t ha^{-1} is far below than other countries. Himachal Pradesh has an area of 6.54 thousand ha with a production of 1.24 thousand tonnes and productivity level of 0.19 t ha^{-1} ; while Uttarakhand has an area of 19.26

thousand ha with a production of 8.73 thousand tonnes and productivity level of 0.45 t/ha and Arunachal Pradesh has an area of 2285 ha with a production of about 51 tonnes and productivity level of 0.022 t/ha.

In the state of Jammu and Kashmir, Anantnag is the leading district both in area as well as production corresponding to an area of 13647 ha and production of 41180 tonnes with a productivity level of 3.01 t ha⁻¹, followed by the Kupwara district that covers an area of 8175 ha with 22103 tonnes production and a productivity level of a 2.70 t ha⁻¹. Kulgam ranks 6th in area and 3rd in production in the J&K state and has the highest productivity of 3.52 t ha⁻¹, which is even higher than that of USA. This indicates that the state has the right type of agro-climatic conditions and vast potential to produce export quality walnut and kernels.

Globally we have to compete with the world's most advanced country viz, USA which has been in search of superior genotype of walnut since 1867. USA started propagating walnuts from 1895-1905 and by 1915 seedling plants were no longer commercially planted. However, after 150 years of breeding work in walnuts the USA has been able to evolve a few genotypes with kernel weight of above 10 g. A selection viz, Suliaman made by SKUAST-K has a kernel weight of more than 11 g. Crop improvement in this crop has been successful in USA with nuts producing

kernels between 11.54 to 13.0 g.

Walnut kernels are very rich in omega-3 essential fatty acid viz., alpha linoleic acid (ALA). It has a high protein content and essential amino acid like glutamic acid, arginin and levine. On an average, kernels contain a protein content of 14.8 to 20.0 per cent, fat content (64.0-69.0%) and carbohydrates around 15.8 per cent. It is a good source of vitamins like A, B₁, B₃, B₅, B₆, E and folic acid. It also provides minerals like P, Fe, K, Ca, Mg and Na, besides fibre and energy from carbohydrates. Kernels are a good source of essential fatty acid like linoleic (49.0%), oleic (25.7%) and linolinic (18.2%) acids (Kuliev *et al.*, 1987). A regular diet of walnut has been reported to prevent cardio-vascular and coronary heart diseases and a type of diabetes. The polyphenolic compounds present in kernels have anticarcinogenic property and a bioavailable form of a hormone (melatonin) which is involved in inducing and regulating sleep. Besides, hulls, shells and leaves also have industrial potential to produce coloring matter, viral and parasite biocides.

The boiled green hull leaves and bark make a good yellow dye which is used (with precaution) for hair dyeing and dyeing of rugs. Other industrial uses include cleaning and polishing of metal glass and plastics; cleaning of jet engines and high quality polishes for jewellery. Ayurvedic

and Unani uses include treatment of throat/tonsil infections, wound healing and against malarial fever. The kernel oil is also used for wood polishing. Walnut timber is prized for making furniture and pieces of artifacts.

Plantation of denuded forest areas, ravines and other areas not suited for agriculture/horticulture crops with fast growing walnut genotypes will be very useful to increase vegetative cover of these denuded areas and also provide quality edible oil from kernels, cakes for animal nutrition and quality timber for small scale industries (furniture and artifacts) in the suitable agro-climatic areas for this crop in Jammu & Kashmir.

Walnut is highly heterozygous plant and each seedling, therefore represents a distinct genotype. In Kashmir walnut population is primarily from seed origin, which has resulted in creating tremendous genetic variability and a vast gene pool. However, this has also reduced the quantum of producing standard export quality nuts and kernels. Besides the impact of standard genotypes on the production of quality nuts and kernels, the climatic conditions (temperature, humidity and altitude) also affect the quality during the developmental stages.

The walnut in Jammu & Kashmir state, due to their origin to non-descriptive type of seedlings, has resulted in the production of a mixed crop that envisages lot of variation in the nut and kernel characteristics (Bhat *et*

al., 1999). The walnut cultivation is mainly based on the conventional method and moreover, the development of walnut cultivation has been constrained by certain limiting factors like the non-availability of sufficient quantity of vegetative propagated plant material of known pedigree, absence of proper classification of local varieties, the long gestation period and less tree density per hectare. This also causes great variability in the nut crop production with low yield. The cultivar evaluation has scientific merits for a system that can ensure the release of genetically distinct elite cultivars. These in turn can promote its productivity, contribute to conservation of genetic resources and also promote breeding strategies that will support both horticulture and plant breeding (Smith and Smith, 1988). The three basic criteria that are used to evaluate the potential of any character for identification of a genotype include environmental stability and inter and intra-varietal variation.

Study of morphological parameters for flowering and nuts envisage detailed assessment of genetic variability from the existing gene pool. Such a study also helps to generate information for the over all characterization of the existing gene pool. This also helps to estimate the genetic diversity in the crop spp, assessing the similarity of cultivated plant groups, determine the impact of domestication that help in identifying unique gene pool

among cultivated types and help to find elite selections for making further crop improvement. The valley of Kashmir is considered the gene bank of seedling walnuts in India that has immense value. Unfortunately no well planned breeding programme on this crop has been initiated so far in the state, thereby improvement has been confined primarily to identification of superior genotypes and selection of a few elite types in the natural population. Study of genetic variability in this crop has also been limited to few areas, thereby leaving the vast potential untapped. Therefore, it is necessary to develop an information to make a characterization of walnuts in the state which would be useful in monitoring the level of genetic diversity present in the existing population.

Micro propagation studies in walnut are not so well established nor any fool proof protocol is yet developed for efficient and faster multiplication of superior plants. The presence of phenolic compounds and entophytic bacteria are still the main limiting factors for establishing plant micro propagation in walnuts. The use of young vegetal material is the usual technique for *in vitro* set up of walnut (Driver and Kuniyuki, 1984; Jay-Allemand *et al.*, 1993). Quality in regeneration of *in vitro* plant material is correlated with maintenance of mother plants in the controlled environments, with regular hormone application and proper choice of physiological stage for collecting materials. The correct temperature in

growth chambers is essential for a proper regeneration as well for subsequent multiplication (Dolcet-Sanjuan *et al.*, 1993). The addition of PVP to the culture medium as well as the substitution of agar by gelrite are the main factors reported for the control of phenolic compounds.

The current methodology of woody crop rooting by a biotapic process is well documented in walnut (Driver *et al.*, 1984) with the use of IBA. Walnut is hard to propagate through micro propagation. Various attempts have been made using different types of explants, media, culture condition and rooting techniques (Driver and Kuniyuki, 1984). Poor proliferation and rooting rate is one of the main obstacles that limit the micro propagation efficiency in walnut. Intensive and well planned research is needed to develop a perfect protocol for micro propagation for this crop. Genotype plays a major role in vegetative propagation, in particular for micro propagation.

In many cases the propagation ratio can be improved by using a stronger cytokinin or increasing its concentration. However, this can sometimes have detrimental effects in the later stages of micro propagation. Micro propagation studies have also been carried out in some other species of nuts and similar trees like hazelnut (Radojevic *et al.*, 1975; Mele and Messeguer, 1983; Perez *et al.*, 1983); chestnut (Vieter and Vieiter, 1980)

and almond (Mehra and Mehra, 1974). But reports on *in vitro* walnut culture are scarce.

In view of the importance of this nut crop within the country and its growing potential, the present study was initiated to document a part of the existing gene pool in some important areas of the valley and a brief study of its *in vitro* morphogenesis. The main objectives of the study were:

- i) Estimation of diagnostic parameters as per the Standard Descriptor of IBPGR,
- ii) Estimation of variability (phenotypic and genotypic) for morphological, maturity, nut characteristics and quality traits,
- iii) Estimation of genetic parameters like variance, phenotypic and genotypic coefficient of variation, range, heritability (broad sense) and genetic gain (% of mean),
- iv) Identification and selection of superior genotypes for commercial vegetative propagation and further improvement, and
- v) *In vitro* morphogenetic response of some walnut selections under different regimes of plant growth regulators (PGRs).

CHAPTER – 2

REVIEW OF LITERATURE

2.1 General

The entire world production of walnut (*Juglans regia* L.) is based primarily on the cultivars which are wild selections and these primary cultivars differ for different production regions. Crop improvement programmes involving the fruit crops are rendered difficult by the long juvenile phase of the plant and the limited ability to precisely identify the gene sources for particular characters. Selecting desirable genotypes from the existing varieties and to use the superior types in breeding programmes is an important tool to increase the productivity in any crop. The extent of genetic diversity determines, to a great extent, the success of a crop improvement programme. The assessment of a viable diversity is basic to the development of improved cultivars. Hence selection from natural population has proved to be a useful tool in the hands of fruit breeders to tap the available genetic variability in these populations.

Characterisation is the depth of the genetic base that has direct bearing on the potential to identify different cultivars with improved performances. Frankel (1947) suggested that the characters of concern to a

plant breeder must be observable or strongly expressed. These could be identified even in a single plant (though very rarely) and must be expressed under growing conditions which are normal for cultivation. The characterisation enables easy and quick discrimination between the phenotypes which are generally highly heritable, can be easily seen by the eye and are quickly expressed in all types of environment (Anonymous, 1984). According to Frankel (1986) characterisation should provide a standardized record of readily assessable plant characters to identify an accession. The requirement of cultivar distinctness has scientific merits for a system that can ensure the release of genetically distinct elite cultivars, promote horticulture productivity and contribute to the conservation of genetic resources (Smith and Smith, 1988).

In Jammu and Kashmir walnut cultivation is mainly based upon conventional methods, with the result all the plantations own their origin to non-descript seedling and therefore, are extremely heterozygous in tree behaviour and quality attributes like nut size, colour and protein contents of kernels (Bhat *et al.*, 1999).

2.2 Genetic Variability

The success of any genetic breeding programme depends on the presence of sufficient genetic variability to make effective selection. It is

important to assess the relative magnitude of components of genetic variability in order to use such information, together with other selection parameters for improvement of the plant type through adoption of effective, breeding methods (Johnson *et al.*, 1955; Hanson *et al.*, 1956; Williams, 1964; Briggs and Knowles, 1967; Li *et al.*, 1991).

It is necessary to divide total phenotypic variance of a character into its components, as these are the basis for a genetic analysis and the dimensions of these components divide the breeding behaviour of the populations. Such selection parameters, particularly genetic coefficient of variability (GCV) helps to choose a potential genotype, whereas heritability (h^2) and expected genetic gain (as per cent of mean) are more useful in predicting the resultant effect for selection of best genotype (Ganesan *et al.*, 1996; Sarvanan and Senthil, 1997). Murty and Arunachalam (1996) stated that genetic drift and selection in different environments could cause greater drift than geographical distance. The value of a phenotype is the result of genotype and environment and their interaction. If no genotype-environment (GxE) interaction is present, the average difference between genotypes estimated through phenotypic stability in different environments is constant. Significant GxE interaction results from changes in the magnitude of the difference among genotypes in different environments or

from changes in sedative ranking of the genotypes. The GxE interaction reduces the correlation between phenotype and genotype and decreases selection progress. Success of new varieties is based on the performance or yield and other essential traits over a wide range of different environmental conditions (Becker, 1981). A genotype showing a constant performance in most of the environments is regarded as stable and having average performance. Such cultivars are useful for areas not suitable for intensive farming conditions.

2.2.1 Morphological characters

In Piemonte-Italy a two year study was conducted with the purpose of verifying the productive adaptability in this environment for seventeen exotic and many local seedling origin cultivars.. The observations revealed that nine cultivars showed least vigor viz, “Sorrentoz”, “Serr”, “Corne”, “Sorrenaz”, “Payne”, “Tehane”, “Magettce”, “Feltrinaz”, and “Chase P9 et.” With regard to canopy shape, the cultivars “Frequettee”, “Marbot”, “Feltrina” and “Surrena” were observed to have upright habit whileas “Amigo”, “Corne”, “Feltrinez”, “Hartley”, “Chaiseoa”, “Eurella”, and “Mayat” were found to have round canopies and “Chico”, “Payne”, “Surrentz”, “Eerr”, “Pedro”, “Sorrenta”, “Vina”, “Parisien”, and “Serr”, were having spreading canopies (Radicati *et al.*, 1990).

Casal *et al.* (2006) reviewed the performance of three seedling origin cultivars of *J. regia* and four *Juglans* commercial hybrids (NG₂₃ x RA; NG.38 x RA; NGMB x HD and MJ209 x RA) at two planting sites in Galicia (Spain). The performance was monitored over eight consecutive years (starting from 1996) for growth habit (apical dominance, stem form and number of branches) and phenology (bud burst and leaf fall). Highly significant difference between sites were found for vigour, bud burst and number of branches and also among (taxa) for vigour, bud burst, number of branches and stem form. The *Juglans* commercial hybrids were the most vigorous among seed source within (taxa) for vigour. At one site the walnuts had better growth with no significant difference among (taxa) for vigour, whereas at another site highly significant difference were found among (taxa) for these traits revealing high G x E interaction.

Godeanu and Botumihai (1997) evaluated 16 selected walnut lines from Oltenia (Romania) for their agro-productive characteristics. Two selections were found to be less vigorous, six had intermediate vigour and remaining eight had highly vigorous trees. Lal *et al.* (1993) studied tree vigor and some other attributes of some walnut cultivars. The cultivars “Turtle-31” and “Blacumore” exhibited maximum tree vigour.

Rough *et al.* (1978) grouped almond cultivars in three categories

according to their time of maturity viz. early, mid and late maturing corresponding to ripening from late August to early September, mid September to late September and October, respectively. Roser (1979) utilised phenological characters for evaluation of some walnut cultivars. He found that the blooming time of pistillate and staminate flowers showed very high degree of coincidence.

Apricot species, groups and cultivars vary greatly in size, vigour and growth habit. Representatives of eco-geographical groups of the common apricot have characteristic growth habit. Extensive study of living collection of 600 forms and cultivars at Yalta and Taskent resulted in the identification of four major groups (Koshtina, 1969). The trees of central Asian group are vigorous and long-lived with small but dense branched crowns. The Irano-Caucasian group are not as vigorous or long lived. It has smaller trees and thicker twigs that come into bearing early and require shorter chilling period. The Zhungar Zailij group is the most primitive with trees smaller than semi-cultivated seedlings of central Asian group but having greater winter hardiness. Most central Asian apricots are self compatible with some notable exceptions like “Perfection”, “Riland”, “Shakha”, and “Chothagold” cultivars. “Charmagz” was found to be most

vigorous and “Ladakhi” the least out of the six varieties evaluated at Mashobra for their cumulative trunk girth, tree height and spread (Sharma, 1976). The apricot cv. “Charmagz” was found most vigorous of the 10 varieties evaluated (Mehbrial, 1990).

Diaz *et al.* (2006) studied genetic variation and genetic x environment interaction in walnuts (inter - and intra - populations) and reported magnificent genetic variability for all the traits. A provenance-progeny test of common walnut (*Juglans regia* L.) was laid at two sites with ten to thirteen open-pollinated families from each of the four local populations viz; “Burgohondo”, “LaGuigueta”, “Pedroso” and “Valadouro”. At both the sites walnut trees were assessed for survival, growth (total height and root collar diameter), Phenology (bud burst, frost damage and leaf fall) and growth habit (stem form and apical dominance) during the first three growing seasons. Significant differences were found between the sites for all traits except for survival rate. At the end of the third growing season the total height was three times higher at Mas–Valero than at Nullem. Significant difference were found among the populations for growth and phenological traits, where as no significant difference was found among the population for heritability (h^2). The heritability (h^2) was over 0.70 for growth traits and leaf fall. ‘Valadouro’ was the most vigorous population at both the sites, while ‘Pedroso’ had the best third growing

season growth increment at the Mas–Valero site. At the best performance site (Mas Valero) significant differences were found among families within populations for growth, growth increment, leaf fall and survival. However, at Nullem the walnut trees had worse performance with no significant difference among families within populations except for second growing season growth.

Casal *et al.* (1996) studied the performance of three seed sources of *Juglans regia*, three seed sources of *Juglans nigra* and four *Juglans* commercial hybrids (NG23 x RA, NG38 x RA, NGMB3 x HD and MJ209 x RA) at two planting sites in Galicia (Spain). The performance was monitored over eight consecutive years (starting 1996) for traits viz. height, diameter increment and growth habit (apical dominance, stem form and number of branches). The *Juglans* commercial hybrids were most vigorous taxon. Significant differences were found among seed sources within taxa for vigour. The Gondomur site had better plant growth but with no significant differences among taxa. At Cartelos site highly significant differences were found among taxa for plant growth.

Solar and Stampar (2006) studied 275 seedling trees from different populations of domestic walnuts. Growth habit parameters were recorded over three years. Majority of genotypes exhibited semi-erect to semi-

spreading growth habit with the exceptions like 'Z-62' (with markedly erect habit) and 'C-6/7' (with spreading branches). 'Remsau' had most vigorous growth with dense and branching canopy.

Ferhatoglu (1989) observed great genetic diversity in walnut populations of Turkey. Of these some trees were upright, spreading and vigorous. Others were upright spreading with normal branches and some had upright spreading trees with dense branching and moderate vigour. Lal *et al.* (1993) studied tree vigour attributes of walnut cultivars viz. 'Tutle-31', 'Blacumore', 'Fraquentta', 'Hartley', 'Xenia', 'Tutle-16', 'Payne', 'Waterloo' and 'K x Giant' and observed that the cultivars, 'Tutle-16' and 'Blacumore' possessed maximum tree vigour.

Totuel *et al.* (1999) evaluated some hazelnut selections from the populations adapted to the ecological conditions in the northeast of Oltenia to identify valuable selections for fruit and rootstock sources. They found that the climatic conditions and the existence of some hazelnut trees with different origins favoured the forming of some landrace populations, from which 25 selections with different agro-biological characteristics were selected. These selections were based on higher vigour.

Layne *et al.* (1996) are of the opinion that the seedling in the nursery with large leaves are vigorous, relatively unbranched shoots are more likely

to produce medium or large sized fruits and be better adapted in an ecological conditions similar to that of the nursery site. While plants with much branching, very thin shoots and small leaves are likely to be late in coming into fruiting and are unadapted.

The apricot leaves vary from elongated to round and round oblate (Yoshida and Yamaishi, 1988). Leaves are serrate, ovate or elliptic, very often cordate and have long reddish petioles with 1-5 glands (Mehlenbach *et al.*, 1990).

Westwood (1993) reported that the hazelnuts (*Corylus* sp.) are perennial deciduous shrubs and rarely trees; with buds obtuse and rarely actuate possessing mostly imbricate scales; leaves are generally ovate, usually double serrate and more or less pubescent.

Gilman and Weston (2006) observed remarkable variability in different morphological characteristics of Turkish filbert (*Corylus colurna* L.) and found that the tree height varied from 12.19 to 18.28 m, with tree spread from 7.62 to 10.66 m and leaf blade length 5.08 to 20.32 cm. Leaf shape varied from ovate to obovate. Branches did not droop.

Physical description of Turkish filbert (*C. colurna* L.) revealed that the plant is deciduous tree with a pyramidal crown. The leaves are up-to 15-24 cm long with alternate leaf arrangement, simple, deciduous leaves,

obovate leaf shape with a cordate leaf base, doubly serrated leaf margins and pubescence on petioles and leaf veins. The trees are monoecious with 5 to 8 cm long catkin bloom in early spring; nut 12.7 mm across enclosed in a volucre and matures in September (Anonymous, 2007)

Ozkan and Cellepe (2001) found that walnut varieties 29/B-4, 170/B-16, 32/B-18 and 198/110 varied in growth and canopy covers. The variation was 3.10-9.10 m for height of trees, 3.60-8.80 m for canopy spread, 28-90 cm for the trunk circumference and 56-115 cm for the first branching height. The tallest (9.10m) tree was 29/B-4 and the shortest (3.10 cm) was 198/110.

2.2.2 Nut and kernel characteristics

The nut and kernel characters have great economic importance. After removal of hull, the nut includes kernel surrounded by the shell. The important shell characteristic from horticultural stand point include hardness of shell, shelling percentage, adherence of the outer shell layer and the degree to which suture is sealed. Shell type vary from hard to papery (Brezik, 1967).

Due to the great demand of the walnut fruit within the country and abroad, it is necessary to have superior and export oriented walnut varieties which give good returns to the growers and also earn foreign exchange for

the country (Nauriyal *et al.*, 1969). Thaper (1969) is of the opinion that at present there are no regular plantations of walnut in our country but in many areas trees are growing in large numbers having lot of variation in their nut and kernel characteristics. There is an urgent need to survey the existing varietal potential and create the valuable germplasm pool for propagation of the superior walnut strains for further distribution and future use in breeding programme (Lal and Singh, 1978). The importance of nut and kernel traits of walnut in the international market has promoted the search for attributes such as nut size, nut thickness, nut colour and its smoothness, shelling percentage and other qualitative traits like kernel colour, fat content and protein percentage (Bhat and Mir, 1992).

Nut size, especially the diameter at suture line is an important attribute for commercial grading. Standard nut diameter not less than 3.00 cm at suture line was found desirable (Serr and Forde, 1956) and have opined that any new selection should have a minimum of fifty per cent shelling. They have prescribed a kernel weight ranging from 5.0 to 7.0 g in commercial cultivars.

Four selections 'P-3', 'P-123', 'V-I' and 'Wossan-3' had a nut diameter ranging between 3.41 and 3.70 cm (Bhat and Mir, 1992). In a study in Kashmir, Bhat and Mir (1992) observed that while the nut weight

of exotic type did not exceed 10.72 g, Wosson-3 recorded the highest nut weight (14.64 g) followed by P-12 (13.84 g), V-6 (13.76 g) and Shalimar-3 (13.39 g). The lowest nut weight (5.22 g) was recorded in P1. The nut length ranged from 32.35 to 40.50 mm in local selection, whereas in exotic types grown at Shalimar it ranged from 30.25 to 39.80 mm and those grown at Caserta (Italy) it ranged from 31.10 to 46.4 mm. The highest nut length of 46.40 mm was recorded in 'FM-6' (a Greece selection) followed by 40.81, 40.50, 39.9, 39.8, 38.5 mm in 'Placenta', 'Wossan-2', 'Pedro', 'Drajnoviski' and 'Chenovo', respectively. It was further propounded that kernel colour was an important character when shelled nuts are to be marketed, as it solely determines the consumer acceptability. Local selection 'Wossan-8' and "Shalimar 10" grown at Shalimar and the cultivar "CC-I" grown at Caserta (Italy) exhibited high kernel colour ranking. Most of the other selections at Shalimar and only few cultivars at Caserta showed medium ranking. Among the local selection, "Wossan-2" had longer nut length (40.50mm) than the range whereas all other selections had nut length within this range. Among the cultivars grown at "Caserta" (Italy), "FM-6", "Placenta" and "Pedro" were found to have a nut size higher than this range.

Guzken and Weiching (1990) is of the opinion that nut weight can

serve as a good parameter for selection as it has both production and commercial value among characters. Qureshi and Dalal (1985) found shelling percentage to be an index of kernel recovery. Nuts with higher shelling percentage assume importance as these can yield higher kernel tonnes per unit area. A shelling percentage of 30-35 per cent was obtained from Kashmir walnuts.

Godeanu and Botumihai (1997) reported a nut size index of 31.2-38.4 mm in walnut. Nauriyal *et al.* (1969) identified some strains of superior quality of walnut. Description of superior strains was made in regard to the pomological characters such as nut size, nut weight and kernel. Lal and Singh (1977) surveyed the area and used bearing as a parameter to select some promising strains of walnut. Kernel characters also formed an important criterion. Ke (1990) evaluated 36 promising clones of walnut. Highest shelling percentage, good nut with soft smooth shell, bright kernel and flavour was recorded in some clones. Radicati *et al.* (1990) found good nut quality in 'Chase-D', 'Franquette', 'Granjean', 'Pedis' and 'Serr' and higher kernel percentage in 'Chico' 'Grandyer' and 'Serr'. Atefi (1997) identified four best selection viz. 'Serr', 'Amingo', 'Chico' and 'Franquette' from the collected population of walnut based on the phenological and pomological characters.

Solar (1990) studied the pomological characteristics of some walnut genotypes. The nut produced by the cultivar 'G-120' was heaviest in weight (13.40 g). The highest percentage of the kernel recovery (55.22%) was found in the cultivar 'Sejnove' and lowest (42.82%) in the cultivar 'G-120'. The best kernel colour was observed in 'MB-24', 'Elit', 'G-139' and 'Meytte'.

Sen (1993) determined the best walnut type on the basis of average nut weight, kernel per cent and shell thickness. In the selected cultivars the average nut weight varied from 15.30 to 20.05 g. the kernel per cent was found between 40.60 to 54.40 and shell thickness between 0.88 and 1.02 mm. Rink *et al.* (1997) obtained data on nut length, nut diameter, nut weight, kernel weight and shell weight of 156 *J. regia* trees and suggested that the nut diameter with longer length could be used as a selection criterion for heavier nuts with maximum nut weight. The correlation between nut weight and shell weight ($r=0.962$) was highly significant.

Sharma *et al.* (2006) studied the variability in nut characters of 134 walnut trees growing in Northern area of district Sirmour and Bharmaur area of district Chamba (Himachal Pradesh). Among 47 seedling trees selected for studies in Northern area, the nut weight varied between 4.85 to 16.59 g, nut width 23.13 to 36.95 mm, shell thickness 0.64 to 2.45 mm and

kernel weight 1.02 to 6.91 g. In Bharmaur area the studies on 87 seedling trees revealed a nut weight range of 6.24 to 23.61 g; nut width 25.13 to 36.18 mm, shell thickness 0.48 to 2.16 mm, kernel weight 2.61 to 8.02 g and kernel recovery percentage 20.74 to 53.51. Significant variation was also reported for nut length, nut shape, etc.

Zhadan and Strvuov (1977) suggested that the shell thickness is most valuable character in selection of superior type walnut. Rathore (1983b) evaluated 22 genotypes and reported great genetic diversity. They made ten promising selections on the basis of morphological and pomological traits. On the basis of fruit morphology viz. nut weight, nut size and shell thickness Mischenko (1986) recommended a number of walnut varieties for breeding and further improvement of walnut.

Kuden *et al.* (1997) reported highest kernel production from Klan-8 (57.39%), Kam-10 (56.01%), Kam-12 (56.35%) and Kam-9 (56.36%). Li *et al.* (1991) released a new promising walnut variety 'Beijing 61' in China. The nuts mature in late August, are elongated round and have 8.6 g average weight, thin shelled, kernel easily extracted, possess yellow white colour and kernel percentage of 65.40.

Sen and Tekinates (1992) evaluated 31 walnuts on the basis of superior nut and other quality parameters. Only three cultivars showed

perfect yellow colour kernels, while the shell colour of only two cultivars was perfect yellow. The shell thickness of 27 walnut types ranged from 0.53 to 1.49 mm. They concluded that about 85 per cent of selected types were suitable from the stand point of shelling thickness.

Yuan and Yang (1998) observed improvement in the quantitative parameter of walnut as harvesting was delayed. They reported that average nut weight and kernel percentage of nuts harvested on 2nd September was 11.94 g and 52.09 per cent, respectively. Progressive increment in nut weight (13.6 and 14.10 g) and kernel percentage (55.96 and 56.31%) was observed when harvesting was delayed by 10 and 20 days, respectively. Deng and Deng (1999) described 15 walnut cultivars on the basis of their morphological and fruit characters. They found most of the cultivars early maturing. Cultivar 'Lunag' and 'Azha 343' produced the largest nuts (15.9 and 16.7 g) and had also high kernel percentage (53.57 and 54.09%), respectively.

Rousuas and Zeugnthinus (2001) evaluated 70 walnut selections and found that trees bearing laterally have good sized nuts ranging from 47.1 to 55.0 mm and further concluded that lateral bearing selections are most promising and suitable.

Longoriet *et al.* (2001) assessed the existing diversity within the

Moroccan walnut germplasm and identified some superior genotypes. It was observed that smaller fruited types had more shrivelled kernels, higher aborted buds and lower fruiting potential as compared to French germplasm. The improved nut and kernel characters could be utilised for a hybridization programme as well as for introgressing their useful traits in the commercial walnut cultivars/selection (Sharma and Sharma, 2001).

Yarilgac *et al.* (2001) identified some promising walnut types on the basis of their morphological, phenological and pomological traits and created a valuable genetic resources on the basis of the fruit characteristics like nut weight, nut diameter, kernel colour, kernel percentage and shell thickness. Bhat *et al.* (2002) studied the genetic variability in natural seedling originated walnut population of Kashmir valley. Most of the nuts were round in shape, had smooth shell surface, light shell colour, intermediate shell seal and intermediate shell strength with light kernel colour. They observed that the natural walnut population of Kashmir valley has great variability in nut shape, shell strength and kernel colour.

Walnut improvement in India is in a very primitive stage. As reported earlier the entire commercial plantation comprises of seedling trees which of course have tremendous variability in the size, shape, colour and shell hardness of nuts as well as colour and flavour of kernels. The

seedling populations of all the walnut growing states have been surveyed and superior genotypes have been identified by many workers (Chauhan and Sharma, 1979; Pandey and Sinha, 1984; Qureshi and Dalal, 1985). Phenotypic variability in walnuts of Kinnaur region of H.P. was evaluated by Chauhan and Sharma (1979). They found highest kernel percentage in selection 'Number 34' (64.3%) followed by the cultivar 'Govind' (52.8%) and selection number 2' (49.2%).

Pandey and Sinha (1984) explored Jouser Bhabhar area of U.P hills and identified fifteen promising walnut seedlings. 'Chakrata-14' was best having very thin shell, good filling and attractive light kernel colour but its highly delicate paper shell might be unsuitable for harvesting and transportation. However, they recommended 'Chakrata - 2, -4, -6 and -13 for commercial cultivation.

Kashmir valley is particularly a very rich source of quality walnut germplasm which needs to be utilised in bringing improvement in walnut. Qureshi and Dalal (1985) identified a collection of 333 quality nut producing seedlings selected after exploring the potential belt of walnut growing areas of Kashmir. Of these 67 seedlings were found promising. They also recorded 67 per cent kernel recovery in certain selected materials as against the highest shelling reported by Forde (1975) of 60 per cent.

Godeanu and Botumihai (1997) studied 900 hybrids obtained by controlled cross or by open pollination. Four hybrids 'VL4', 'VL54', 'VL57' and 'VL102' were characterized by high productivity, terminal bearing and resistance to different diseases. More than 50 per cent population has large nut size with uniform good kernel shell ratio (more than 50%), good quality of kernels and nuts. Sutyemez and Kaska (2006) evaluated 19 local and 8 exotic walnut cultivars. The local cultivars were selected from different parts of Turkey in an earlier study. Early leafing was observed in 'Serr' and 'Sebin', whereas the latest leafing occurred in 'Franquette'. Earliest male flowers to open were found in 'Sebin' and 'Yalova-4'. In 'Bileiu' and 'Kr-2' the female flowers became receptive earlier than the other cultivars. 'Maras-18' and 'Pedro' appeared to be homogamous cultivars. The heaviest nuts were obtained from 'Bacu' (10.56 g) and Kaplan-86 (12.07 g). During the studies one of the locally selected cultivars 'Maras-18' seemed to be the best adapted cultivar to 'K-Maras' ecological conditions.

Kazankaya and Koyunc (2001) evaluated 38 selections and found that most selections had fruit weight over 11-12 g, kernel weight over 6-7 g with light coloured kernels. Rathore (1983b) evaluated twenty two genotypes of walnut and reported a great genetic diversity. He made ten promising selections on the basis of their various morphological and

pomological traits.

Agabbie *et al.* (1984) reported variability in shelling percentage, nut shape, weight, shell thickness, kernel weight in 92 almond varieties. Anonymous (1984) reported variability in nut shape, shell hardness, shelling percentage, kernel shape, size and weight in 75 almond types. Five varieties were recommended on the basis of desirable nut and kernel characters. On the basis of fruit morphology viz. nut weight, nut size and thin shell, Mishchenko (1986) recommended a number of walnut varieties for breeding and concluded that these could be important source materials for improvement of walnut.

Sofi *et al.* (1992) studied various nut and kernel characters such as nut length, width, shell type, shell seal, nut appearance, kernel length, kernel colour, shelling percentage etc. of some indigenous promising type and exotic cultivars of almond at Shalimar, Kashmir and suggested that the shell seal is an important parameter for bleaching and insect damage, and also opined that kernel colour governs the consumer acceptability of shelled almond. Highest kernel recovery (61.3%) was observed in indigenous germplasm 'H-15' and all the cultivars except 'Pranyaj' had very fair to moderate good nut appearance, whereas the nut appearance of all indigenous selections vary from fair to moderate good.

1215 seedling trees from different population of domestic walnut were analysed “*in situ*” in Himachal Pradesh (Sen and Tekinates, 1989). The main selection criteria were “late leafing”, intermediate and lateral bearing, potential resistance to walnut blight and nut quality. The most perspective cultivars were late leafing genotypes ‘Z-62’, ‘Krne’, and ‘Z-60’ with leaf out in the beginning of May. The kernel colour of ‘Z-59’, ‘Eryavee’ and ‘Z-60’ was much brighter than others. For the in shell marketing, the most appropriate is ‘Puksic’ with round nuts, bright and very smooth shells.

Radicati *et al.* (1990) evaluated 19 walnut cultivars in Piemonte (Italy) for fruit quality. ‘Franquette’, ‘Hartley’ and ‘Pedro’ appeared particularly valuable for in-shell consumption. ‘Chico’, ‘Grandjean’ and ‘Serr’ were well suited for the shelled nut market on account of their high kernel percentage.

Sharma and Sharma (2001) recorded variation for nut and kernel characters in the seedling trees. Nut weight varied from 6.40 to 20.55 g; nut width from 21.03 to 38.76 mm, nut length from 27.05 to 47.15 mm, nut thickness 21.79 to 41.93 mm, index of roundness from 0.50 to 1.09, pad width between 2.30 to 6.95 mm, pad thickness from 0.60 to 2.60 mm, mean kernel weight of nut from 1.50 to 7.10 g, kernel width 13.79 to 33.37 mm,

kernel length from 21.94 to 36.31 mm, kernel thickness from 18.31 to 47.33 mm and kernel recovery percentage from 12.0 to 62.5. Lal and Singh (1978) reported superior strains of walnut in “Chakrata hills”. A large number of trees growing in different areas were examined during the fruiting season. On the basis of the bearing and quality of the nuts nine strains were selected. Strain ‘I’ and ‘III’ were of very superior quality. All these nine strains could be recommended for commercial cultivation in this region. All genotypes were grouped into 16 different clusters. The clustering pattern of walnut genotypes belonging to the same location revealed that their distribution was more than in one cluster exhibiting non-parallelism between geographic and genetic diversity. Genotypes of cluster-5 and -8 were highly diverse from each other. Mean nut weight and kernel weight were highest in the cluster -8 and -14, respectively. The highest mean kernel percentage and minimum shell thickness were observed in ‘cluster 13’.

Sharma and Kumar (1984) found that the nature and extent of existing variation for various phenological characters present prospects for future genetic improvement of this nut crop and export potential as well.

Akea and Sen (2001) studied genetic variability and selected superior walnut types within seedling population of Hizam (Turkey). He

found that seedling population exhibited tremendous genetic variation in pomological and phenological characteristics. Fruitfulness was highest in selected types that were lateral bearing and in which leafing time was very late. Nut quality of these types was not so good as compared to any standard walnut cultivar. But these types need to be collected and propagated. The nut weight varied between 7.49 ± 0.75 to 13.93 ± 0.75 g, kernel weight between 2.61 ± 0.13 to 5.73 ± 1.13 g, shell thickness between 1.32 ± 0.33 mm to 2.45 ± 0.44 mm and nut diameter between 22.30 ± 1.12 to 32.26 ± 2.95 mm. Range of variation in nut colour was dark brown to dark yellow.

Sharma and Sharma (1998) studied 125 seedling trees selected from a population of 500 trees. In these the nut weight varied from 6.3 to 18.6 g, nut length 2.3 to 4.8 cm, nut width 2.1 to 3.9 cm, index of roundness 0.6 to 1.3, pad width 0.2 to 0.9 cm, pad thickness 0.2 to 0.7 cm and shell thickness 0.07 to 0.5 cm. The variation was also observed in nut shape, shape of base and apex of nut and pistil point position of pad on suture.

Sharma and Chauhan (1980) evaluated fourteen seedling walnut trees and found that the highest average nut weight (14.76 g) was obtained in seedling '5' and minimum (4.33g) in seedling '4'. However, the seedling '1' produced the highest number of nuts. In respect of nut length the

seedling '1' produced the longest nut (4.15 cm), whereas seedling '20' produced the smallest nut (3.22 cm). It was also observed that the highest diameter was obtained in seedling '6' (3.7 cm), whereas least was obtained in seedling '9' and '11'. Seedling '11' produced the nuts with highest percentage of kernels (56.65%) whereas the lowest kernel percentage (24.08) was obtained in seedling '7'.

Atefi (1997) identified five genotypes 'B21', '1/3', 'G3', '263' and 'E60' from the collected population of walnut (*J. regia*) based on the fruit and kernel characters viz. lateral bearing, weight of nut, leafing and percentage of kernel. In Anatolia (Turkey) walnut populations from seed origin possess lot of variability and promising selections have been made with high nut quality and lateral fruit bearing.

Lal *et al.* (1993) evaluated different cultivars of walnut viz., 'Tutle-31', 'Blaumore', 'Franquette', 'Hartley', 'Xenin', 'Tutle-16', 'Payne', 'Waterloo' and 'KX' for many traits. Maximum nut and kernel weights were recorded in 'Xenin' and 'Blaumore' (10.97-11.55 nut weight) and (4.71 to 4.82 g kernel weight), respectively. Kernel recovery per cent was higher in 'Blaumore' (43.93%) and 'Tutle-31' (46.58%). On the basis of overall performance 'Blaumore', 'Tutle-31' and 'Xenin' cultivars were found very promising for commercial cultivation in the region.

Neriman and Taki (2006) evaluated thirteen promising walnut genotypes from selected genotypes. The nut length ranged from 34.48-60.42 mm, nut width (30.38-45.24 mm), nut cheek (32.54-47.96 mm), nut size (33.97-50.80 mm), nut weight (11.84-24.19 g), kernel weight (6.39-10.73 g), kernel recovery per cent (47.76-60.55), kernel shrivel (1.25-9.38%), per cent defective kernel (2.08-18.75) and shell thickness (0.98-1.65 mm). Shell colour was light for most of the genotypes. Kernel removal was very easy or easy, shell connection was found to be very strong or strong. Maximum genotypes were found to possess smooth or medium nut texture.

2.2.3 Harvesting

Walnuts in Kashmir valley are mostly harvested in the months of September and October (earlier at lower altitudes and later at higher altitudes). Some nuts fall on to the ground after splitting of the hull in a natural way while others are forced to drop down by beating with long sticks. The complete harvest is achieved in two or three harvestings carried at intervals. The fallen nuts (naturally and manually) are collected in huge heaps and covered with leaves and/or grass for hull fermentation which takes about 9-15 days. Dehulling, washing and cleaning are carried out manually causing staining of hands, clothes and nuts. Sometimes in order to

improve the appearance of nuts these are bleached either with alkali or acid solution. After cleaning the nuts are dried under sun for number of days by spreading them on a canvas sheet or hard floor. Occasionally the nut drying is not complete due to lesser number of sunny hours and lack of adequate warmth in the autumn sunshine. The dried nuts are graded according to the size and colour. Ungraded product fetches lower price in the market.

In walnut there is a great diversity both for leafing out and flowering. In certain cultivars the anthesis of catkins is delayed up-to 3rd week of May. The female flowers bloom normally from 2nd week of April to 3rd week of May. However, fruit harvesting is usually completed by mid of September (Rathore, 1983b).

Sharma and Chauhan (1980) evaluated fourteen seedling walnut cultivars for maturity and found that harvesting started from 2nd week of August and continued till last week of September. The cultivar “Seedling - 1” was the earliest to be harvested, whereas the “seedling-14” ripened last of all. Therefore, variability in respect of harvesting period was an established fact.

Rathore (1983b) observed a great genetic diversity in walnut for maturity. He found that the fruit harvesting was usually completed from

30th August to 15th September. McGranahan and Leslie (2006) studied three new walnut cultivars ‘Sexton’, Gillet’ and ‘Forde’. These cultivars developed through controlled pollination are characterised by low blight score and early maturity as compared to cv. ‘Chandler’.

2.2.4 Yield

A great variation is found in the yield of walnut, which is influenced by difference in tree age, size, soil, fertilization, irrigation and management practices. A 40-year old tree with a good canopy can produce as much as 175 kg nuts as compared to the average yield of only around 40 kg (Metzer, 1977). Commercial bearing commences after 18-20 years in seedling trees and after 8-10 years in grafted trees. The nut yield of about two and half tonnes per hectare is obtained from 8 or 9 year old trees in USA under quadruple planting system (Serr, 1969). Yarılgac *et al.* (2000) studied yield potential of *Juglans regia*. Selections were evaluated over a period of two years in the district Van (Turkey). A formula based on per cent production and average number of pistillate flowers on terminal and lateral shoots was used. Yield index scores of promising selections in terms of fruit characteristics were calculated. Yield potentials were classified into four groups viz. low, medium, good and very good. Based on these scores five selections were considered as very good for yield potential.

Paunovic (1990) studied large number of indigenous populations of walnut as pre-selection '*in-situ*' and finally selected around 200 accessions. These accessions had pretty good biological and commercial characteristics with regard to time of blooming, ripening, regular bearing and good yields.

Jamal (1990) studied more than 250 genotypes of walnuts in different production areas of Turkey and found that regression line equation between pair of characters could be an important aid for selection of cultivars. He also recorded behavioural characters which are important for cultivation of Persian walnut cultivars. The yield of nuts was governed by bearing habit, bearing surface, fruitset and nut size. Two types of bearing habits have been observed in walnut cultivars viz. cultivars having pistillate flowers only on shoot terminals and subterminal buds which usually do not have more than two flowers and the cultivars that have laterals with negligible pistillate flowers which hamper complete pollination and is a cause of reduced yields.

Akcea and Sen (2001) examined genetic variability so as to select superior walnut trees within seedling populations around Vanelaku (Turkey). In this study they examined over 25,000 walnut trees with high heterozygosity for yield and tolerance to anthracnose. Twenty three trees were identified on the basis of yield and tolerance to anthracnose disease

and pests that could be used as *per se* or hybridization programmes.

Lal *et al.* (1993) evaluated different cultivars of walnut viz. 'Tuttle-31', 'Blaumore', 'Franquette', 'Hartley', 'Xenin', 'Tuttle-16', 'Payne', 'Waterloo' and 'Krihart'. The cultivars 'Tuttle31' and 'Blaumore' were highest in nut yield. Ferhatoglu (1989) studied some walnut selections from the large gene pool maintained in Turkey and identified many cultivars on account of their high yield and homogenous ripening. Ozkan and Cellepe (2001) evaluated walnut varieties 29/B-4, 170/B-16, 32-B-18 and 198/110 for their potential yield indices. The yield indices of these varieties were found between 129.0 to 402.8. It was also found that there was a correlation between yield and vegetative growth.

Solar and Stampar (2006) studied 1215 *in situ* seedling trees from different domestic walnut populations and found that lateral bearing potential was well expressed in 'Erjavee' that had very regular bearing and expressed the highest yield. Crop production was also very stable in 'Z-59' which had intermediate bearing habit.

Sharma and Chauhan (1980) evaluated fourteen seedling walnut trees in the approximate age range of 20-25 years. Their yields ranged from 15.0 kg in "Seedling-1" to 40.0 kg in "Seedling -14".

Newman (2006) found that tree management and intercropping

system had a major effect on walnut yield. A yield of 1 tonne per hectare was obtained from selected trees of varieties '139' and '286' of 10 years age. The economic impact of improving precocity was modelled.

McGranahan and Leslie (2006) reported on three new cultivars 'Sexton', 'Gillet', and 'Forde' developed through controlled pollination that were characterized by high yields and early harvesting.

Picirillo and Petriccinone (2000) studied 69 walnut accessions from a germplasm collection set up in Pignataro Maggiore district of Castra, Italy. They observed that the Californian accessions were the early ones, the Spanish mid early, the Italian and Greek intermediate and the French late in blooming. Fruit ripening of all accessions took about 30 days. All accessions were protandrous, except two that were protogynous. Kernel yields showed variability not related to the origin of the accessions.

2.3 Chemical characters

2.3.1 Kernel oil content

Walnut oil is almost colourless or pale yellow with pleasant aroma. It is used for culinary purposes, in artists oil colours, varnishes and soap manufacturing. The walnut kernels contain about 60-75 per cent of fat and oil, the major components of which are linoleic (49.2%), oleic (25.7%) and linolenic (18.2%) acids (Kuliev *et al.*, 1987). It has a specific gravity of

0.923, refractive index 1.475, iodine number 158.5, saponification number 194.5, saturated acids 5.34 per cent and unsaturated acids 89.74 per cent (Jacobs, 1957). Both the kernels and its oil reduce considerably serum cholesterol, triglycerides, phospholipids and total lipids in Cholestemic rats (George *et al.*, 1986).

Bhat and Mir (1992) reported that the fat content of exotic varieties grown in Kashmir ranged between 51.5 to 59.0 per cent. Bugaria and Orgus (1985) have reported a fat content of 61.0 to 72.0 per cent in 48 specimens of walnut kernels.

Savage and Neil (2001) reported that walnut kernels contain between 63 to 70 per cent oil and more than 90 per cent of this oil is unsaturated fatty acid, with oleic acid content ranging from 12 to 20 per cent. Vitamin E is also found dissolved in the oil fraction and these alongwith oleic acid are the most positive nutritional features of walnut.

Sharma and Sharma (2001) analysed 229 seedling trees of Persian walnut growing naturally in four districts of Himachal Pradesh for 15 nut and kernel characters. All genotypes were grouped into 16 different clusters. The highest mean value for fat content was recorded in nuts of the cluster 3.

Ferhatoglu (1989) evaluated 116 cultivars of walnut for various

quality parameters and identified 9 cultivars having kernel oil content between 69.0 to 70.00 per cent.

Yarilgac *et al.* (2001) studied walnut plantation, growing *in situ*, for different quantitative and qualitative traits over several years. They recorded kernel oil content between 55.74 to 68.52 per cent in the promising selections.

2.3.2 Protein Content

The walnut kernels are very rich in protein (14.8 g) and carbohydrates (15.8 g). Kernels are also a good source of vitamins (B-group) with vitamin B₆ being maximum as compared to other nuts such as almond, Brazil nut, chestnut, pecan nut etc. (Polansuy and Murphy, 1966).

Furuchi *et al.* (1981) reported 13.2 per cent protein in walnut kernels, whereas 10.0 to 19.5 per cent was reported by Bugaria and Orgus (1985) and 15.0 per cent by Easayan and Besseган (1984).

Bhat and Mir (1992) revealed that the protein content of the exotic and local types of walnut did not differ much. In one exotic type of *Juglans* from Drajnoviski the protein content was 18.7 per cent, while among the local types it ranged from 17 to 20 per cent. Yarilgac *et al.* (2001) recorded a protein content of 16.21 to 21.86 per cent in promising walnut selections.

2.4 Shoot morphogenetic response to plant growth regulators

Tissue culture has rapidly evolved as a dynamic and important field of propagation during last two decades. Spectacular developments in the various areas relating to *in vitro* culture since 1975 resulted in the production and regeneration of many plant species. In addition, major advances have been made in the field of genetic engineering and biotechnology which has given further inputs to research in the field of tissue culture.

Tissue culture techniques have been used to develop new cultivars of strawberries in Egypt, dates in Israel, peaches and grapes in North Africa (Hopper, 1989), which has enabled these countries to move quickly and realize advantages of new marketing opportunities in Europe.

The widespread use of *in vitro* cloning has resulted in many advantages and achievements. This scientific achievement owns its genesis to the concept of ‘totipotency’ of plant cells (Haberland, 1902) and relentless efforts of pioneer investigations (White, 1934; Nobecourt, 1939; Skoog and Miller 1957; Reinert, 1958) which laid the foundation of *in vitro* culture techniques.

The number of species cultured *in vitro* has steadily increased over past two decades and has been reviewed by Murashige (1974, 1977a, 1977b

and 1978); de-Fossard (1977); Hussey (1978, 1980, 1983 and 1988); Vasil and vasil (1980); Congre (1981); Lane (1982); Bonga and Durzan (1987) and Geroge and Sherrington (1984). Vasil *et al.* (1979) reported that the techniques of micro-propagation have been standardised in about 332 crop species.

According to George and Sherrington (1984) the studies on *in vitro* culture systems were progressing at least in 1051 crop species of which less than 94 were fruit crops including walnuts. However, the available information on tissue culture multiplication of plants has been very much unbalanced between herbaceous genera and that of woody perennials including fruit plants, placing the former group in an advantageous position. Although earlier tissue culture studies were done with tree species like *Ulmas* (Gautheret, 1940) yet tissue culture studies of trees have lagged behind. The work done with herbaceous plants have been more convenient and result oriented than the woody tree species because of their peculiar problems. Tissue culture of woody perennials including forest and fruit trees has been extensively reviewed by Skirvin (1981); Zimmerman (1986); Swartz and Lindstorm (1986); McCown (1986); Dirr and Heusar (1987) and D'Souza (1988); Litz and Conover (1978); Singh (1982); Grusel and Boxes (1990).

2.4.1 *In vitro* cloning systems

Rapid asexual multiplication can be achieved by either enhancing the release of axillary buds from the apical dominance (shoot tip culture) or by producing adventitious buds/shoots through organogenesis or somatic embryogenesis (Murashige, 1974). The main advantage of the first route of regeneration is greater progeny uniformity by maintenance of the genetic integrity of the initial material (Chand and Roy, 1980) while in the second route, though multiplication is faster, product uniformity cannot be assured as the process involves callus mediated somatic organogenesis. The third route namely somatic embryogenesis is reported to be successful only in limited plant species (Vasil and Vasil, 1984) which is most rapid mode of regeneration and with higher rate of some clonal variation (Evans *et al.*, 1981). The tissue culture techniques mostly used for micro-propagation and crop improvement involve the meristem/shoot tip culture and also somatic embryogenesis for rapid propagation of selected genotypes, recovery of useful variants/mutants, elimination of pathogens especially virus, phytosanitary transport, exchange and long term storage of germplasm.

2.4.2 Shoot tip culture

Long shoot tips are considered as ideal material for mass propagation but its realization take considerable time. Motti (1981) was the

first to publish the procedure of using excised stem tips to render orchids (*Cymbidium* spp.) free of viruses. Since then shoot tip and lateral bud cultures have been tried by several workers in gooseberry (Jones, 1986), strawberry and mulberry (Ozkan, 1993), apple (Dutcher and Powell, 1972), grapes (Skene and Barlass, 1978, Chee and Pool, 1983; Stamp and Meredith, 1988), papaya (Rajeevan, 1983; Litz, 1985), almond (Mehra and Mehra, 1974) and pistacio (Barghchi and Alderson, 1985). By reducing the explant to the smallest size that would survive and grow in culture, the correlative controls imposed by other tissues and neighbouring cells needed to be reduced (Bonga, 1987). Shoot tip explants as small as 0.1 to 1.0 mm apices (Chee and Pool, 1982) and as large as 10 to 20 mm microcutting (Dawn, 1987) have been used in *in vitro* culture of grapevine.

Rodriguez *et al.* (1989) observed that *J. regia* juvenile material proliferated by applying concentration of BAP ($1-4 \text{ mg l}^{-1}$) to a constant concentration of IBA (0.1 mg l^{-1}). After 10 days culture on media all the existing buds developed simultaneously attaining an average growth of about 0.5 cm, which was found useful for rooting and primary source of explants for establishing a multiplying chain. Shoot tip culture of actively growing seedling was tried in *J. regia* by Somers *et al.* (1982). It was found that presence of BAP (1 mg l^{-1}) and IBA (0.1 mg l^{-1}) on high MS salt

concentration stimulated growth of axillary shoot buds. Heile and Gaffiney (1984) achieved multiple shoot bud formation on WP medium by applying BAP (2-5 mg l⁻¹). In general, it is possible to affirm that the hormonal balance and the *in vitro* requirements for simultaneous shoot bud development on shoot bud proliferation depends on two main factors viz., *Popysis* and shoot diameter, as also reported for *J. regia* and other walnut hybrids (Jay-Allemand, 1982). Results indicate that apical shoot segments require higher amount of cytokinins in media than basal shoot portions, further that the hormonal ratio required decrease when kinetin instead of IBA is applied (Rodriguez *et al.*, 1984). Driver and Kuniyuki (1984) using shoot segments of paradox actively growing walnut seedling, reported an excellent rate of proliferation (five shoots per node) after 56 days of culture. The results were obtained in a new medium DKW in the presence of BA (1.01 mg l⁻¹) and IBA (0.1 mg l⁻¹). These combinations did not give the same results in *J. regia* when K (h) or MS were used.

2.4.3 Culture establishment

Explant is cultured in establishment medium containing different growth regulators. Constitution of media varies from species to species. Apple meristem tips were established on a medium including macronutrients (Quirin *et al.*, 1977), MS-micronutrients (Murashige and

Skoog, 1962) and vitamins (Walkey, 1972). Shoot tip establishment of *Vitis* species was found to be excellent on Murashige and Skoog (1962) medium substituted with 3 μ M thiamine HCl, 5 μ M benzylaminopurine, 0.5 μ M naphthalene acetic acid, 3 per cent sucrose and 0.8 per cent 'Bacto Agar' (Chee *et al.*, 1984). Walkey (1972) reported that establishment of apple shoot tip was facilitated by a few days culture in flasks containing liquid medium tilted on their sides and rotating in a vertical plane.

2.5 Occurrence of phenols and browning mechanism of *in vitro* cultures

2.5.1 Plant systems

Plant phenols are present in abundant quantities in plants. Lignin is a good example of ubiquity of phenolic compounds in plant kingdom. It is now evident that biosynthesis and productivity of phenolic compounds are regulated by variety of external and biological factors (Ibrahim, 1987).

According to the Loomis and Battaile (1966) most phenolic compounds belong either to the flavonoid compounds (including condensed tannins) or to the group of compounds where the 6-carbon ring has 1 or 3 carbon side chains and their derivatives e.g. cofferic acid, gallaicacids, hydrolysable tannins, tyrosine and lignin. Chemically plant phenolic compounds are extremely heterogenous and range from simple monomers to a very large polymers (Haslam, 1989).

No tissue lacks phenolic compounds and high concentration can be found in actively growing cells (Esau, 1977). Phenolic compounds can be found in the cytoplasm, vacuoles, cell walls and these cells form a connected system that occurs in many families e.g. *Ericacea*, *Fabacea*, *Myrtaceu*, *Roseaevitaceae* (Compton and Preece 1986).

Walnut is an important nut crop which is difficult to propagate and where browning and death of explants has been reported. Browning has been found to be mainly due to phenolic compounds that get oxidised (Catherine *et al.* 1986). Steffan and Milikan (1983) also reported that meristems were not suitable for walnut micropropagation. Kevers (1997) reported that lower multiplication and rooting rate was due to the presence of phenolic compounds and endophytic bacteria contamination and were main limiting factors to the industrial plant micropropagation set up in this crop.

Driver *et al.* (1984) investigated constraints in 8 varieties (Chico, Franquette, Hartley, Lara, Melanaisa, Rego, Serr and Trinta) and 2 rootstocks (Nigra and Dagia) of walnut. The use of young vegetal material was the usual technique for *in vitro* set up. The quality in regeneration of *in vitro* plant material was correlated with maintenance of mother plants in controlled environments, with regular hormone application and proper

choice of physiological stage for collecting the material. The correct temperature regime in growth chamber was essential for a proper regeneration as well as for subsequent multiplication. The addition of PVP to the culture medium as well as the substitution of agar by gelrite were the main factors reported for the control of phenolic compound production (Ponchi *et al.*, 1993).

Vahdati *et al.* (2009) compared the proliferation and rooting ability of some low vigour and early mature seedlings of Persian walnut with those of semi-and high-vigour Persian walnut seedlings in *in vitro* condition. For each vigour group model the explants of newly grown shoots of 5 year old seedlings were cultured on PKW medium. These explants were subcultured after every month and up to 13 times to increase the number of microshoots. Results showed that the number of axillary shoots arising from the microshoots were highest in the dwarf and semi-dwarf genotypes as compared to the high vigour ones. The low vigour genotypes also showed the highest number of nodes for a given size of shoot, smaller shoot size (2.6 vs. 4.5 cm), lower callus formation and higher rooting percentage (63.5 vs. 37.1%). Moreover, these genotypes showed *in vitro* flowering on microshoots which were consistent with the field observations. These results provided the consistency of low vigour, precocity, basotonic growth

tendency and easy rooting of dwarf and precocious genotypes under *in vitro* conditions. This study recommended simultaneous recurrent selection programme for both dwarfing and rooting ability to utilise their advantages in high density orchard systems.

Fatima *et al.* (2006) studied embryonal axes in *in vitro* formed shoot apices. Nodal segments of walnut (*J. regia*) were cultured on MS medium of Murashige and Skoog (1962) that was supplemented with different concentrations and combinations of plant growth regulators. Varying response of cultured embryos to various phytohormones were observed and the best results were obtained when combination of BAP and IAA were used. Stimulation and development of multiple shoot buds (6-8) on both sides of embryonal axes were observed after 10 weeks of culture on half strength MS medium augmented with IAA (11.4 μM) and BAP (8.8 μM). Multiplication continued for months together. The materials were transferred to fresh medium after every 4-6 weeks to avoid decrease in proliferation. Remarkable elongation of these microshoots was noticed after 8-10 weeks of culture when combination of auxin and cytokinin was used. Different trials for rooting were conducted on the cultures raised from *in vitro* elongated shoots using different rooting media which resulted in well developed root initials at cut end after 6-8 weeks of culture period.

Robert and Phillippe (1990) studied the micropropagation of different species of Juglans viz. *J. regia*, *J. nigra*, *J. cinera* and a rootstock called Paradox. The initiation of the culture was obtained by using a modified MS medium containing BAP (0.5 mg l^{-1}). Proliferation increased when increased quantity of macronutrients and BAP ($0.8\text{-}1.0 \text{ mg l}^{-1}$) were used. The proliferated clusters were transferred to an 'elongation' medium for 3 weeks. The microcuttings were transferred to DKW medium containing IBA (2.0 mg l^{-1}) and riboflavin (1.0 mg l^{-1}). The culture tubes were kept in darkness for a period of 10 days. After 4-5 weeks the plantlets were transferred to a compost and sand mix (2:1) and covered with plastic for hardening.

Kaur *et al.* (2006) standardized the medium and culture conditions for embryo culture of five cultivars of walnut viz. ACO38853, "Nectar Akhrot", "Gobind", "Solding" and "Blackmore". Embryo from mature fruits were aseptically excised and cultured on MS medium supplemented with different combinations of BAP, kinetin and GA_3 . Best performance was obtained in MS medium containing 0.5 mg l^{-1} kinetin, 0.5 mg l^{-1} BAP and 2 mg l^{-1} GA_3 resulting in 66.6 per cent germination in "Necktar Akhrot" after 12 days of cutting. Per cent germination of excised embryos was higher when GA_3 and cold treatments were simultaneously applied as

compared to those when applied separately. “Nectar Akhrot” was found to be the best responding cultivar, which had a range of 25.0-66.6 per cent embryo germination under different culture conditions. Plantlets with shoots and roots were obtained in “Nectar Akhrot” and “ACO38853” and subsequently were transferred to soil media after hardening.

Cheema and Mehra (1982) observed callus formation in seed endosperm (mature and immature) on MS medium containing IBA (2.0 mg l^{-1}) and NAA (0.2 mg l^{-1}). The cultures developed roots and shoots when the media was also supplemented with cytokinins such as Kn (2 mg l^{-1}). Rodriguez *et al.* (1983) studied *in vitro* propagation of 5 to 7 week old plants. Callus formation preceded root or multiple shoot bud induction. The morphology varied in accordance with the auxins present in the medium of culture. Callus induction in miniature cotyledons when cultured on K (h) medium supplemented with 2,4-D ($0.1\text{-}10 \text{ mg l}^{-1}$) and Kn ($0.1\text{-}1 \text{ mg l}^{-1}$) was rapid. The callus was nodular and compact with a tendency of browning which increased gradually as the 2,4-D concentration increased and the period of explant culture was prolonged. No organ induction was possible from the callus formed in the presence of this growth regulator. However, callus induction on the same media but supplemented with NAA ($0.1\text{-}10 \text{ mg l}^{-1}$) or IBA (0.1 mg l^{-1}) was rapid and more morphogenetic. The callus

obtained was fragile, greenish, with less tendency to browning, of more rapid growth and produced a greater number of anthocyanins especially in presence of NAA (100 mg l^{-1}) and Kn (0.5 mg l^{-1}). Greater root initiation was also observed on this medium.

Rodriguez (1982) had undertaken studies with embryos from mature seeds, and from leaves of adult plants. Callus was induced from both kinds of explants on MS medium supplemented with auxins (2,4-D or NAA or IBA), and in certain cases when cytokinins (Kn or BAP) were added. It was found that auxin induced callus formation. The presence of cytokinin was not essential but increased the effect of auxin when applied at low concentration. He also observed that callus morphology induced from mature seeds or from leaves was not different from that obtained from cotyledons. Those induced by 2,4-D were not morphogenetic. Calli induced by NAA or IBA were morphogenetic although they had lesser development in IBA than in NAA.

Jay-Allemand (1982) reported that BAP in combination with IBA on K(h) or MS media gave promising results but the same response did not occur in higher concentrations of mineral salts in other tested media. When BAP or other cytokinins were applied alone or in combination with IBA, respectively, proliferation decreased forming only shoot buds or callus. It

was also found that BAP (1,5, 10 mg l^{-1}) in combination with IBA (0.1 mg l^{-1}) was useful for simultaneous shoot bud development in model shoot segments. The optimal cytokinin level being around 1 mg l^{-1} . Higher concentration of BAP (5 to 10 mg l^{-1}) induced callus formation which in a short period proliferated. The new shoots formed new proliferations as in juvenile materials. Apical shoot segments gave rise to a single shoot in all the concentrations tested. Thus, more topophysis, leaf development and apical dominance were shown to have a great influence on morphogenetic response.

Somers *et al.* (1982) studied shoot tip culture of actively growing seedlings of *J. nigra*. The presence of BAP (1 mg l^{-1}) and IBA (0.1 mg l^{-1}) on high MS salt concentration stimulated axillary shoot bud development. Heile and Gaffney (1984) also reported the multiple shoot bud formation on WP medium containing BAP (2-5 mg l^{-1}).

Meynier (1984) reported that the shoot bud development was obtained when meristems of about 0.5 mm were cultured on half strength MS medium. BAP (1 mg l^{-1}) seems to be effective when the culture period was not more than 60 days with transfer after every 7 days.

2.5.2 Tissue injury and oxidative browning

Phenols and other secondary metabolites play principal role in the

ecological context (Wink, 1987). They serve as defence compounds against micro-organisms (Levin, 1976; Harbone, 1982), Phytophagous animals and other plants (allelopathy). In contrast to mechanical defence (thorns, spikes and trichomes), chemical defence mechanism involves the production of phenols (and other secondary metabolites) and hydrolytic or oxidative enzymes. In woody plants phenolic compounds are also thought to be strongly involved in compartmentalization, a process in which plants seal off the damage of tissue by establishing chemical barrier or walls to guard against infection and decay (Harris, 1983). When plant tissues are injured, they respond chemically by oxidation of endogenous phenols or by production of either mono or polyphenolic compounds (Haslam, 1989). Key enzymes are then activated that lead to the accumulation of either simple phenols such as chlorogenic acid or polymeric compounds such as lignin (Rhodes and Wooltorton, 1978). There is however, ambiguity in the literature about the relationship between oxidised phenolic compounds and disease resistance (Mayer and Harel, 1979). Terpenoid metabolism is also affected by tissue injury. Work with potato (Kahl, 1974) has shown that as soon as the tissue is injured carbon dioxide is released and respiration increases resulting in terpenoid synthesis and its accumulation.

2.5.3 Oxidative browning in tissue cultures of various woody perennials

Phenolic compounds produced in *in vitro* cultures may function to protect injured tissue from invasion of various pests. In *in vitro* the oxidation products are autoinhibitory to the explants. The autoinhibitory phenomenon of oxidative browning is encountered in many species especially woody plants which include fruit, forest and plantation trees and woody ornamentals.

Fanizza *et al.* (1984) cultured shoot tips of mature field grown vines and observed extensive browning. Highly browned explants turned necrotic and died within two days. Yu and Meredith (1986) also observed tissue browning in cutlrued grape explants which affected *in vitro* explant establishment adversely. Explants of apple excreted yellowish brown exudates into the medium which not only inhibited growth of explatns but also adversely affected their survival (Cheema and Sharma, 1983). Explants, however, vary with respect to the amount of total phenol exudates in the medium (Chadha and Sharma, 1988). Zaid (1987) reported inhibition in growth of date palm explants due to browning. Success in the attempts of tissue culture in cashew (Nambiar and Iyer, 1988) and its fellow species mango (Doreswamy, 1988) and pistachio (Barghchi and Alderson, 1985) has been rather limited mainly because of the problem of polyphenols

which rapidly arrest growth of explants in *in vitro*. The difficulties in establishment of aseptic culture from mature explants of coconut (Padmanabhan, 1988), walnut (Lenartowicz and Millikan, 1977) and chestnut (Chevere *et al.*, 1983) are associated with browning of explants. This has limited the progress in the development of *in vitro* propagation in these species.

Lloyd and McCown (1980) observed tissue breakdown and a dark exudation into the medium after dividing and subculturing the basal shoot mass of mountain laurel (*Kalmia latifolia* L.) shoot tips. Christiansen and Fonnesbech (1975) reported greater problems with browning of the agar medium that resulted in the subsequent death of *Hamamelis intermedia* Rehd shoot tips. They measured product of the width and depth of the brown zone underneath the explant, and called it “browning intensity”. McCown and Amos (1979) observed exudation from the cut surface of birch stem tips and node sections that were removed from actively growing plants. Rodriguez (1982), while working with *Juglans regia* L., observed that calli were incapable of organ induction possibly because of the increased production of tannins. According to Skirvin *et al.* (1990) the principal factors which limit growth in rose tissue culture is the browning and contamination.

Anderson (1975) observed that portions of *Rhododendron* shoot tip that were in contact with medium rapidly turned brown. The browning gradually progressed up the stem until the explants were completely killed. Ettinger and Preece (1985) observed a dark exudate that diffused into the medium from *Rhododendron* 'P.J.M. Hybrid' shoot tip cultures. Explants from *Exbury azalea* cultured on Anderson medium developed stem and leaf necrosis at the base that moved up the explant (Fordham *et al.*, 1982).

2.5.4 Stock plant *in vitro* ecology and manipulations in relation to oxidative browning

The age of the donor plant and location on the stem from which the explants are removed can greatly affect the establishment of tissue in *in vitro*. Muhitch and Fletcher (1985) reported that the younger growth of Paul's scarlet rose was easier to establish in *in vitro* than older growth. The concentration of phenols in older (more basal) stems was higher than in more apical (young) stems. In addition, older stems contained a greater variety of phenolic compounds than younger stems. Chevere *et al.* (1983) observed that the amount of phenolic compounds from chestnut was less in 3 month old seedlings than in the buds from twigs of 5 year old trees. They also concluded that for explants in *in vitro* culture the youngest growth should be utilized to minimise exudation. Yu and Meredith (1986) reported that phenolic content of explants derived from terminal position fully

exposed to sun was relatively higher than those from shaded axillary positions in grapevine. Welsh *et al.* (1979) found that phenolic secretion was especially more evident from buds which were taken from trees of *Pseudotsuga menziesii*, and which decreased with increasing age (Everse *et al.*, 1988). Fanniza *et al.* (1984) observed much more browning and necrosis in cultured shoot tips from field grown grapevines than from those grown either in the shade house or in greenhouse. Physiological state of source plant and its physiological age (juvenile or adult phase) has profound affect on culture performance of explants (Lane, 1982).

Research on the response of explants and their relationship to stock plant treatments has been limited. Attempts to improve *in vitro* performance of explants by the treatment of stock plants with growth regulators have been made by various workers (Read *et al.*, 1978; Preece, 1987) with varying degree of success. Variable species specific response to rejuvenation with hormonal sprays, pruning and grafting on seedling stock have been obtained (Debergh and Maene, 1985). However, physical methods to induce rejuvenation in mature trees of 50 year old *Sequoia sempervirens* by pruning and lopping were used with success. According to Debergh and Maene (1985) stock plant preparation for tissue culture propagation can be helpful in minimising browning problems. Forcing of

stock plant preparation for *in vitro* culture of woody species has been studied by Read and his coworkers (Read *et al.*, 1984; Read *et al.*, 1986), but they did not report effectiveness of this treatment to control oxidative browning.

2.5.5 Hormones in the culture medium

Presence of certain hormonal substance in the nutrient media might bring about browning in cultured tissues either directly or indirectly (Zaid, 1987). There is sufficient evidence to indicate that the type and concentration of auxin and cytokinins, as well as their relative ratios in the culture medium, control the biosynthesis and accumulation of phenols (Davies, 1972; Ibrahim, 1987; Zaprometov, 1988). The accumulation of simple phenolics, coumarins, depsides and lignins has been reported to be stimulated in the presence of low auxin levels especially naphthalene acetic acid (King, 1976) or 2,4-D (Sugano *et al.*, 1975). However, increasing the auxin concentration either stimulated (Zenk *et al.*, 1977) or inhibited (Ibrahim and Edgar, 1976) phenolic production.

Treutter *et al.* (1985) reported that addition of benylaminopurine (BAP) to the media of *Prunus* tissue cultures resulted in an increased synthesis of eriodictyal-7-glycosides and particularly prunin. Both these compounds have antagonistic effects on growth (Stenlid, 1963). Concerning

hormonal implications, it is revealed that pruning has been found to cause growth inhibition in excised tissue in *in vitro* (Corgan, 1967). While working with walnut cotyledons (Rodriguez, 1982) attributed the failure of organogenesis in the callus induced by 2,4-D and kinetin to production of phenolic compounds.

2.5.6 Antioxidants and phenol binding agents

The use of antioxidants is one method that has been used to control media and explant browning. Christiansen and Fonnesbech (1975) found that more *Hamamelis* explants survived and developed by increasing polyvinylpyrrolidone (PVP) concentration in culture medium. The best development occurred when there was no browning which was accomplished by adding 1-2 per cent PVP into the agar medium. PVP is a polymer that is a strong hydrogen acceptor but cannot act as a hydrogen donor (Forrest, 1969). This compound adsorbs phenols and inhibits polyphenol oxidase (Vaugh and Duke, 1984). Somer *et al.* (1982) observed that PVP may have inhibited shoot proliferation of black walnut (*J. regia* L.) shoot tip, and that the medium with PVP was darker than medium without PVP. Chevere *et al.* (1983) reported that treating the medium with caffeine and PVP as antioxidants proved to be ineffective in preventing the browning of chestnut cultures. Thus, PVP may be effective only with

selected species. The soaking of explants in water as pre-treatment reduced the browning in walnuts and chestnuts (Rodriguez, 1982). Monaco *et al.* (1977) suggested that coffee explants should be pretreated with an antioxidants (e.g. cysteine-HCl, ascorbic acid) to reduce browning. Terminal and axillary buds of *Strelitiza reginae* treated with antioxidant solution by total submergence and cultured on agar medium controlled browning.

2.5.7 Reduction of salts

Modification of media composition by reducing salts has been an effective means of reducing media browning. Anderson (1975) observed that the browning problem of *Rhododendron* could be overcome by reducing, by half, the concentration of nitrogen containing compounds in the MS medium. Chevere *et al.* (1983) observed that using a media low in salts worked best for reducing browning of the medium in chestnut axillary bud cultures. Gould and Murashige (1985) observed that media that contained MS salts in concentration of 0.003 and 0.1 times than the normal formation contained lower concentration of ber-berine, while higher concentration was observed when the medium contained salts at full strength.

CHAPTER – 3

MATERIALS AND METHODS

The present investigation entitled “studies on intra-specific biodiversity of horticultural traits and *in vitro* shoot morphogenetic response in walnut (*Juglans regia* L) germplasm” was carried out during the crop seasons of 2008 and 2009. The studies comprised two clusters of germplasm extending over the main geographical distribution of cultivation in the Jammu and Kashmir state.

3.1 Materials studied

Genetic variability studies and diversity were estimated in the natural walnut population of Kashmir valley forming two cluster populations. Two standard check cultivars (Sulaiman and Hamdaan) were used for comparison.

3.1.1 Cluster-I

Plant materials in this cluster comprised 75 *in situ* earmarked seedling raised plants that were identified after detailed survey of the areas having large concentration of the crop in the districts of Kupwara and Baramulla (Appendix – I-II).

3.1.2 Cluster-II

In this cluster plant materials also comprised 75 *in situ* earmarked seedling raised plants that were identified after extensive survey of promising materials in the Pulwama and Shopian districts of South Kashmir and Budgam district of central Kashmir (Appendix – I-II). The data of both the clusters (over 2 years) was pooled together for statistical analyses.

Ex-situ elite plant materials of walnut were also studied for response to shoot morphogenesis and comparative influence of different concentrations of growth regulators. Based on the earlier reports, one basic growth media was selected and different concentration of IBA and BAP were tried.

3.2 Methods adopted

3.2.1 Phenological Descriptor

For the purpose of collecting the data, the individual trees in the study area were earmarked after preliminary visual observations. Each tree was given a number and its location, owners name and other identification marks. Data were recorded as per the “International Buru Plant Genetic Resources” (IBPGR for Walnut). Apart from recording all the descriptive and quantitative aspects of the available germplasm, the data generated were also numerically coded as per the Descriptor.

Each of the tagged walnut (*Juglans regia* L.) tree, in both the clusters, was studied for the below mentioned phenological characters in both the years as per the phenological character descriptor of walnut (Anonymous, 1994). For each trait of phenological character, four branches of each tagged tree spread in four directions i.e North, East, South and West were taken for observations. The detailed procedure is presented as under:

3.2.1.1 Harvesting date

Walnut is mostly harvested in the autumn season in the Kashmir valley. The total fruit is harvested in two to three pickings within a few days interval. Two maturity indices were used for determining the harvesting date of the walnut viz, when nut was tightly covered with a thick hull which splits and falls on to the ground naturally, and when about 80 per cent of the hulls had cracked and resulted in the exposure of nuts.

3.2.1.2 Tree Vigour

Growth rate of seedling trees based on height and stem diameter was recorded and scored as per the descriptor.

Low	-	3
Intermediate	-	5
High	-	7

3. 2.1.3 Growth habit

Uprightness of vigorous current season shoots was taken for this trait.

Erect - 1

Semi erect - 2

Spreading - 3

3.2.1.4 Branching

The branching habit of the tree was recorded as per the relative degree of branching.

Sparse - 3

Intermediate - 5

Dense - 7

3.2.2 Leaf Descriptor

Average of 10 fully expanded representative leaves were taken from each earmarked tree in both the years and average worked out.

3.2.2.1 Leaflet shape

Narrow elliptic - 1

Elliptic - 2

Broad elliptic - 3

3.2.2.2 Leaflet margin

Entire	-	1
Serrate	-	2
Dentate	-	3

3.2.2.3 Leaf colour

Light green	-	3
Green	-	5
Dark green	-	7

3.2.3 Shoot colour

Green	-	3
Brown	-	5
Black	-	7

3.2.4 Pomological Characters

For nut and kernel characters a random sample of 20 nuts was selected from the harvested lot of each earmarked tree in both the years and data recorded after proper drying of the nuts. Following characters were studied as per the Descriptor.

3.2.4.1 Nut shape

The selected nuts were scored as per their shape.

Round	-	1
Triangular	-	2
Broad ovate	-	3
Ovate	-	4
Short trapezoid	-	5
Long trapezoid	-	6
Broad elliptic	-	7
Elliptic	-	8
Cordate	-	9

3.2.4.2 Shell texture

The shell strength of the nuts was graded as per the following score:

Papery	-	1
Weak	-	3
Intermediate	-	5
Strong	-	7

3.2.4.3 Shell integrity

The shell integrity was graded as follows :

Incomplete shell	-	1
Intermediate	-	2
Complete shell with no holes	-	3

3.2.4.4 Kernel shrivel

Kernel shrivel percentage was graded as under:

1. Kernel exhibiting tip shrivel
2. Kernel exhibiting <50% shrivel
3. Kernel exhibiting 50% or more shrivel

3.2.4.5 Kernel fill

The kernel fill in the nuts was classified as follows :

Poor	-	3
Moderate	-	5
Well	-	7

3.2.4.6 Ease of removal of kernel halves

Ease of removal of kernel halves was recorded as follows:

Very easy	-	1
Easy	-	3
Moderate	-	5
Difficult	-	7

3.2.4.7 Kernel colour

Colour of the kernels was scored as

Extra light	-	1
Light	-	2
Light amber	-	3
Amber	-	4

3.3 Canopy spread and stem diameter

3.3.1 Canopy spread

The spread was measured in terms of the extent of the canopy in two different directions i.e North South and East West and mean of two was expressed in metres (m).

3.3.2 Stem girth

The girth of the plant was measured above the ground level and expressed in metres (m).

3.4 Leaf size

3.4.1 Leaf Length

The length was measured with the help of scale and expressed in centimetres.

3.3.5 Leaf width

The width of the leaf was measured with the help of scale and expressed in centimetres.

3.5 Nut Characters

3.5.1 Nut weight (g)

The weight of 20 randomly selected dried nuts from the harvested lot of each tree was recorded on a high precision technical balance and the average expressed in grams.

3.5.2 Nut diameter

The diameter of randomly selected dried nuts taken for nut weight was measured by using vernier calliper and expressed in millimetres both

for suture and cheek.

3.5.3 Nut length

The length from distal to top proximal end was measured for each of the 20 randomly selected dried nuts by using vernier calliper and expressed in millimetres.

3.5.4 Kernel weight

Weight of 20 sound and unbroken dried kernels was recorded on a high precision technical balance and the average expressed in grams.

3.5.5 Kernel recovery percentage

The kernel recovery was recorded as per the following formula and expressed in percentage.

$$\text{Kernel percentage} = \frac{\text{Weight of the kernels (g)}}{\text{Weight of the nuts (g)}} \times 100$$

3.6 Nut Yield

The nut yield was obtained by taking weight of all the harvested nuts. The nuts were cleaned and all the hulls removed before weighing and then dried to a moisture content of 8-10 per cent. The total nut yield per tree was expressed in kg. The yield/tree was grouped into different age groups to minimize the variation.

3.7 Qualitative analysis

3.7.1 Protein content

The total nitrogen content was estimated by Micro-Kjeldahl's method and nitrogen content so observed was converted into protein ($N \times 6.25$) as crude protein content, which also included non-protein nitrogen. However, to get true protein content the method given by Sandasivam and Manickam (1992) was followed.

3.7.2 Kernel oil content

The oil content was extracted as per the method suggested by Sandasivam and Manickam (1992).

3.8 Shoot morphogenetic studies

The investigation was carried out at the Tissue Culture Laboratory of the Division of Pomology, SKUAST (K), Shalimar during the year 2008-09. The investigation involved shoot morphogenetic response in walnut to different treatment combinations of growth hormones.

3.8.1 Plant materials used

The mature and bearing elite genotypes of walnut selections viz., SKUAST-002, SKUAST-008 and SKUAST-010 growing at the Experimental Farm of the Division of Polmology at the main campus

Shalimar were used as stock plants in the present study. The plant materials were subjected to uniform cultural practices.

3.8.2 Chemicals used

The major and minor elements required for the preparation of media were obtained from the Hi Media Pvt. Ltd.. The amino acids, vitamins and plant growth regulators used were obtained from the Sigma Chemicals.

3.8.3 Glass wares

The glass ware used for the experiment was obtained from the Borosil Glass-ware. Before use the glassware was soaked in potassium dichromate- nitric acid solution for six hours followed by thorough washing in a jet of tap water so as to completely remove all traces of dichromate solution. They were then soaked in detergent solution (Teepol 1%) overnight and were thoroughly washed in tap water and rinsed twice with double distilled water. The glass-ware was then dried in hot air oven at 100°C for 24 h and later stored under aseptic conditions till use.

3.8.4 Culture media

3.8.4.1 Selection of culture media

Murashige and Skoog's (1962) medium was used for the present investigation.

Composition and preparation of stock solutions for Murashige and Skoog (1962) medium

Stock solution	Components	Chemical formula	Quantity (mg l ⁻¹)	Quantity for 20 litres	Quantity of stock solution prepared	Conc. of stock solution
A	Ammonium nitrate	NH ₄ NO ₃	1650	33.00 g	200 ml	100 X
B	Potassium nitrate	KNO ₃	1900	38.00 g	200 ml	100 X
C	Potassium iodide	KI	0.83	16.6 mg	200 ml	100 X
	Boric acid	H ₃ BO ₃	6.2	124 mg		
	Potassium phosphate, monobasic	KH ₂ PO ₄	170	3.4 g		
	Molybdic acid (Sodium salt) 2H ₂ O	Na ₂ MoO ₄ ·2 H ₂ O	0.25	5 mg		
	Cobalt chloride hexahydrate	CoCl ₂ ·6H ₂ O	0.025	0.5 mg		
D	Calcium chloride, dihydrate	CaCl ₂ ·2H ₂ O	440	8.8 g	200 ml	100 X
E	Magnesium sulphate, heptahydrate	MgSO ₄ ·7H ₂ O	370	7.4 g	200 ml	100 X
	Zinc sulphate, heptahydrate	ZnSO ₄ ·7H ₂ O	8.6	172 mg		
	Managanese sulfatem tetrahydrate	MnSO ₄ ·4H ₂ O	22.3	446 mg		
	Cupric sulfate pentahydrate	CuSO ₄ ·5H ₂ O	0.025	0.5 mg		
F	Ethylenediaminetera acetic acid disodium salt	Na ₂ EDTA·2H ₂ O	37.3	746	200 ml	100 X
	Ferrous sulfate heptahydrate	FeSO ₄ ·7H ₂ O	27.8	556 mg		
G	Glycine	-	2.00	40 mg	200 ml	100 X
	Nicotinic acid	-	0.5	10		
	Pyridoxine HCl	-	0.5	10		
	Thiamine HCl	-	0.1	2		

Inositol (100mg), sucrose (30g) and agar (7.0g) required for one litre culture medium were added directly at the time of preparation of culture media.

3.8.4.2 Adjuvants to the basal medium

The MS basal medium was appropriately supplemented with vitamins, aminoacids and growth regulators. Stock solution of organics (vitamins and amino acids) were prepared in double distilled water.

3.8.4.3 Preparation of the culture media

The required quantity of sucrose was dissolved in double distilled water and to this stock solution macronutrients, micronutrients, vitamins and growth substance were added as per treatment required. The media of various formulations were prepared.

The pH of the medium was adjusted to 5.5 with HCl or 0.1 N NaOH prior to the addition of agar. Agar (7.0 g^{-1}) was dissolved in boiling distilled water and added to the medium and the volume was made up with distilled water. The medium was boiled and the hot medium was then immediately dispensed in the test tubes or conical flasks which were plugged tightly with non-absorbent cotton plugs and covered with aluminum foil. The medium was autoclaved at 15 psi (121°C) for 15 minutes (Dodds and Robert, 1982).

3.8.4.4 Transfer area and aseptic manipulations

All the aseptic manipulations like surface sterilization, preparation and inoculation of explants and subsequent subculturing were carried out

under hood of clean laminar air flow chamber. The working table of the laminar air flow chamber was first surface sterilized with absolute alcohol followed by ultra violet light for 30 minutes. The forceps and scalpel used for the inoculation as well as the Petri dishes were first steam sterilized in an autoclave at 121⁰C for 20 minutes and later flame sterilized before each inoculation. The hands were cleaned and wiped with alcohol before working. Further, the standard general procedure for sterile technique suggested by Street (1977) was followed.

3.8.4.5 Incubation chamber

The cultures were incubated at 24±1⁰C in an air condition culture room with a 16 h photoperiod (3500 lux).

3.8.4.6 Collection and preparation of explant

3.8.4.6.1 Forcing of stock plant

This experiment consisted of field grown adult shoots and artificially forced walnut cuttings of the genotypes SKUAST-002, SKUAST-008, and SKUAST-010 of walnut. For forcing the dormant cuttings of 15-20 cm length were collected in January. Dormant cuttings were treated with 0.2 per cent captan and stored in cold store at 4±3⁰C in polythene bags for two months. Cuttings were withdrawn from cold store as and when required and placed in jars containing distilled water and kept in incubation chamber.

The cuttings were incubated at $24\pm 1^{\circ}\text{C}$ in an air condition room with 16 hours of Photoperiod (3.5 lux). Sprouting of dormant buds occurred after one month of transferring to incubation chamber which served as explant source. Shoot tips from all the three genotypes were excised and cultured on semi-solid and stationery liquid medium for establishment. Each treatment combination consisted of 25 explants and was replicated 3 times for each genotype.

3.8.4.6.2 Surface sterilization and inoculation

The explants were surface sterilized with 0.1 per cent (w/v) mercuric chloride solution for 10 minutes and then washed thoroughly with double distilled sterile water (5-6 times) under aseptic conditions in the laminar flow chamber. The explants were then placed on the medium in such a manner that conformed to the original polarity and were exposed above the surface of medium.

3.8.5 Details of *in vitro* studies

3.8.5.1 Details of growth regulators

The explants were cultured in Murashige and Skoogs (1962) basal medium (MS) supplemented with different concentrations of growth regulators. The details of the combinations of different concentration of growth regulators are presented below :

Growth regulators		Concentration used			
BAP	0.3 mg l^{-1}	0.6 mg l^{-1}	0.9 mg l^{-1}	1.2 mg l^{-1}	
IBA	0.1 mg l^{-1}	-	-	-	

3.8.5.2 Treatment combinations used

$$\begin{aligned}
 T_1 &= \text{BAP } 0.3\text{mg}l^{-1} + \text{IBA } 0.1\text{mg}l^{-1} \\
 T_2 &= \text{BAP } 0.6 \text{ mg}l^{-1} + \text{IBA } 0.1 \text{ mg}l^{-1} \\
 T_3 &= \text{BAP } 0.9 \text{ mg}l^{-1} + \text{IBA } 0.1 \text{ mg}l^{-1} \\
 T_4 &= \text{BAP } 1.2 \text{ mg}l^{-1} + \text{IBA } 0.1 \text{ mg}l^{-1}
 \end{aligned}$$

The data recorded on various parameters of tissue culture studies were put to statistical analyses. Values in per cent were transformed to arc sin (angular) or square root transformation before analysis. The mean performance of the cultivars and growth regulator combinations was presented for experimental findings alongwith their interaction (cultivars x growth regulator combination).

3.8.5.3 Survival of shoot apices

Survival of explants was recorded within 8 days of inoculation in each treatment combination for each cultivar and expressed as per cent as follows :

$$\text{Per cent survival of explants} = \frac{\text{Number of explants survived}}{\text{Total number of explants cultured}} \times 100$$

3.8.5.4 Per cent growing cultures

Number of explants that showed growth were also recorded within 3 weeks of inoculation and expressed in per cent.

$$\text{Per cent growing cultures} = \frac{\text{Number of explants showing growth}}{\text{Total number of explants cultured}} \times 100$$

3.8.5.5 Explants showing browning

Explants that showed browning within 8 days were recorded and expressed as per cent for each treatment.

$$\text{Per cent explant browning} = \frac{\text{Number of explants browned}}{\text{Total number of explants cultured}} \times 100$$

3.8.5.6 Mean browning score per explant

Explants that showed browning were scored for browning intensity as under;-

- | | | |
|---|---|--|
| 0 | = | No browning |
| 1 | = | Browning on excised wound tissue. |
| 2 | = | Partial browning on outer tissue of explant |
| 3 | = | Complete browning of the outer tissue of explant |
| 4 | = | Browning of the entire explant tissue. |

3.8.5.7 Explants showing substrates browning

Substrate browning (irrespective of explant browning) was recorded for each culture treatment in a genotype and expressed as per cent.

$$\text{Per cent substrate browning} = \frac{\text{Number of culture tubes showing media browning}}{\text{Total number of explants cultured}} \times 100$$

3.8.5.8 Volume of browned media per culture

Volume of the browned media was calculated on the basis of diameter of browned media surrounding the explant and depth of the browned media by the following relationship.

$$V = \pi r^2 \times d$$

Where,

V = Volume in cubic millimetres.

r = radius of the browned media in millimetres

d = depth of the browned media in millimetres.

Browning score of the explant and volume of browning media in each treatment was worked out per explant basis. The values were expressed finally as volume of browned media (mm)³ x 1/100 per explant.

Observation on growth rating of explant and percentage of callused cultures was recorded after 30 days of culture initiation and growing culture

were again transferred to fresh medium of the same composition.

3.8.5.9 Growth rating of explants

Explants that revived and showed growth were scored for growth vigour as under :

- 1 = Low growth rate
- 2 = Medium growth rate
- 3 = High growth rate

3.8.5.10 Percentage of callused cultures

Explants that survived and revealed growth were scored for callusing and expressed as per cent.

$$\text{Per cent cultivars showing callusing} = \frac{\text{Number of explants expressing callus}}{\text{Number of surviving and growing explants}} \times 100$$

3.9 Statistical analysis of the data

The data recorded for phenotypic variability of trait were put to statistical analyses and different parameters estimated.

3.9.1 Genetic variability studies

Data for each trait of each earmarked *in-situ* tree was recorded over two crop seasons (*Kharief* 2008 and *Kharief* 2009) and statically analyzed.

3.9.2 Analysis of variance

Source of variation	df	Expected mean	Sum of squares	Variance ratio
Replications (years)	y-1	MS ₁	$\sigma^2_e + g\sigma^2_y$	MS ₁ /MS ₃
Cultivars (genotypes)	g-1	MS ₂	$\sigma^2_e + y\sigma^2_g$	MS ₂ /MS ₃
Replications x Cultivars	(y-1) (g-1)	MS ₃	σ^2_e	

3.9.3 Estimation of variability parameters

Different variability parameters estimated were as under :

3.9.3.1 Mean and range

The population mean and range of each trait was expressed in the same units as that of the trait.

3.9.3.2 Phenotypic coefficient of variation

Phenotypic coefficient of variation (PCV) was expressed as

$$PCV = \frac{\sqrt{\sigma^2_p}}{\bar{X}} \times 100$$

3.9.3.3 Genotypic coefficient of variation

Genotypic coefficient of variation (GCV) was expressed as

$$GCV = \frac{\sqrt{\sigma^2_g}}{\bar{X}} \times 100$$

3.9.3.4 Components of variance

The variance for each trait comprised variance arising from the

cultivars difference (genotypic variance) and that arising from the environmental factor (in this case years). The expected mean sum of squares would be as under:

$$\text{MS for cultivars} = \sigma^2_e + y \sigma^2_g = \text{MS}_2$$

$$\text{MS for error} = \sigma^2_e = \text{MS}_3$$

$$\text{Genotypic variance, } \sigma^2_g = \frac{\text{MS}_2 - \text{MS}_3}{Y}$$

$$\text{Phenotypic variance} = \sigma^2_P = \sigma^2_g + \sigma^2_e$$

3.9.3.5 Heritability (broad sense)

It was measured as the ratio of genotypic variance to phenotypic variance.

$$h^2(\text{bs}) = \sigma^2_g / \sigma^2_P$$

3.9.3.6 Genetic gain

Genetic gain (per cent of mean) was expressed as

$$\text{Genetic gain} = \frac{h^2(\text{b.s}) \times \sigma_P \times K}{\bar{X}}$$

Where,

$h^2(\text{b.s})$ = Heritability in broad sense,

σ_P = Phenotypic standard deviation

K = Selection intensity (the value of k at 5% was taken as 2.06), and

$\bar{X}$ = Population mean of the trait.

CHAPTER – 4

EXPERIMENTAL FINDINGS

4.1 General

The present investigation comprised one hundred fifty (150) seedling genotypes found growing in various regions of Kashmir valley together with two standard checks (Sulaiman and Hamdan). Most of the seedling trees were indigenous of Kashmir valley. Tremendous variation in configuration of land surface, vegetation aspect, meteorology and soil type was encountered during the study.

The geographical variation has resulted in sizeable genetic diversity in walnuts. The seedlings identified and catalogued in this study represent a cross section of walnut germplasm available in Kashmir. An attempt has been made to evaluate this germplasm in respect of various descriptive and quantitative characters and measures their diversity.

4.2 Date of harvesting

The harvesting of mature walnut genotypes extended from 29th August to 17th September over a period of 23 days (range over 2 years). The mature nuts ready for harvest were first in district Budgam (29th August) followed by Baramulla (5th to 8th September) and Kupwara (10th to 12th

September), while it was last in Pulwama and Shopian (15th to 20th September). The mature nuts were first harvested from selection WS-128 on 29th August which worked out as 178 days taken from full bloom followed by 180 days in WS-130 on 29th August. Most of the remaining cultivars were harvested after 190-197 days after full bloom.

4.3 Morphological and Phenological characters

4.3.1 Tree Vigour

Studies on tree vigour revealed substantial variability among the seedling raised walnuts genotypes in Kashmir valley (Table-1). Tree vigour ranged from low to high. Out of 152 genotypes 43 genotypes (28.30%) possessed low tree vigour, 45 genotypes (29.60%) were intermediate and 64 genotypes (42.10%) exhibited high tree vigour. Walnut selections WS-9, WS-23, WS-24, WS-25, WS-26, WS-27, WS-28, WS-29, WS-30, WS-32, WS-33, WS-35, WS-36, WS-37, WS-38, WS-39, WS-40, 148 had low tree vigour; walnut selections WS-08, WS-10, WS-13, WS-15, WS-16, WS-28, WS-31, WS-34, WS-44, WS-49, WS-52, WS-54, WS-57, WS-62, WS-68, WS-71, WS-73, WS-75, WS-76, WS-77, WS-78, WS-79, WS-81, WS-91, WS-92, WS-93, WS-95, WS-98, WS-99, WS-100, WS-101, WS-106, WS-109, WS-110, WS-111, WS-121, WS-122, WS-123, WS-124, WS-134, WS-146, WS-149, WS-150 and Sulaiman were intermediate in tree vigour;

Table-1: Diversity for tree vigour in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession number	Total	Per cent of the population
Low	3	WS-09, WS-23, WS-24, WS-25, WS-26, WS-27, WS-29, WS-30, WS-32, WS-33, WS-35, WS-36, WS-37, WS-38, WS-39, WS-40, WS-42, WS-43, WS-45, WS-47, WS-50, WS-56, WS-59, WS-60, WS-65, WS-67, WS-69, WS-83, WS-84, WS-102, WS-103, WS-107, WS-108, WS-118, WS-119, WS-120, WS-131, WS-135, WS-136, WS-140, WS-142, WS-143 and WS-148	43	28.30
Intermediate	5	WS-08, WS-10, WS-13, WS-14, WS-15, WS-16, WS-28, WS-31, WS-34, WS-44, WS-49, WS-52, WS-54, WS-57, WS-62, WS-68, WS-71, WS-73, WS-75, WS-76, WS-77, WS-78, WS-79, WS-81, WS-91, WS-92, WS-93, WS-95, WS-98, WS-99, WS-100, WS-101, WS-106, WS-109, WS-110, WS-111, WS-121, WS-122, WS-123, WS-124, WS-134, WS-146, WS-149, WS-150 and Sulaiman	45	29.60
High	7	WS-01, WS-02, WS-03, WS-04, WS-05, WS-06, WS-07, WS-11, WS-12, WS-17, WS-18, WS-19, WS-20, WS-21, WS-22, WS-41, WS-46, WS-48, WS-51, WS-53, WS-55, WS-58, WS-61, WS-63, WS-64, WS-66, WS-70, WS-72, WS-74, WS-80, WS-82, WS-85, WS-86, WS-87, WS-88, WS-89, WS-90, WS-94, WS-96, WS-97, WS-104, WS-105, WS-112, WS-113, WS-114, WS-115, WS-116, WS-117, WS-125, WS-126, WS-127, WS-128, WS-129, WS-130, WS-132, WS-133, WS-137, WS-138, WS-139, WS-141, WS-144, WS-145, WS-147 and Hamdan	64	42.10

*As per the IBPGR Descriptor for Walnut

whileas, walnut selections WS-1, WS-2, WS-3, WS-4, WS-5, WS-6, WS-7, WS-11, WS-12, WS-17, WS-18, WS-19, WS-20, WS-21, WS-22, WS-41, WS-46, WS-48, WS-51, WS-53, WS-55, WS-58, WS-61, WS-63, WS-64, WS-66, WS-70, WS-72, WS-74, WS-80, WS-82, WS-85, WS-86, WS-87, WS-88, WS-89, WS-90, WS-94, WS-96, WS-97, WS-104, WS-105, WS-112, WS-113, WS-114, WS-115, WS-116, WS-117, WS-125, WS-126, WS-127, WS-128, WS-129, WS-130, WS-132, WS-133, WS-137, WS-138, WS-139, WS-141, WS-144, WS-145, WS-147 and Hamdan were found to have high tree vigour. As the seedling trees were never supplied with nutrients the vigour exhibited was inherent and natural.

4.3.2 Tree growth habit

Seedling raised natural population of walnut trees of Kashmir valley exhibited great variability for this traits (Table-2). Fifty four genotypes (35.52%) had erect growth habit and another 54 genotypes (35.52%) had semi-erect growth habit, while remaining 44 genotypes (28.96%) had spreading growth habit. The genotypes WS-11, WS-12, WS-23, WS-24, WS-25, WS-25, WS-27, WS-29, WS-32, WS-33, WS-35, WS-38, WS-40, WS-43, WS-44, WS-45, WS-46, WS-52, WS-53, WS-54, WS-,62 WS-67, WS-69, WS-72, WS-76, WS-89, WS-90, WS-91, WS-93, WS-94, WS-97, WS-101, WS-104, WS-107, WS-111, WS-115, WS-122, WS-124, WS-127,

Table-2: Diversity for growth habit in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession number	Total	Per cent of the population
Erect	1	WS-11, WS-12, WS-23, WS-24, WS-25, WS-25, WS-27, WS-29, WS-32, WS-33, WS-35, WS-38, WS-40, WS-43, WS-44, WS-45, WS-46, WS-52, WS-53, WS-54, WS-,62 WS-67, WS-69, WS-72, WS-76, WS-89, WS-90, WS-91, WS-93, WS-94, WS-97, WS-101, WS-104, WS-107, WS-111, WS-115, WS-122, WS-124, WS-127, WS-129, WS-130, WS-131, WS-132, WS-134, WS-135, WS-137, WS-140, WS-142, WS-143, WS-145, WS-146, WS-148, WS-149 and WS-150	54	35.52
Semi-erect	2	WS-01, WS-02, WS-03, WS-06, WS-07, WS-08, WS-09, WS-10, WS-16, WS-17, WS-21, WS-28, WS-31, WS-36, WS-42, WS-49, WS-50, WS-55, WS-57, WS-59, WS-60, WS-64, WS-68, WS-71, WS-73, WS-75, WS-77, WS-78, WS-81, WS-83, WS-84, WS-85, WS-86, WS-88, WS-89, WS-92, WS-95, WS-96, WS-98, WS-99, WS-100, WS-102, WS-103, WS-106, WS-114, WS-120, WS-121, WS-123, WS-125, WS-126, WS-133, WS-147 and Sulaiman	54	35.52
Spreading	3	WS-04, WS-05, WS-13, WS-14, WS-15, WS-18, WS-19, WS-20, WS-22, WS-30, WS-34, WS-37, WS-39, WS-41, WS-47, WS-48, WS-51, WS-56, WS-58, WS-61, WS-63, WS-,65 WS-66, WS-70, WS-74, WS-80, WS-82, WS-87, WS-105, WS-108, WS-109, WS-110, WS-112, WS-113, WS-116, WS-117, WS-118, WS-120, WS-136, WS-138, WS-139, WS-141, WS-144 and Hamdan.	44	28.96

*As per the IBPGR Descriptor for Walnut

WS-129, WS-130, WS-131, WS-132, WS-134, WS-135, WS-137, WS-140, WS-142, WS-143, WS-145, WS-146, WS-148, WS-149 and WS-150 had erect growth habit, genotypes WS-01, WS-02, WS-03, WS-06, WS-07, WS-08, WS-09, WS-10, WS-16, WS-17, WS-21, WS-28, WS-31, WS-36, WS-42, WS-49, WS-50, WS-55, WS-57, WS-59, WS-60, WS-64, WS-68, WS-71, WS-73, WS-75, WS-77, WS-78, WS-81, WS-83, WS-84, WS-85, WS-86, WS-88, WS-89, WS-92, WS-95, WS-96, WS-98, WS-99, WS-100, WS-102, WS-103, WS-106, WS-114, WS-120, WS-121, WS-123, WS-125, WS-126, WS-133, WS-147 and Sulaiman had semi-erect growth habit; while WS-04, WS-05, WS-13, WS-14, WS-15, WS-18, WS-19, WS-20, WS-22, WS-30, WS-34, WS-37, WS-39, WS-41, WS-47, WS-48, WS-51, WS-56, WS-58, WS-61, WS-63, WS-65, WS-66, WS-70, WS-74, WS-80, WS-82, WS-87, WS-105, WS-108, WS-109, WS-110, WS-112, WS-113, WS-116, WS-117, WS-118, WS-120, WS-136, WS-138, WS-139, WS-141, WS-144 and Hamdan had spreading growth habit. Since no training or pruning had ever been practiced in this natural population therefore, we can safely consider it as their natural habit.

4.3.3 Branching habit

In this study it was found (Table 3) that 30 genotypes (19.73%) possessed sparse branching habit, 57 genotypes (37.50%) exhibited

intermediate branching habit and 65 genotypes (42.77%) had dense branching habit. Branching habit of the genotypes WS-24, WS-25, WS-27, WS-29, WS-31, WS-33, WS-35, WS-40, WS-42, WS-49, WS-50, WS-52, WS-54, WS-59, WS-60, WS-67, WS-69, WS-71, WS-77, WS-83, WS-84, WS-90, WS-91, WS-100, WS-108, WS-112, WS-118, WS-119, WS-120 and WS-142 was sparse. The genotypes, WS-08, WS-09, WS-11, WS-12, WS-15, WS-20, WS-21, WS- WS-23, WS-26, WS-28, WS-32, WS-34, WS-38, WS-44, WS-45, WS-48, WS-53, WS-55, WS-57, WS-64, WS-65, WS-68, WS-72, WS-73, WS-75, WS-78, WS-79, WS-81, WS-89, WS-92, WS-94, WS-97, WS-99, WS-103, WS-104, WS-107, WS-110, WS-111, WS-115, WS-116, WS-117, WS-122, WS-123, WS-124, WS-125, WS-127, WS-129, WS-130, WS-131, WS-136, WS-140, WS-141, WS-145, WS-148, WS-149, WS-150 and Sulaiman were intermediate in branching habit, while the genotypes WS-01, WS-02, WS-03, WS-04, WS-05, WS-06, WS-07, WS-10, WS-13, WS-14, WS-16, WS-17, WS-18, WS-19, WS-22, WS-30, WS-36, WS-37, WS-39, WS-41, WS-43, WS-46, WS-47, WS-51, WS-56, WS-58, WS-61, WS-62, WS-63, WS-66, WS-70, WS-74, WS-76, WS-80, WS-82, WS-85, WS-86, WS-87, WS-88, WS-93, WS-95, WS-96, WS-98, WS-101, WS-102, WS-105, WS-106, WS-109, WS-113, WS-114, WS-121, WS-126, WS-128, WS-132, WS-133, WS-134, WS-135, WS-137, WS-138, WS-139, WS-143, WS-144, WS-146, WS-147 and Hamdan were found to have dense branching habit.

Table-3: Diversity for branching habit in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession number	Total	Per cent of the population
Sparse	3	WS-24, WS-25, WS-27, WS-29, WS-31, WS-33, WS-35, WS-40, WS-42, WS-49, WS-50, WS-52, WS-54, WS-59, WS-60, WS-67, WS-69, WS-71, WS-77, WS-83, WS-84, WS-90, WS-91, WS-100, WS-108, WS-112, WS-118, WS-119, WS-120 and WS-142	30	19.73
Intermediate	5	WS-08, WS-09, WS-11, WS-12, WS-15, WS-20, WS-21, WS- WS-23, WS-26, WS-28, WS-32, WS-34, WS-38, WS-44, WS-45, WS-48, WS-53, WS-55, WS-57, WS-64, WS-65, WS-68, WS-72, WS-73, WS-75, WS-78, WS-79, WS-81, WS-89, WS-92, WS-94, WS-97, WS-99, WS-103, WS-104, WS-107, WS-110, WS-111, WS-115, WS-116, WS-117, WS-122, WS-123, WS-124, WS-125, WS-127, WS-129, WS-130, WS-131, WS-136, WS-140, WS-141, WS-145, WS-148, WS-149, WS-150 and Sulaiman	57	37.50
Dense	7	WS-01, WS-02, WS-03, WS-04, WS-05, WS-06, WS-07, WS-10, WS-13, WS-14, WS-16, WS-17, WS-18, WS-19, WS-22, WS-30, WS-36, WS-37, WS-39, WS-41, WS-43, WS-46, WS-47, WS-51, WS-56, WS-58, WS-61, WS-62, WS-63, WS-66, WS-70, WS-74, WS-76, WS-80, WS-82, WS-85, WS-86, WS-87, WS-88, WS-93, WS-95, WS-96, WS-98, WS-101, WS-102, WS-105, WS-106, WS-109, WS-113, WS-114, WS-121, WS-126, WS-128, WS-132, WS-133, WS-134, WS-135, WS-137, WS-138, WS-139, WS-143, WS-144, WS-146, WS-147 and Hamdan	65	42.77

*As per the IBPGR Descriptor for Walnut

4.3.4 Leaflet shape

Leaflet shape in the seedling raised natural population of walnut in the Kashmir valley (Table 4) varied from narrow elliptic to broad elliptic. During the study it was observed that 46 genotypes (30.26%) had narrow elliptic Leaflets, 39 genotypes (25.65%) had elliptic leaflet and 67 genotypes (44.09%) were with broad elliptic leaflet shape. The walnut genotypes WS-11, WS-12, WS-18, WS-25, WS-26, WS-27, WS-29, WS-30, WS-31, WS-32, WS-33, WS-34, WS-37, WS-38, WS-40, WS-43, WS-44, WS-46, WS-47, WS-53, WS-54, WS-57, WS-58, WS-61, WS-67, WS-69, WS-75, WS-80, WS-81, WS-82, WS-89, WS-91, WS-94, WS-104, WS-108, WS-119, WS-120, WS-130, WS-131, WS-132, WS-133, WS-134, WS-137, WS-137, WS-140 and WS-141 had narrow elliptic leaflet shape; the genotypes WS-21, WS-24, WS-36, WS-39, WS-42, WS-45, WS-48, WS-50, WS-52, WS-55, WS-59, WS-60, WS-62, WS-64, WS-65, WS-83, WS-84, WS-98, WS-101, WS-102, WS-106, WS-107, WS-109, WS-113, WS-114, WS-115, WS-118, WS-121, WS-122, WS-123, WS-124, WS-125, WS-126, WS-129, WS-142, WS-143, WS-144, WS-149 and WS-150 had elliptic leaflet shape, while the genotypes WS-01, WS-02, WS-03, WS-04, WS-05, WS-06, WS-07, WS-08, WS-09, WS-10, WS-13, WS-14, WS-15, WS-16, WS-17, WS-19, WS-20, WS-22, WS-23, WS-28, WS-35, WS-41,

Table-4: Diversity for leaflet shape in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession number	Total	Per cent of the population
Narrow elliptic	1	WS-11, WS-12, WS-18, WS-25, WS-26, WS-27, WS-29, WS-30, WS-31, WS-32, WS-33, WS-34, WS-37, WS-38, WS-40, WS-43, WS-44, WS-46, WS-47, WS-53, WS-54, WS-57, WS-58, WS-61, WS-67, WS-69, WS-75, WS-80, WS-81, WS-82, WS-89, WS-91, WS-94, WS-104, WS-108, WS-119, WS-120, WS-130, WS-131, WS-132, WS-133, WS-134, WS-137, WS-137, WS-140 and WS-141,	46	30.26
Elliptic	2	WS-21, WS-24, WS-36, WS-39, WS-42, WS-45, WS-48, WS-50, WS-52, WS-55, WS-59, WS-60, WS-62, WS-64, WS-65, WS-83, WS-84, WS-98, WS-101, WS-102, WS-106, WS-107, WS-109, WS-113, WS-114, WS-115, WS-118, WS-121, WS-122, WS-123, WS-124, WS-125, WS-126, WS-129, WS-142, WS-143, WS-144, WS-149 and WS-150	39	25.65
Broad elliptic	3	WS-01, WS-02, WS-03, WS-04, WS-05, WS-06, WS-07, WS-08, WS-09, WS-10, WS-13, WS-14, WS-15, WS-16, WS-17, WS-19, WS-20, WS-22, WS-23, WS-28, WS-35, WS-41, WS-49, WS-51, WS-56, WS-63, WS-66, WS-68, WS-70, WS-71, WS-72, WS-73, WS-74, WS-76, WS-77, WS-78, WS-79, WS-85, WS-86, WS-87, WS-88, WS-90, WS-92, WS-93, WS-95, WS-96, WS-97, WS-99, WS-100, WS-103, WS-105, WS-110, WS-111, WS-112, WS-116, WS-117, WS-127, WS-128, WS-135, WS-136, WS-138, WS-139, WS-145, WS-146, WS-147, WS-148, Hamdan and Sulaiman	67	44.09

*As per the IBPGR Descriptor for Walnut

WS-49, WS-51, WS-56, WS-63, WS-66, WS-68, WS-70, WS-71, WS-72, WS-73, WS-74, WS-76, WS-77, WS-78, WS-79, WS-85, WS-86, WS-87, WS-88, WS-90, WS-92, WS-93, WS-95, WS-96, WS-97, WS-99, WS-100, WS-103, WS-105, WS-110, WS-111, WS-112, WS-116, WS-117, WS-127, WS-128, WS-135, WS-136, WS-138, WS-139, WS-145, WS-146, WS-147, WS-148, Hamdan and Sulaiman were found to have broad elliptic leaflet shape.

4.3.5 Leaf margin

No variability in leaf margin was recorded in the entire population under study (Table 5). All the 152 genotype (100%) walnut selection in the present study had entire leaflet margin.

Table-5: Diversity for leaflet margin in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession number	Total	Per cent of the population
Entire	1	WS-001-WS-150 and Sulaiman and Hamdan	152	100.00
Serate	2	-	Nil	Nil
Dentate	3	-	Nil	Nil

*As per the IBPGR Descriptor for Walnut

4.3.6 Leaf colour

It is evident from the Table-6 that leaf colour score of the population studied ranged from 3 to 7 (light green to dark green). Of the population studied, 35 genotypes (23.03%) were having light green coloured leaves, 114 genotypes (75%) were having green coloured leaves, while only 3 genotypes (1.97%) had dark green leaf colour. The genotypes WS-19, WS-20, WS-22, WS-23, WS-24, WS-28, WS-32, WS-33, WS-36, WS-39, WS-44, WS-48, WS-50, WS-54, WS-56, WS-57, WS-58, WS-61, WS-62, WS-64, WS-66, WS-68, WS-70, WS-71, WS-72, WS-75, WS-80, WS-81, WS-82, WS-91, WS-97, WS-98, WS-131, WS-132 and WS-136 had light green colour; the genotypes WS-01, WS-02, WS-03, WS-04, WS-05, WS-06, WS-07, WS-08, WS-09, WS-10, WS-11, WS-12, WS-13, WS-14, WS-15, WS-16, WS-17, WS-18, WS-21, WS-25, WS-26, WS-27, WS-29, WS-30, WS-31, WS-34, WS-35, WS-37, WS-38, WS-40, WS-41, WS-42, WS-43, WS-45, WS-46, WS-47, WS-49, WS-51, WS-52, WS-53, WS-55, WS-59, WS-60, WS-63, WS-65, WS-67, WS-69, WS-73, WS-74, WS-76, WS-77, WS-78, WS-79, WS-85, WS-86, WS-87, WS-88, WS-89, WS-90, WS-92, WS-93, WS-94, WS-95, WS-96, WS-99, WS-100, WS-101, WS-102, WS-103, WS-104, WS-105, WS-106, WS-107, WS-109, WS-110, WS-111, WS-112, WS-113, WS-114, WS-115, WS-116, WS-117, WS-118,

Table-6: Diversity for leaf colour in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession number	Total	Per cent of the population
Light green	3	WS-19, WS-20, WS-22, WS-23, WS-24, WS-28, WS-32, WS-33, WS-36, WS-39, WS-44, WS-48, WS-50, WS-54, WS-56, WS-57, WS-58, WS-61, WS-62, WS-64, WS-66, WS-68, WS-70, WS-71, WS-72, WS-75, WS-80, WS-81, WS-82, WS-91, WS-97, WS-98, WS-131, WS-132 and WS-136	35	23.03
Green	5	WS-01, WS-02, WS-03, WS-04, WS-05, WS-06, WS-07, WS-08, WS-09, WS-10, WS-11, WS-12, WS-13, WS-14, WS-15, WS-16, WS-17, WS-18, WS-21, WS-25, WS-26, WS-27, WS-29, WS-30, WS-31, WS-34, WS-35, WS-37, WS-38, WS-40, WS-41, WS-42, WS-43, WS-45, WS-46, WS-47, WS-49, WS-51, WS-52, WS-53, WS-55, WS-59, WS-60, WS-63, WS-65, WS-67, WS-69, WS-73, WS-74, WS-76, WS-77, WS-78, WS-79, WS-85, WS-86, WS-87, WS-88, WS-89, WS-90, WS-92, WS-93, WS-94, WS-95, WS-96, WS-99, WS-100, WS-101, WS-102, WS-103, WS-104, WS-105, WS-106, WS-107, WS-109, WS-110, WS-111, WS-112, WS-113, WS-114, WS-115, WS-116, WS-117, WS-118, WS-119, WS-120, WS-121, WS-122, WS-123, WS-124, WS-125, WS-126, WS-127, WS-128, WS-129, WS-130, WS-133, WS-134, WS-135, WS-137, WS-138, WS-139, WS-140, WS-141, WS-142, WS-143, WS-144, WS-145, WS-146, WS-147, WS-148, WS-149, WS-150, Hamdan and Sulaiman	114	75.00
Dark green	7	WS-83, WS-84 and WS-108	03	1.97

*As per the IBPGR Descriptor for Walnut

WS-119, WS-120, WS-121, WS-122, WS-123, WS-124, WS-125, WS-126, WS-127, WS-128, WS-129, WS-130, WS-133, WS-134, WS-135, WS-137, WS-138, WS-139, WS-140, WS-141, WS-142, WS-143, WS-144, WS-145, WS-146, WS-147, WS-148, WS-149, WS-150, Hamdan and Sulaiman had green leaf colour; and only three genotypes WS-83, WS-84, and WS-108 had dark green leaf colour.

4.3.7 Shoot colour

Perusal of the Table-7 revealed that the shoot colour in the seedling raised natural population of walnut ranged from green to black. Thirteen genotypes (8.56%) were having green shoot colour while majority of the trees i.e. 115 genotypes (75.66%) had brown shoot colour and 24 genotypes (15.78%) had black coloured shoots. The shoot colour of WS-19, WS-20, WS-35, WS-44, WS-54, WS-62, WS-65, WS-91, WS-108, WS-119, WS-127, WS-131 and WS-136 was green; the genotypes WS-01, WS-02, WS-03, WS-04, WS-05, WS-06, WS-07, WS-08, WS-09, WS-10, WS-11, WS-12, WS-13, WS-14, WS-15, WS-16, WS-17, WS-18, WS-26, WS-27, WS-28, WS-29, WS-30, WS-31, WS-32, WS-33, WS-34, WS-36, WS-37, WS-38, WS-40, WS-41, WS-42, WS-43, WS-45, WS-46, WS-47, WS-48, WS-49, WS-51, WS-53, WS-55, WS-57, WS-58, WS-61, WS-63, WS-64, WS-67, WS-68, WS-69, WS-70, WS-71, WS-73, WS-74, WS-75,

Table-7: Diversity for shoot colour in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession number	Total	Per cent of the population
Green	3	WS-19, WS-20, WS-35, WS-44, WS-54, WS-62, WS-65, WS-91, WS-108, WS-119, WS-127, WS-131 and WS-136	13	8.56
Brown	5	WS-01, WS-02, WS-03, WS-04, WS-05, WS-06, WS-07, WS-08, WS-09, WS-10, WS-11, WS-12, WS-13, WS-14, WS-15, WS-16, WS-17, WS-18, WS-26, WS-27, WS-28, WS-29, WS-30, WS-31, WS-32, WS-33, WS-34, WS-36, WS-37, WS-38, WS-40, WS-41, WS-42, WS-43, WS-45, WS-46, WS-47, WS-48, WS-49, WS-51, WS-53, WS-55, WS-57, WS-58, WS-61, WS-63, WS-64, WS-67, WS-68, WS-69, WS-70, WS-71, WS-73, WS-74, WS-75, WS-76, WS-77, WS-78, WS-79, WS-80, WS-81, WS-82, WS-85, WS-86, WS-87, WS-88, WS-89, WS-90, WS-92, WS-93, WS-94, WS-95, WS-96, WS-101, WS-102, WS-105, WS-107, WS-109, WS-110, WS-111, WS-112, WS-113, WS-114, WS-115, WS-116, WS-117, WS-118, WS-121, WS-122, WS-123, WS-124, WS-125, WS-126, WS-128, WS-129, WS-130, WS-132, WS-133, WS-134, WS-135, WS-137, WS-138, WS-139, WS-140, WS-141, WS-142, WS-143, WS-144, WS-145, WS-146, WS-148, WS-149, WS-150, Hamdan and Sulaiman	115	75.66
Black	7	WS-21, WS-22, WS-23, WS-24, WS-25, WS-39, WS-50, WS-52, WS-56, WS-59, WS-60, WS-66, WS-72, WS-83, WS-84, WS-97, WS-98, WS-99, WS-100, WS-103, WS-104, WS-106, WS-120 and WS-147	24	15.78

*As per the IBPGR Descriptor for Walnut

WS-76, WS-77, WS-78, WS-79, WS-80, WS-81, WS-82, WS-85, WS-86, WS-87, WS-88, WS-89, WS-90, WS-92, WS-93, WS-94, WS-95, WS-96, WS-101, WS-102, WS-105, WS-107, WS-109, WS-110, WS-111, WS-112, WS-113, WS-114, WS-115, WS-116, WS-117, WS-118, WS-121, WS-122, WS-123, WS-124, WS-125, WS-126, WS-128, WS-129, WS-130, WS-132, WS-133, WS-134, WS-135, WS-137, WS-138, WS-139, WS-140, WS-141, WS-142, WS-143, WS-144, WS-145, WS-146, WS-148, WS-149, WS-150, Hamdan and Sulaiman had brown shoot colour, while the genotypes WS-21, WS-22, WS-23, WS-24, WS-25, WS-39, WS-50, WS-52, WS-56, WS-59, WS-60, WS-66, WS-72, WS-83, WS-84, WS-97, WS-98, WS-99, WS-100, WS-103, WS-104, WS-106, WS-120 and WS-147 had black shoot colour.

4.4 Nut characters

Among nut characteristics the nut shape, nut colour and nut texture were studied as these have commercial impact. The diversity observed is described as follows:

4.4.1 Nut shape

Perusal of the Table-8 revealed that nut shape varied from round to cordate. Majority of the genotypes (56) that represented 36.85 per cent population had round nut shape followed by 36 genotypes (23.69%) that had ovate nut shape; 8 genotypes (5.27%) had triangular nut shape; 10

genotypes (6.58%) had broad ovate nut shape; 7 genotypes (4.60%) had short trapezoid nut shape, 3 genotypes (1.97%) had long trapezoid nut shape, 16 genotypes (10.52%) had broad elliptic nut shape and the remaining 13 genotypes (8.55%) had elliptic nut shape.

Long trapezoid and cordate shaped nuts were the least and found only in 3 genotypes (1.97% each). The walnut selection WS-01, WS-02, WS-03, WS-09, WS-12, WS-20, WS-21, WS-23, WS-24, WS-25, WS-30, WS-37, WS-39, WS-40, WS-41, WS-43, WS-45, WS-47, WS-54, WS-55, WS-56, WS-61, WS-65, WS-66, WS-67, WS-68, WS-76, WS-79, WS-85, WS-86, WS-90, WS-91, WS-93, WS-96, WS-99, WS-100, WS-101, WS-106, WS-107, WS-112, WS-115, WS-121, WS-126, WS-129, WS-130, WS-131, WS-133, WS-135, WS-136, WS-138, WS-139, WS-140, WS-142, WS-145, WS-146 and WS-150 had round nut shape; the selections WS-07, WS-52, WS-69, WS-71, WS-88, WS-98, WS-103 and WS-110 had triangular nut shape; the selections WS-04, WS-05, WS-16, WS-17, WS-31, WS-87, WS-111, WS-128, WS-143 and WS-147 had broad ovate nut shape; while the selections WS-08, WS-11, WS-18, WS-22, WS-28, WS-29, WS-32, WS-33, WS-44, WS-48, WS-49, WS-51, WS-53, WS-57, WS-58, WS-62, WS-63, WS-64, WS-70, WS-74, WS-75, WS-78, WS-80, WS-81, WS-82, WS-89, WS-94, WS-105, WS-109, WS-114, WS-124, WS-127, WS-134, WS-137, WS-148 and Sulaiman had ovate nut shape.

Table-8: Diversity for nut shape in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession numbers	Total	Per cent of the population
Round	1	WS-01, WS-02, WS-03, WS-09, WS-12, WS-20, WS-21, WS-23, WS-24, WS-25, WS-30, WS-37, WS-39, WS-40, WS-41, WS-43, WS-45, WS-47, WS-54, WS-55, WS-56, WS-61, WS-65, WS-66, WS-67, WS-68, WS-76, WS-79, WS-85, WS-86, WS-90, WS-91, WS-93, WS-96, WS-99, WS-100, WS-101, WS-106, WS-107, WS-112, WS-115, WS-121, WS-126, WS-129, WS-130, WS-131, WS-133, WS-135, WS-136, WS-138, WS-139, WS-140, WS-142, WS-145, WS-146 and WS-150	56	36.85
Triangular	2	WS-07, WS-52, WS-69, WS-71, WS-88, WS-98, WS-103 and WS-110	08	5.27
Broad ovate	3	WS-04, WS-05, WS-16, WS-17, WS-31, WS-87, WS-111, WS-128, WS-143 and WS-147	10	6.58
Ovate	4	WS-08, WS-11, WS-18, WS-22, WS-28, WS-29, WS-32, WS-33, WS-44, WS-48, WS-49, WS-51, WS-53, WS-57, WS-58, WS-62, WS-63, WS-64, WS-70, WS-74, WS-75, WS-78, WS-80, WS-81, WS-82, WS-89, WS-94, WS-105, WS-109, WS-114, WS-124, WS-127, WS-134, WS-137, WS-148 and Sulaiman	36	23.69
Short trapezoid	5	WS-36, WS-77, WS-108, WS-119, WS-120, WS-125 and WS-149	07	4.60
Long trapezoid	6	WS-06, WS-46 and WS-141	03	1.97
Broad elliptic	7	WS-10, WS-13, WS-14, WS-15, WS-26, WS-34, WS-38, WS-42, WS-73, WS-92, WS-95, WS-117, WS-118, WS-122, WS-123 and Hamdan	16	10.52
Elliptic	8	WS-19, WS-50, WS-59, WS-60, WS-72, WS-83, WS-84, WS-97, WS-102, WS-104, WS-113, WS-116 and WS-144	13	8.55
Cordate	9	WS-27, WS-35 and WS-132	03	1.97

*As per the IBPGR Descriptor for Walnut



Round



Triangular



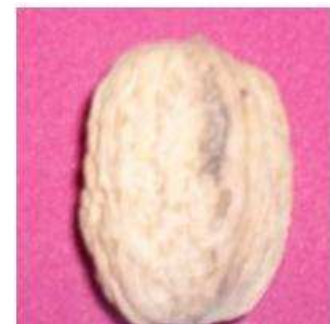
Broad ovate



Ovate



Short trapezoid



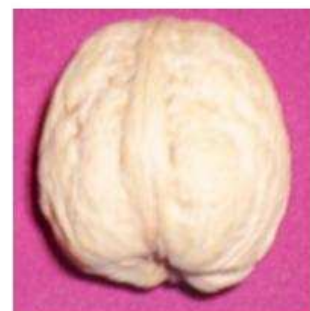
Long trapezoid



Broad elliptic



Elliptic



Cordate

Plate-1 : Phenotypic variability in the shape of nuts recorded in the seedling raised walnut (*Juglans regia* L.) population in the Kashmir valley

The genotypes WS-36, WS-77, WS-108, WS-119, WS-120, WS-125 and WS-149 had short trapezoid nut shape; while WS-06, WS-46 and WS-141 had long trapezoid shape. Similarly, the genotypes WS-10, WS-13, WS-14, WS-15, WS-26, WS-34, WS-38, WS-42, WS-73, WS-92, WS-95, WS-117, WS-118, WS-122 and WS-123 had broad elliptic nuts, while WS-19, WS-50, WS-59, WS-60, WS-72, WS-83, WS-84, WS-97, WS-102, WS-104, WS-113, WS-116, WS-144 and Hamdan had elliptic nuts; and three genotypes viz, WS-27, WS-35 and WS-132 had ordate nut shape.

4.4.2 Shell texture

Perusal of the Table-9 revealed that shell texture of walnut genotypes under the present study varied from very smooth to rough shell texture. Seventeen genotypes (11.18%) were found to possess rough shell texture. Seventy five genotypes (49.34%) had smooth shell texture, 52 genotypes (34.21%) had medium shell texture and 8 genotypes (5.27%) were very smooth shell textured. Thus, most of the genotypes possessed acceptable shell texture. Walnuts selections WS-68, WS-69, WS-76, WS-79, WS-93, WS-107, WS-115 and Hamdan had very smooth nut shell texture. Walnut selection WS-01, WS-03, WS-06, WS-09, WS-11, WS-12, WS-13, WS-14, WS-15, WS-20, WS-21, WS-23, WS-25, WS-26, WS-34, WS-35, WS-36, WS-37, WS-38, WS-40, WS-41, WS-42, WS-43, WS-47, WS-48, WS-50,

WS-51, WS-52, WS-53, WS-54, WS-55, WS-59, WS-60, WS-61, WS-62, WS-63, WS-64, WS-71, WS-72, WS-73, WS-74, WS-84, WS-85, WS-89, WS-90, WS-91, WS-92, WS-94, WS-97, WS-99, WS-102, WS-110, WS-111, WS-112, WS-116, WS-117, WS-118, WS-120, WS-123, WS-129, WS-130, WS-131, WS-133, WS-135, WS-136, WS-138, WS-140, WS-141, WS-142, WS-143, WS-144, WS-145, WS-146, WS-150 and Sulaiman had smooth shell texture. Walnut selections WS-02, WS-05, WS-08, WS-16, WS-17, WS-18, WS-19, WS-22, WS-24, WS-27, WS-28, WS-29, WS-30, WS-32, WS-33, WS-39, WS-45, WS-46, WS-49, WS-56, WS-57, WS-58, WS-65, WS-67, WS-70, WS-75, WS-77, WS-78, WS-80, WS-81, WS-82, WS-83, WS-86, WS-96, WS-100, WS-101, WS-106, WS-108, WS-109, WS-113, WS-114, WS-119, WS-121, WS-122, WS-125, WS-126, WS-132, WS-134, WS-137, WS-139, WS-147 and WS-148 were found to have medium nut shell texture, while the selections WS-04, WS-07, WS-10, WS-31, WS-44, WS-66, WS-87, WS-88, WS-95, WS-98, WS-103, WS-104, WS-105, WS-124, WS-127, WS-128 and WS-149 were found to have rough nut shell texture.

Table-9: Diversity for shell texture in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession number	Total	Per cent of the population
Very smooth	1	WS-68, WS-69, WS-76, WS-79, WS-93, WS-107, WS-115 and Hamdan	08	5.27
Smooth	3	WS-01, WS-03, WS-06, WS-09, WS-11, WS-12, WS-13, WS-14, WS-15, WS-20, WS-21, WS-23, WS-25, WS-26, WS-34, WS-35, WS-36, WS-37, WS-38, WS-40, WS-41, WS-42, WS-43, WS-47, WS-48, WS-50, WS-51, WS-52, WS-53, WS-54, WS-55, WS-59, WS-60, WS-61, WS-62, WS-63, WS-64, WS-71, WS-72, WS-73, WS-74, WS-84, WS-85, WS-89, WS-90, WS-91, WS-92, WS-94, WS-97, WS-99, WS-102, WS-110, WS-111, WS-112, WS-116, WS-117, WS-118, WS-120, WS-123, WS-129, WS-130, WS-131, WS-133, WS-135, WS-136, WS-138, WS-140, WS-141, WS-142, WS-143, WS-144, WS-145, WS-146, WS-150 and Sulaiman	75	49.34
Medium	5	WS-02, WS-05, WS-08, WS-16, WS-17, WS-18, WS-19, WS-22, WS-24, WS-27, WS-28, WS-29, WS-30, WS-32, WS-33, WS-39, WS-45, WS-46, WS-49, WS-56, WS-57, WS-58, WS-65, WS-67, WS-70, WS-75, WS-77, WS-78, WS-80, WS-81, WS-82, WS-83, WS-86, WS-96, WS-100, WS-101, WS-106, WS-108, WS-109, WS-113, WS-114, WS-119, WS-121, WS-122, WS-125, WS-126, WS-132, WS-134, WS-137, WS-139, WS-147 and WS-148	52	34.21
Rough	7	WS-04, WS-07, WS-10, WS-31, WS-44, WS-66, WS-87, WS-88, WS-95, WS-98, WS-103, WS-104, WS-105, WS-124, WS-127, WS-128 and WS-149	17	11.18

*As per the IBPGR Descriptor for Walnut

4.4.3 Shell colour

The colour of walnuts in the present study (Table-10) varied from very light (14 genotypes), light (93 genotypes), medium (42 genotypes) to dark (3 genotypes) shell colour corresponding to 9.22, 61.18, 27.63 and 1.97 per cent, respectively. Walnut selections WS-01, WS-23, WS-76, WS-77, WS-85, WS-93, WS-107, WS-112, WS-116, WS-117, WS-118, WS-129, WS-145 and WS-146 possessed very light shell colour; the selections WS-02, WS-03, WS-06, WS-07, WS-09, WS-11, WS-12, WS-13, WS-14, WS-15, WS-16, WS-17, WS-18, WS-19, WS-20, WS-21, WS-25, WS-26, WS-27, WS-28, WS-29, WS-31, WS-32, WS-33, WS-34, WS-35, WS-36, WS-38, WS-41, WS-42, WS-43, WS-44, WS-46, WS-48, WS-51, WS-52, WS-53, WS-55, WS-57, WS-58, WS-62, WS-63, WS-67, WS-69, WS-72, WS-73, WS-74, WS-75, WS-79, WS-80, WS-81, WS-82, WS-86, WS-88, WS-89, WS-90, WS-92, WS-94, WS-96, WS-97, WS-98, WS-99, WS-100, WS-102, WS-103, WS-105, WS-106, WS-108, WS-110, WS-111, WS-113, WS-114, WS-115, WS-121, WS-123, WS-125, WS-127, WS-130, WS-131, WS-132, WS-133, WS-134, WS-135, WS-136, WS-137, WS-139, WS-139, WS-140, WS-141, WS-143, WS-148, Hamdan and Sulaiman had light nut shell colour; the selections WS-04, WS-05, WS-08, WS-10, WS-22, WS-24, WS-30, WS-37, WS-39, WS-40, WS-45, WS-47, WS-49, WS-50,

Table-10: Diversity for shell colour in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession number	Total	Per cent of the population
Very light	1	WS-01, WS-23, WS-76, WS-77, WS-85, WS-93, WS-107, WS-112, WS-116, WS-117, WS-118, WS-129, WS-145 and WS-146	14	9.22
Light	3	WS-02, WS-03, WS-06, WS-07, WS-09, WS-11, WS-12, WS-13, WS-14, WS-15, WS-16, WS-17, WS-18, WS-19, WS-20, WS-21, WS-25, WS-26, WS-27, WS-28, WS-29, WS-31, WS-32, WS-33, WS-34, WS-35, WS-36, WS-38, WS-41, WS-42, WS-43, WS-44, WS-46, WS-48, WS-51, WS-52, WS-53, WS-55, WS-57, WS-58, WS-62, WS-63, WS-67, WS-69, WS-72, WS-73, WS-74, WS-75, WS-79, WS-80, WS-81, WS-82, WS-86, WS-88, WS-89, WS-90, WS-92, WS-94, WS-96, WS-97, WS-98, WS-99, WS-100, WS-102, WS-103, WS-105, WS-106, WS-108, WS-110, WS-111, WS-113, WS-114, WS-115, WS-121, WS-123, WS-125, WS-127, WS-130, WS-131, WS-132, WS-133, WS-134, WS-135, WS-136, WS-137, WS-139, WS-139, WS-140, WS-141, WS-143, WS-148, Hamdan and Sulaiman	93	61.18
Medium	5	WS-04, WS-05, WS-08, WS-10, WS-22, WS-24, WS-30, WS-37, WS-39, WS-40, WS-45, WS-47, WS-49, WS-50, WS-54, WS-56, WS-59, WS-60, WS-61, WS-64, WS-66, WS-68, WS-70, WS-71, WS-78, WS-83, WS-84, WS-87, WS-91, WS-95, WS-101, WS-104, WS-109, WS-120, WS-122, WS-124, WS-126, WS-128, WS-142, WS-144, WS-147 and WS-149	42	27.63
Dark	7	WS-65, WS-119 and WS-150	03	1.97

*As per the IBPGR Descriptor for Walnut

WS-54, WS-56, WS-59, WS-60, WS-61, WS-64, WS-66, WS-68, WS-70, WS-71, WS-78, WS-83, WS-84, WS-87, WS-91, WS-95, WS-101, WS-104, WS-109, WS-120, WS-122, WS-124, WS-126, WS-128, WS-142, WS-144, WS-147 and WS-149 had medium nut shell colour and the selections WS-65, WS-119 and WS-150 had dark nut shell colour.

4.4.4 Shell seal

Shell seal is important characteristic for protection of the Kernels during the development and maturity. Shell seal of the walnut genotypes (Table 11) varied from weak to strong. Forty five genotypes (29.60%) were found to have weak shell seal; 60 genotypes (39.48%) had intermediate shell seal and 47 genotypes (30.92%) exhibited strong shell seal. The walnut selections WS-01, WS-09, WS-10, WS-16, WS-21, WS-22, WS-23, WS-25, WS-35, WS-37, WS-41, WS-45, WS-47, WS-51, WS-52, WS-56, WS-63, WS-65, WS-72, WS-79, WS-85, WS-87, WS-90, WS-95, WS-97, WS-102, WS-105, WS-107, WS-110, WS-112, WS-113, WS-114, WS-115, WS-116, WS-123, WS-124, WS-125, WS-129, WS-131, WS-135, WS-136, WS-144, WS-145, WS-150 and Sulaiman had weak shell seal; the selections WS-02, WS-03, WS-04, WS-05, WS-06, WS-08, WS-12, WS-17, WS-24, WS-26, WS-28, WS-29, WS-33, WS-39, WS-40, WS-42, WS-49, WS-50, WS-54, WS-57, WS-58, WS-60, WS-61, WS-64,

Table-11: Diversity for shell seal in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession number	Total	Per cent of the population
Weak	3	WS-01, WS-09, WS-10, WS-16, WS-21, WS-22, WS-23, WS-25, WS-35, WS-37, WS-41, WS-45, WS-47, WS-51, WS-52, WS-56, WS-63, WS-65, WS-72, WS-79, WS-85, WS-87, WS-90, WS-95, WS-97, WS-102, WS-105, WS-107, WS-110, WS-112, WS-113, WS-114, WS-115, WS-116, WS-123, WS-124, WS-125, WS-129, WS-131, WS-135, WS-136, WS-144, WS-145, WS-150 and Sulaiman	45	29.60
Intermediate	5	WS-02, WS-03, WS-04, WS-05, WS-06, WS-08, WS-12, WS-17, WS-24, WS-26, WS-28, WS-29, WS-33, WS-39, WS-40, WS-42, WS-49, WS-50, WS-54, WS-57, WS-58, WS-60, WS-61, WS-64, WS-66, WS-67, WS-69, WS-70, WS-71, WS-74, WS-75, WS-76, WS-77, WS-78, WS-80, WS-81, WS-82, WS-84, WS-86, WS-91, WS-96, WS-98, WS-101, WS-103, WS-104, WS-111, WS-117, WS-118, WS-120, WS-121, WS-126, WS-127, WS-128, WS-132, WS-133, WS-134, WS-137, WS-138, WS-146 and Hamdan	60	39.48
Strong	7	WS-07, WS-11, WS-13, WS-14, WS-15, WS-18, WS-19, WS-20, WS-27, WS-30, WS-31, WS-32, WS-34, WS-36, WS-38, WS-43, WS-44, WS-46, WS-48, WS-53, WS-55, WS-59, WS-62, WS-68, WS-73, WS-83, WS-88, WS-89, WS-92, WS-93, WS-94, WS-99, WS-100, WS-106, WS-108, WS-109, WS-119, WS-122, WS-130, WS-139, WS-140, WS-141, WS-142, WS-143, WS-147, WS-148 and WS-149	47	30.92

*As per the IBPGR Descriptor for Walnut

WS-66, WS-67, WS-69, WS-70, WS-71, WS-74, WS-75, WS-76, WS-77, WS-78, WS-80, WS-81, WS-82, WS-84, WS-86, WS-91, WS-96, WS-98, WS-101, WS-103, WS-104, WS-111, WS-117, WS-118, WS-120, WS-121, WS-126, WS-127, WS-128, WS-132, WS-133, WS-134, WS-137, WS-138, WS-146 and Hamdan had intermediate shell seal and the remaining selections WS-07, WS-11, WS-13, WS-14, WS-15, WS-18, WS-19, WS-20, WS-27, WS-30, WS-31, WS-32, WS-34, WS-36, WS-38, WS-43, WS-44, WS-46, WS-48, WS-53, WS-55, WS-59, WS-62, WS-68, WS-73, WS-83, WS-88, WS-89, WS-92, WS-93, WS-94, WS-99, WS-100, WS-106, WS-108, WS-109, WS-119, WS-122, WS-130, WS-139, WS-140, WS-141, WS-142, WS-143, WS-147, WS-148 and WS-149 had strong shell seal.

4.4.5 Shell strength

Perusal of the Table-12 revealed that shell strength of walnut genotypes under study varied from papery to strong. Ten genotypes (6.58%) were found having papery shell, 41 genotypes (26.98%) had weak shell strength, 76 genotypes (50.00%) possessed intermediate shell strength and 25 genotypes (16.44%) were found to have strong shell strength. The walnut selections WS-90, WS-102, WS-110, WS-112, WS-119, WS-123, WS-129, WS-135, WS-136 and WS-145 had papery shell strength; the selections WS-03, WS-09, WS-16, WS-17, WS-21, WS-22, WS-23, WS-

25, WS-35, WS-41, WS-43, WS-45, WS-47, WS-50, WS-52, WS-56, WS-60, WS-61, WS-62, WS-65, WS-66, WS-67, WS-68, WS-71, WS-79, WS-84, WS-87, WS-91, WS-101, WS-105, WS-111, WS-113, WS-115, WS-116, WS-125, WS-127, WS-130, WS-131, WS-142, WS-144 and WS-146 had weak shell strength; the selections WS-01, WS-02, WS-04, WS-05, WS-06, WS-08, WS-10, WS-12, WS-18, WS-19, WS-20, WS-24, WS-28, WS-29, WS-33, WS-34, WS-36, WS-37, WS-38, WS-39, WS-40, WS-42, WS-49, WS-51, WS-54, WS-55, WS-57, WS-58, WS-59, WS-63, WS-69, WS-70, WS-72, WS-74, WS-75, WS-76, WS-77, WS-78, WS-80, WS-81, WS-82, WS-83, WS-85, WS-86, WS-93, WS-95, WS-96, WS-97, WS-98, WS-99, WS-100, WS-103, WS-104, WS-107, WS-109, WS-117, WS-118, WS-120, WS-121, WS-122, WS-124, WS-126, WS-128, WS-132, WS-133, WS-134, WS-137, WS-138, WS-139, WS-141, WS-143, WS-147, WS-148, WS-150, Hamdan and Sulaiman had intermediate shell strength and the selections WS-07, WS-11, WS-13, WS-14, WS-15, WS-26, WS-27, WS-30, WS-31, WS-32, WS-44, WS-46, WS-48, WS-53, WS-64, WS-73, WS-88, WS-89, WS-92, WS-94, WS-106, WS-108, WS-114, WS-140 and WS-149 had strong shell strength.

Table-12: Diversity for shell strength in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession number	Total	Per cent of the population
Papery	1	WS-90, WS-102, WS-110, WS-112, WS-119, WS-123, WS-129, WS-135, WS-136 and WS-145	10	6.58
Weak	3	WS-03, WS-09, WS-16, WS-17, WS-21, WS-22, WS-23, WS-25, WS-35, WS-41, WS-43, WS-45, WS-47, WS-50, WS-52, WS-56, WS-60, WS-61, WS-62, WS-65, WS-66, WS-67, WS-68, WS-71, WS-79, WS-84, WS-87, WS-91, WS-101, WS-105, WS-111, WS-113, WS-115, WS-116, WS-125, WS-127, WS-130, WS-131, WS-142, WS-144 and WS-146	41	26.98
Intermediate	5	WS-01, WS-02, WS-04, WS-05, WS-06, WS-08, WS-10, WS-12, WS-18, WS-19, WS-20, WS-24, WS-28, WS-29, WS-33, WS-34, WS-36, WS-37, WS-38, WS-39, WS-40, WS-42, WS-49, WS-51, WS-54, WS-55, WS-57, WS-58, WS-59, WS-63, WS-69, WS-70, WS-72, WS-74, WS-75, WS-76, WS-77, WS-78, WS-80, WS-81, WS-82, WS-83, WS-85, WS-86, WS-93, WS-95, WS-96, WS-97, WS-98, WS-99, WS-100, WS-103, WS-104, WS-107, WS-109, WS-117, WS-118, WS-120, WS-121, WS-122, WS-124, WS-126, WS-128, WS-132, WS-133, WS-134, WS-137, WS-138, WS-139, WS-141, WS-143, WS-147, WS-148, WS-150, Hamdan and Sulaiman	76	50.00
Strong	7	WS-07, WS-11, WS-13, WS-14, WS-15, WS-26, WS-27, WS-30, WS-31, WS-32, WS-44, WS-46, WS-48, WS-53, WS-64, WS-73, WS-88, WS-89, WS-92, WS-94, WS-106, WS-108, WS-114, WS-140 and WS-149	25	16.44

*As per the IBPGR Descriptor for Walnut

4.4.6 Shell integrity

Perusal of the Table-13 revealed that shell integrity of walnut genotypes under study varied from incomplete to complete. Six genotypes (3.95%) had incomplete shell integrity, 95 genotypes (62.50%) were found to have intermediate shell integrity and the remaining 51 genotypes (33.55%) had complete shell integrity. The selections WS-26, WS-28, WS-40, WS-99, WS-145 and WS-150 had incomplete shell integrity; the selections WS-03, WS-04, WS-05, WS-07, WS-08, WS-09, WS-13, WS-14, WS-15, WS-17, WS-19, WS-20, WS-22, WS-23, WS-24, WS-27, WS-29, WS-30, WS-32, WS-33, WS-34, WS-35, WS-36, WS-37, WS-38, WS-39, WS-41, WS-42, WS-43, WS-44, WS-45, WS-47, WS-48, WS-50, WS-52, WS-54, WS-55, WS-59, WS-60, WS-61, WS-62, WS-64, WS-65, WS-66, WS-67, WS-70, WS-71, WS-72, WS-78, WS-83, WS-84, WS-87, WS-88, WS-90, WS-91, WS-97, WS-98, WS-100, WS-101, WS-104, WS-105, WS-106, WS-108, WS-109, WS-111, WS-117, WS-118, WS-119, WS-121, WS-122, WS-123, WS-124, WS-125, WS-126, WS-127, WS-129, WS-130, WS-132, WS-133, WS-135, WS-136, WS-137, WS-138, WS-139, WS-140, WS-141, WS-142, WS-143, WS-144, WS-146, WS-147, WS-148, WS-149, Hamdan and Sulaiman had intermediate shell integrity and the remaining selections viz, WS-01, WS-02, WS-06, WS-10, WS-11, WS-12, WS-16,

Table-13: Diversity for shell integrity in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession number	Total	Per cent of the population
Incomplete shell	1	WS-26, WS-28, WS-40, WS-99, WS-145 and WS-150	6	3.95
Intermediate shell	2	WS-03, WS-04, WS-05, WS-07, WS-08, WS-09, WS-13, WS-14, WS-15, WS-17, WS-19, WS-20, WS-22, WS-23, WS-24, WS-27, WS-29, WS-30, WS-32, WS-33, WS-34, WS-35, WS-36, WS-37, WS-38, WS-39, WS-41, WS-42, WS-43, WS-44, WS-45, WS-47, WS-48, WS-50, WS-52, WS-54, WS-55, WS-59, WS-60, WS-61, WS-62, WS-64, WS-65, WS-66, WS-67, WS-70, WS-71, WS-72, WS-78, WS-83, WS-84, WS-87, WS-88, WS-90, WS-91, WS-97, WS-98, WS-100, WS-101, WS-104, WS-105, WS-106, WS-108, WS-109, WS-111, WS-117, WS-118, WS-119, WS-121, WS-122, WS-123, WS-124, WS-125, WS-126, WS-127, WS-129, WS-130, WS-132, WS-133, WS-135, WS-136, WS-137, WS-138, WS-139, WS-140, WS-141, WS-142, WS-143, WS-144, WS-146, WS-147, WS-148, WS-149, Hamdan and Sulaiman	95	62.50
Complete shell	3	WS-01, WS-02, WS-06, WS-10, WS-11, WS-12, WS-16, WS-18, WS-21, WS-25, WS-31, WS-46, WS-49, WS-51, WS-53, WS-56, WS-57, WS-58, WS-63, WS-68, WS-69, WS-73, WS-74, WS-75, WS-76, WS-77, WS-79, WS-80, WS-81, WS-82, WS-85, WS-86, WS-89, WS-92, WS-93, WS-94, WS-95, WS-96, WS-102, WS-103, WS-107, WS-110, WS-112, WS-113, WS-114, WS-115, WS-116, WS-120, WS-128, WS-131 and WS-134	51	33.55

*As per the IBPGR Descriptor for Walnut

WS-18, WS-21, WS-25, WS-31, WS-46, WS-49, WS-51, WS-53, WS-56, WS-57, WS-58, WS-63, WS-68, WS-69, WS-73, WS-74, WS-75, WS-76, WS-77, WS- 79, WS-80, WS-81, WS-82, WS-85, WS-86, WS-89, WS-92, WS-93, WS-94, WS-95, WS-96, WS-102, WS-103, WS-107, WS-110, WS-112, WS-113, WS-114, WS-115, WS-116, WS-120, WS-128, WS-131 and WS-134 possessed complete shell integrity.

4.5 Kernel characteristics

4.5.1 Kernel shrivel

Perusal of the Table- 14 revealed that 48 genotypes (31.57%) had tip Kernel shrivel, 87 genotypes (57.33%) demonstrated < 50.0 per cent kernel shrivel and 17 genotypes (11.18%) exhibited ≥ 50.0 per cent kernel shrivel. The walnut selections WS-01, WS-02, WS-04, WS-06, WS-09, WS-16, WS-17, WS-18, WS-21, WS-24, WS-25, WS-26, WS-28, WS-33, WS-34, WS-43, WS-46, WS-49, WS-51, WS-54, WS-55, WS-57, WS-63, WS-66, WS-71, WS-73, WS-75, WS-77, WS-81, WS-85, WS-86, WS-87, WS-90, WS-91, WS-92, WS-96, WS-104, WS-105, WS-107, WS-110, WS-112, WS-129, WS-130, WS-133, WS-139, WS-145, WS-150 and Hamdan exhibited tip kernel shrivel. The selections WS-03, WS-05, WS-07, WS-08, WS-10, WS-11, WS-13, WS-14, WS-15, WS-19, WS-20, WS-22, WS-23, WS-27, WS-30, WS-32, WS-35, WS-36, WS-37, WS-38, WS-41, WS-42,

Table-14: Diversity for kernel shrivel in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession number	Total	Per cent of the population
Kernels exhibiting tip shrivel	1	WS-01, WS-02, WS-04, WS-06, WS-09, WS-16, WS-17, WS-18, WS-21, WS-24, WS-25, WS-26, WS-28, WS-33, WS-34, WS-43, WS-46, WS-49, WS-51, WS-54, WS-55, WS-57, WS-63, WS-66, WS-71, WS-73, WS-75, WS-77, WS-81, WS-85, WS-86, WS-87, WS-90, WS-91, WS-92, WS-96, WS-104, WS-105, WS-107, WS-110, WS-112, WS-129, WS-130, WS-133, WS-139, WS-145, WS-150 and Hamdan	48	31.57
Kernels exhibiting <50% shrivel	2	WS-03, WS-05, WS-07, WS-08, WS-10, WS-11, WS-13, WS-14, WS-15, WS-19, WS-20, WS-22, WS-23, WS-27, WS-30, WS-32, WS-35, WS-36, WS-37, WS-38, WS-41, WS-42, WS-44, WS-45, WS-47, WS-48, WS-50, WS-52, WS-53, WS-59, WS-60, WS-61, WS-65, WS-67, WS-68, WS-69, WS-70, WS-72, WS-76, WS-78, WS-79, WS-82, WS-83, WS-84, WS-88, WS-89, WS-93, WS-94, WS-95, WS-97, WS-98, WS-99, WS-100, WS-101, WS-102, WS-103, WS-108, WS-109, WS-111, WS-113, WS-114, WS-115, WS-116, WS-117, WS-118, WS-119, WS-120, WS-121, WS-122, WS-124, WS-125, WS-126, WS-127, WS-128, WS-131, WS-134, WS-135, WS-136, WS-137, WS-141, WS-142, WS-144, WS-146, WS-147, WS-148, WS-149 and Sulaiman	87	57.33
Kernels exhibiting 50% or more shrivel	3	WS-29, WS-31, WS-39, WS-40, WS-56, WS-58, WS-62, WS-64, WS-74, WS-80, WS-106, WS-12, WS-143, WS-123, WS-132, WS-138 and WS-140	17	11.18

*As per the IBPGR Descriptor for Walnut

WS-44, WS-45, WS-47, WS-48, WS-50, WS-52, WS-53, WS-59, WS-60, WS-61, WS-65, WS-67, WS-68, WS-69, WS-70, WS-72, WS-76, WS-78, WS-79, WS-82, WS-83, WS-84, WS-88, WS-89, WS-93, WS-94, WS-95, WS-97, WS-98, WS-99, WS-100, WS-101, WS-102, WS-103, WS-108, WS-109, WS-111, WS-113, WS-114, WS-115, WS-116, WS-117, WS-118, WS-119, WS-120, WS-121, WS-122, WS-124, WS-125, WS-126, WS-127, WS-128, WS-131, WS-134, WS-135, WS-136, WS-137, WS-141, WS-142, WS-144, WS-146, WS-147, WS-148, WS-149 and Sulaiman had kernel shrivel less than 50 per cent; while the selections WS-29, WS-31, WS-39, WS-40, WS-56, WS-58, WS-62, WS-64, WS-74, WS-80, WS-106, WS-12, WS-143, WS-123, WS-132, WS-138 and WS-140 exhibited ≥ 50.0 per cent kernel shrivel.

4.5.2 Kernel fill

Majority of the genotypes (99) had moderate kernel fill, 27 genotypes had well filled kernels and remaining 26 genotypes had poor kernel fill (Table 15) corresponding to 65.14, 17.76 and 17.10 per cent of the population, respectively. The walnut selections WS-12, WS-17, WS-22, WS-26, WS-40, WS-50, WS-51, WS-58, WS-61, WS-63, WS-69, WS-71, WS-74, WS-80, WS-82, WS-84, WS-96, WS-101, WS-104, WS-117, WS-119, WS-122, WS-123, WS-126, WS-132, WS-140 and WS-147 had poor

Table-15: Diversity for kernel fill in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession number	Total	Per cent of the population
Poor	3	WS-12, WS-17, WS-22, WS-26, WS-40, WS-50, WS-51, WS-58, WS-61, WS-63, WS-69, WS-71, WS-74, WS-80, WS-82, WS-84, WS-96, WS-101, WS-104, WS-117, WS-119, WS-122, WS-123, WS-126, WS-132, WS-140 and WS-147	27	17.76
Moderate	5	WS-01, WS-02, WS-03, WS-05, WS-06, WS-07, WS-08, WS-10, WS-11, WS-13, WS-14, WS-15, WS-16, WS-18, WS-19, WS-20, WS-23, WS-24, WS-28, WS-29, WS-30, WS-31, WS-32, WS-35, WS-36, WS-37, WS-38, WS-39, WS-41, WS-42, WS-44, WS-45, WS-47, WS-48, WS-49, WS-53, WS-55, WS-56, WS-57, WS-60, WS-64, WS-70, WS-72, WS-73, WS-75, WS-76, WS-77, WS-78, WS-81, WS-85, WS-86, WS-87, WS-88, WS-89, WS-90, WS-91, WS-92, WS-93, WS-94, WS-95, WS-97, WS-98, WS-99, WS-100, WS-102, WS-103, WS-106, WS-107, WS-108, WS-109, WS-110, WS-111, WS-113, WS-114, WS-115, WS-116, WS-118, WS-120, WS-121, WS-124, WS-125, WS-127, WS-128, WS-131, WS-133, WS-134, WS-135, WS-136, WS-137, WS-138, WS-139, WS-141, WS-142, WS-143, WS-144, WS-145, WS-146, WS-148 and WS-149	99	65.14
Well	7	WS-04, WS-09, WS-21, WS-25, WS-27, WS-33, WS-34, WS-43, WS-46, WS-52, WS-54, WS-59, WS-62, WS-65, WS-66, WS-67, WS-68, WS-79, WS-83, WS-105, WS-112, WS-129, WS-130, WS-150 and Hamdan, Sulaiman	26	17.10

*As per the IBPGR Descriptor for Walnut

kernel fill; the selections WS-01, WS-02, WS-03, WS-05, WS-06, WS-07, WS-08, WS-10, WS-11, WS-13, WS-14, WS-15, WS-16, WS-18, WS-19, WS-20, WS-23, WS-24, WS-28, WS-29, WS-30, WS-31, WS-32, WS-35, WS-36, WS-37, WS-38, WS-39, WS-41, WS-42, WS-44, WS-45, WS-47, WS-48, WS-49, WS-53, WS-55, WS-56, WS-57, WS-60, WS-64, WS-70, WS-72, WS-73, WS-75, WS-76, WS-77, WS-78, WS-81, WS-85, WS-86, WS-87, WS-88, WS-89, WS-90, WS-91, WS-92, WS-93, WS-94, WS-95, WS-97, WS-98, WS-99, WS-100, WS-102, WS-103, WS-106, WS-107, WS-108, WS-109, WS-110, WS-111, WS-113, WS-114, WS-115, WS-116, WS-118, WS-120, WS-121, WS-124, WS-125, WS-127, WS-128, WS-131, WS-133, WS-134, WS-135, WS-136, WS-137, WS-138, WS-139, WS-141, WS-142, WS-143, WS-144, WS-145, WS-146, WS-148 and WS-149 had moderate kernel fill and the remaining selections WS-04, WS-09, WS-21, WS-25, WS-27, WS-33, WS-34, WS-43, WS-46, WS-52, WS-54, WS-59, WS-62, WS-65, WS-66, WS-67, WS-68, WS-79, WS-83, WS-105, WS-112, WS-129, WS-130, WS-150, Hamdan and Sulaiman had well kernel fill.

4.5.3 Ease of kernel removal

Perusal of the Table-16 revealed that in the natural population of walnut under study 17.11 per cent (26 genotypes) had removal of kernels as

Table-16: Diversity for ease of removal of kernels from nuts in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession number	Total	Per cent of the population
Very easy	1	WS-12, WS-22, WS-33, WS-49, WS-50, WS-52, WS-56, WS-60, WS-61, WS-62, WS-63, WS-65, WS-67, WS-68, WS-71, WS-76, WS-84, WS-93, WS-96, WS-102, WS-110, WS-129, WS-130, WS-131, WS-132 and WS-138	26	17.11
Easy	3	WS-01, WS-02, WS-03, WS-04, WS-13, WS-14, WS-15, WS-16, WS-17, WS-18, WS-20, WS-21, WS-23, WS-24, WS-25, WS-27, WS-28, WS-30, WS-34, WS-35, WS-36, WS-37, WS-38, WS-39, WS-40, WS-42, WS-43, WS-45, WS-46, WS-47, WS-51, WS-57, WS-58, WS-64, WS-66, WS-69, WS-74, WS-75, WS-77, WS-79, WS-80, WS-81, WS-82, WS-85, WS-86, WS-87, WS-90, WS-98, WS-99, WS-101, WS-103, WS-104, WS-105, WS-106, WS-107, WS-109, WS-111, WS-112, WS-113, WS-114, WS-115, WS-116, WS-117, WS-118, WS-121, WS-122, WS-123, WS-125, WS-126, WS-128, WS-133, WS-134, WS-135, WS-136, WS-137, WS-141, WS-144, WS-145, WS-146, Hamdan and Sulaiman	81	53.29
Moderate	5	WS-05, WS-06, WS-07, WS-08, WS-09, WS-10, WS-11, WS-19, WS-26, WS-29, WS-32, WS-41, WS-44, WS-48, WS-53, WS-55, WS-59, WS-70, WS-72, WS-73, WS-78, WS-83, WS-88, WS-89, WS-97, WS-100, WS-108, WS-119, WS-120, WS-124, WS-127, WS-139, WS-140, WS-142, WS-143, WS-147, WS-148, WS-149 and WS-150	39	25.66
Difficult	7	WS-31, WS-54, WS-91, WS-92, WS-94, WS-95	6	3.94

*As per the IBPGR Descriptor for Walnut

very easy, 53.29 per cent (81 genotypes) had easy removal of kernels, 25.66 per cent (39 genotypes) were found to be moderate in ease of kernel removal and the remaining 3.94 per cent (6 genotypes) had difficult removal of kernels.

The walnut genotypes WS-12, WS-22, WS-33, WS-49, WS-50, WS-52, WS-56, WS-60, WS-61, WS-62, WS-63, WS-65, WS-67, WS-68, WS-71, WS-76, WS-84, WS-93, WS-96, WS-102, WS-110, WS-129, WS-130, WS-131, WS-132 and WS-138 had very easy removal of kernels; the genotypes WS-01, WS-02, WS-03, WS-04, WS-13, WS-14, WS-15, WS-16, WS-17, WS-18, WS-20, WS-21, WS-23, WS-24, WS-25, WS-27, WS-28, WS-30, WS-34, WS-35, WS-36, WS-37, WS-38, WS-39, WS-40, WS-42, WS-43, WS-45, WS-46, WS-47, WS-51, WS-57, WS-58, WS-64, WS-66, WS-69, WS-74, WS-75, WS-77, WS-79, WS-80, WS-81, WS-82, WS-85, WS-86, WS-87, WS-90, WS-98, WS-99, WS-101, WS-103, WS-104, WS-105, WS-106, WS-107, WS-109, WS-111, WS-112, WS-113, WS-114, WS-115, WS-116, WS-117, WS-118, WS-121, WS-122, WS-123, WS-125, WS-126, WS-128, WS-133, WS-134, WS-135, WS-136, WS-137, WS-141, WS-144, WS-145, WS-146, Hamdan and Sulaiman had easy removal of kernels; the genotypes WS-05, WS-06, WS-07, WS-08, WS-09, WS-10, WS-11, WS-19, WS-26, WS-29, WS-32, WS-41, WS-44, WS-48, WS-53,

WS-55, WS-59, WS-70, WS-72, WS-73, WS-78, WS-83, WS-88, WS-89, WS-97, WS-100, WS-108, WS-119, WS-120, WS-124, WS-127, WS-139, WS-140, WS-142, WS-143, WS-147, WS-148, WS-149 and WS-150 had moderate removal of kernels and the genotypes WS-31, WS-54, WS-91, WS-92, WS-94 and WS-95 had difficult removal of kernels.

4.5.4 Kernel colour

Kernel colour in the present study (Table 17) varied from extra light to amber. Twenty four genotypes (15.79%) were having extra light coloured kernels; 100 genotypes (65.79%) were found to have light coloured kernels; 18 genotypes (11.85%) had light amber coloured kernels and remaining 10 genotypes (6.57%) had amber kernel colour. The walnut genotypes WS-04, WS-09, WS-11, WS-21, WS-33, WS-43, WS-46, WS-53, WS-54, WS-62, WS-76, WS-79, WS-89, WS-90, WS-91, WS-93, WS-94, WS-105, WS-112, WS-114, WS-138, WS-139, WS-141 and WS-145 had extra light kernel colour; the genotypes WS-01, WS-02, WS-03, WS-07, WS-08, WS-10, WS-13, WS-14, WS-15, WS-17, WS-18, WS-19, WS-20, WS-23, WS-24, WS-25, WS-26, WS-27, WS-28, WS-29, WS-32, WS-34, WS-35, WS-38, WS-39, WS-40, WS-48, WS-49, WS-50, WS-51, WS-52, WS-55, WS-56, WS-57, WS-58, WS-60, WS-61, WS-63, WS-64, WS-65, WS-66, WS-67, WS-68, WS-69, WS-71, WS-72, WS-73, WS-74, WS-75, WS-77, WS-78, WS-80, WS-81, WS-82, WS-83, WS-84, WS-85, WS-86, WS-87,

Table-17: Diversity for kernel colour in the natural population of walnut (*Juglans regia* L.) in the Kashmir valley

Descriptor*	Score*	Accession numbers	Total	Per cent of the population
Extra light	1	WS-04, WS-09, WS-11, WS-21, WS-33, WS-43, WS-46, WS-53, WS-54, WS-62, WS-76, WS-79, WS-89, WS-90, WS-91, WS-93, WS-94, WS-105, WS-112, WS-114, WS-138, WS-139, WS-141, WS-145	24	15.79
Light	2	WS-01, WS-02, WS-03, WS-07, WS-08, WS-10, WS-13, WS-14, WS-15, WS-17, WS-18, WS-19, WS-20, WS-23, WS-24, WS-25, WS-26, WS-27, WS-28, WS-29, WS-32, WS-34, WS-35, WS-38, WS-39, WS-40, WS-48, WS-49, WS-50, WS-51, WS-52, WS-55, WS-56, WS-57, WS-58, WS-60, WS-61, WS-63, WS-64, WS-65, WS-66, WS-67, WS-68, WS-69, WS-71, WS-72, WS-73, WS-74, WS-75, WS-77, WS-78, WS-80, WS-81, WS-82, WS-83, WS-84, WS-85, WS-86, WS-87, WS-88, WS-92, WS-95, WS-98, WS-99, WS-100, WS-101, WS-102, WS-103, WS-104, WS-106, WS-107, WS-108, WS-110, WS-111, WS-113, WS-115, WS-116, WS-117, WS-118, WS-120, WS-121, WS-122, WS-123, WS-124, WS-126, WS-128, WS-131, WS-132, WS-133, WS-134, WS-135, WS-136, WS-137, WS-146, WS-147, WS-149, WS-150, Hamdan, Sulaiman	100	65.79
Light amber	3	WS-05, WS-06, WS-12, WS-16, WS-22, WS-30, WS-31, WS-36, WS-41, WS-42, WS-44, WS-70, WS-97, WS-109, WS-125, WS-127, WS-129, WS-130	18	11.85
Amber	4	WS-37, WS-45, WS-47, WS-96, WS-119, WS-140, WS-142, WS-143, WS-144, WS-149	10	6.57

*As per the IBPGR Descriptor for Walnut

WS-88, WS-92, WS-95, WS-98, WS-99, WS-100, WS-101, WS-102, WS-103, WS-104, WS-106, WS-107, WS-108, WS-110, WS-111, WS-113, WS-115, WS-116, WS-117, WS-118, WS-120, WS-121, WS-122, WS-123, WS-124, WS-126, WS-128, WS-131, WS-132, WS-133, WS-134, WS-135, WS-136, WS-137, WS-146, WS-147, WS-149, WS-150, Hamdan and Sulaiman had light colour kernels; the genotypes WS-05, WS-06, WS-12, WS-16, WS-22, WS-30, WS-31, WS-36, WS-41, WS-42, WS-44, WS-70, WS-97, WS-109, WS-125, WS-127, WS-129 and WS-130 had light amber kernel colour and remaining genotypes WS-37, WS-45, WS-47, WS-96, WS-119, WS-140, WS-142, WS-143, WS-144 and WS-149 possessed amber kernel colour.

4.6 Estimation of the components of variation

4.6.1 Morphological characters

4.6.1.1 Canopy spread and stem girth

The data on canopy spread of the tagged walnut genotypes in the present study (Table-18) revealed a population mean of 6.25 m which ranged from 1.20-12.50 m. The maximum canopy spread was recorded in the walnut selection WS-011 (12.50 m) whereas, the lower tree spread was recorded in the walnut selection WS-036 (1.20 m). Analysis of variance revealed a phenotypic variance of 5.656 and the genotypic variance of 2.852. The environmental variance was 2.803. Based on the estimates of variance (phenotypic and genotypic), the coefficients of variation (phenotypic and genotypic) were recorded as 37.98 and 26.97 per cent, respectively. Heritability (broad sense) was 50.42 per cent and expected genetic gain as 39.4 per cent of mean.

Table-18: Estimation of variability and genetic parameters for canopy spread and stem girth in the seedling raised natural walnut (*Juglans regia* L.) population in the Kashmir valley

(Pooled values over 2 years)

Parameters estimated	Canopy spread (m)	Stem girth (m)
Population mean	6.25 ±1.37	1.37 ±0.58
Range	1.20-12.50	0.3-3.60
Phenotypic variance (σ^2_p)	5.656	0.687
Genotypic variance (σ^2_g)	2.852	0.452
Environmental variance (σ^2_e)	2.803	0.235
Phenotypic coefficient of variation (PCV)	37.98%	60.40%
Genotypic coefficient of variation (GCV)	26.97%	49.07%
Heritability broad sense (h^2)	50.42%	65.79%
Expected genetic gain (Per cent of mean) at 5% selection intensity ($k = 2.06$)	39.47	82.18

Stem girth (Table 18) revealed a population mean of 1.37 m that ranged from 0.3-3.60 m. The maximum tree girth was recorded in the walnut selection WS-106 (3.60m) and the minimum in the walnut selection WS-024 (0.3 m). Analysis of variance revealed phenotypic variance to the extent of 0.687 and genotypic variance as 0.452. The environmental variance was 0.235. Phenotypic and genotypic co-efficients of variation worked out as 60.40 and 49.07 per cent, respectively. The heritability (broad sense) was 65.79 per cent and the expected genetic gain as 82.18 per cent of mean.

4.6.1.2 Leaf length and width

Estimates of variability and other genetic parameters with regard to leaf length and width are presented in the Table-19. The leaf length had a population mean 12.156 cm and a range of 5.25-24.50 cm. The maximum leaf length was recorded in the walnut selection WS-108 (24.50 cm) followed by the walnut selection WS-111 (23.10cm), WS-49 (23.0 cm), WS-114 (22.25 cm), WS-60 (21.50) and WS-52 (20 cm), whereas, the minimum leaf length was recorded in the walnut selection WS-33 (5.25cm).

Analysis of variance revealed phenotypic variance of 19.502, genotypic variance 12.163 and environmental variance 7.339. The phenotypic and genotypic coefficients of variation were as 36.32 and 28.69 per cent, respectively. Heritability (broad sense) was 62.30 per cent and expected genetic gain was 46.67 (per cent of mean).

Table-19: Estimation of variability and genetic parameters for leaf length and leaf width in the seedling raised natural walnut (*Juglans regia* L.) population in the Kashmir valley

(Pooled values over 2 years)

Parameters estimated	Leaf length (cm)	Leaf width (cm)
Population mean	12.156 ±1.909	6.490 ±1.466
Range	5.25-24.50	2.55-13.50
Phenotypic variance (σ^2_p)	19.502	8.433
Genotypic variance (σ^2_g)	12.163	4.102
Environmental variance (σ^2_e)	7.339	4.330
Phenotypic coefficient of variation (PCV)	36.32%	44.74%
Genotypic coefficient of variation (GCV)	28.69%	31.20%
Heritability broad sense (h^2)	62.30%	48.06%
Expected genetic gain (Per cent of mean) at 5% selection intensity ($k = 2.06$)	46.67	44.84

The leaf width had a population mean of 6.490 cm with a range of 2.55 and 13.50 cm. Maximum leaf width was noticed in the walnut selection WS-076 (13.50 cm) followed by the walnut selection WS-081 (11.75 cm) and the minimum leaf width (2.55 cm) was noticed in the selection WS-36. Analysis of variance revealed a phenotypic variance of 8.433, genotypic variance of 4.102 and the environmental variance of 4.330. The phenotypic and genotypic coefficients of variance were 44.74 and 31.20 per cent, respectively. Heritability (broad sense) was 48.06 per cent. Expected genetic gain (per cent of mean) would be 44.84.

4.6.2 Nut characters

Perusal of the Table-20 revealed that nut weight had a population mean of 14.48 g with a range of 8.50 to 35.0 g. The highest nut weight was recorded in the selection WS-10 (35.0 g) followed by the selection WS-147 (30 g), WS-148 (26 g), WS-28 (24.50 g) and WS-18 (24.0 g) and the lowest was recorded in the selection WS-42 (8.50 g).

Analysis of variance revealed a phenotypic variance of 18.785 and genotypic variance of 12.760. The environmental variance was 6.025. Based on the estimates of Phenotypic and genotypic variance, their coefficients of variation worked out to 27.98 and 23.02 per cent, respectively. Heritability (broad sense) was 67.9 per cent and expected genetic gain as 39.15 per cent of the mean.

Table-20: Estimation of variability and genetic parameters for nut characters (weight, diameter and length) in the seedling raised natural walnut (*Juglans regia* L.) population in the Kashmir valley

(Pooled values over 2 years)

Parameters estimated	Nut weight (g)	Nut diameter		Nut length (mm)
		Suture (mm)	Cheek (mm)	
Population mean	14.48 ±2.54	33.73 ±2.82	34.63 ±2.92	55.62 ±3.28
Range	8.50-35.00	24.50-50.50	25.50-50.00	30.00-60.00
Phenotypic variance (σ^2_p)	18.785	18.544	19.457	605.14
Genotypic variance (σ^2_g)	12.760	10.421	12.338	427.56
Environmental variance (σ^2_e)	6.025	8.122	17.209	177.58
Phenotypic coefficient of variation (PCV)	27.98%	12.76%	12.76%	44.25%
Genotypic coefficient of variation (GCV)	23.02%	9.53%	10.13%	37.17%
Heritability broad sense (h^2)	67.9%	56.2%	63.1%	70.65%
Expected genetic gain (Per cent of mean) at 5% selection intensity ($k = 2.06$)	39.15	14.77	16.59	64.36

Nut suture expressed a population mean of 33.73mm with a range of 24.50-50.50 mm. Maximum nut suture was recorded in the selection WS-139 (50.50 mm), followed by WS28 (42.5 mm) and WS-60 (42.00 mm), while the minimum nut suture (24.50 mm) was recorded in the WS-40. Analysis of the components of variance revealed phenotypic variance of 18.544, genotypic variance of 10.421 and environmental variance of 8.122. Based on the estimates of phenotypic and genotypic components of variance, the phenotypic and genotypic coefficients of variation worked out to be 12.76 and 9.53 per cent respectively. Heritability (broad sense) was 56.2 per cent and expected genetic gain as 14.77 per cent of the mean.

Nut cheek had a population mean of 34.63 mm with a range of 25.50-50.0 mm. The maximum nut cheek was recorded in the walnut selection WS-046 (50.0mm) followed by the walnut selection WS-142 (44.00 mm) and WS-14 (43.00 mm), whileas the minimum nut cheek was recorded in the walnut selection WS-034 (25.50 mm). Analysis of variance revealed phenotypic variance of 19.457, genotypic variance of 12.338 and the environmental variance of 17.209. Estimation of components of variance exhibited phenotypic and genotypic coefficients of variation as 12.76 and 10.13 per cent, respectively. Heritability (broad sense) was 63.1 per cent and expected genetic gain as 16.59 per cent of the mean.

The nut length in the population ranged from 30.00-60.00 mm around the population mean of 55.62 mm. The maximum nut length of 60.00 mm was found in the selection WS-028 followed by WS-084 (52.50 mm), WS-110 (51.25 mm), WS-113 (50.0 mm), WS-121 (50.0 mm) and WS-114 (49.00 mm), whileas the minimum nut length was found in the selection WS-042 (30.00 mm). Analysis of variance revealed a phenotypic variance of 605.14, genotypic variance of 427.56 and environmental variance of 177.58. Estimation of phenotypic and genotypic coefficients of variation were 44.25 and 37.17 per cent, respectively. Heritability (broad sense) was 70.65 per cent and the expected genetic gain as 64.36 per cent of the mean.

4.6.3 Kernel characters

Analysis of variance with respect to kernel characters (weight and kernel recovery) are presented in the Table-21. The kernel weight per nut in the population varied considerably from 3.50 to 15.0 g with a mean of 7.75 g. The highest kernel weight (15.0 g) was recorded in the selection WS-147, whereas the lowest kernel weight (3.50 g) was recorded in selection the WS-042. Similarly, the kernel recovery percentage ranged from 31.03 to 70.0 in the present population with a mean of 50.04 per cent. The genotype WS-116 had the highest (70%) kernel recovery followed by walnut

Table-21: Estimation of variability and genetic parameters for kernel traits (weight and per cent recovery) in seedling raised natural walnut (*Juglans regia* L.) population in the Kashmir valley

(Pooled values over 2 years)

Parameters estimated	Kernel weight nut⁻¹ (g)	Kernel recovery Per centage
Population mean	7.75 ±1.54	50.04 ±5.10
Range	3.50-15.00	31.03-70.00
Phenotypic variance (σ^2_p)	6.149	54.280
Genotypic variance (σ^2_g)	3.329	31.917
Environmental variance (σ^2_e)	2.819	22.372
Phenotypic coefficient of variation (PCV)	31.99 %	41.72 %
Genotypic coefficient of variation (GCV)	23.54 %	11.29 %
Heritability broad sense (h^2)	54.14 %	58.88 %
Expected genetic gain (Per cent of mean) at 5% selection intensity (k = 2.06)	35.68	17.85

selection WS-004 (65.2 %), WS-12 (65%) and WS-17 (60%). The lowest kernel recovery (31.03%) was recorded in the genotype WS-74. The phenotypic and genotypic components of variance were estimated as 6.149 and 3.329 for kernel weight nut^{-1} and 54.28 and 31.917 per cent for kernel recovery, respectively. The Phenotypic coefficient of variation for kernel weight was 31.99 per cent while genotypic coefficient of variation for this character was 23.54 per cent. For kernel recovery percentage the corresponding values of phenotypic and genotypic coefficients of variation were 41.72 and 11.29 per cent, respectively. Heritability (broad sense) for kernel weight nut^{-1} was 54.14 per cent and the expected genetic gain was 35.68 per cent; whileas for kernel recovery per cent the heritability (broad sense) was 58.88 and expected genetic gain was 17.85 per cent of the mean.

4.7 Qualitative Characters

4.7.1 Protein Content

The population mean of kernel protein content (Table-22) was 17.967 per cent which ranged from 14.15 to 24.25 per cent (Table 22). Maximum protein content (24.25%) was recorded in kernels of WS-112 and minimum (14.15%) in the WS-149. Analysis of variance revealed a phenotypic variance of 4.315, genotypic variance 1.985 and the environmental variance of 2.329. Based on the components of variance, phenotypic and genotypic coefficients of variation worked out to be 11.56 and 7.84 per cent, respectively. Heritability (broad sense) was 46.02 per cent and expected genetic gain (as per cent of the mean) was 10.960.

Table-22: Estimation of variability and genetic parameters for kernel quality traits (protein and oil content) in the seedling raised natural walnut (*Juglans regia* L.) population in the Kashmir valley

(Pooled values over 2 years)

Parameters estimated	Protein (%)	Oil content (%)
Population mean	17.967 ±1.075	62.102 ±3.989
Range	14.15-24.25	50.22-70.00
Phenotypic variance (σ^2_p)	4.315	29.027
Genotypic variance (σ^2_g)	1.985	8.546
Environmental variance (σ^2_e)	2.329	20.48
Phenotypic coefficient of variation (PCV)	11.56 %	8.67 %
Genotypic coefficient of variation (GCV)	7.84 %	4.70 %
Heritability broad sense (h^2)	46.02 %	47.43 %
Expected genetic gain (Per cent of mean) at 5% selection intensity ($k = 2.06$)	10.960	5.261

4.7.2 Kernel oil content

Perusal of the Table-22 revealed that the oil content of kernels had a population mean of 62.102 per cent with a range of 54.93-70.00 per cent. Maximum kernel oil content (70%) was found in the walnut selection WS-114 and minimum (50.22%) in the WS-150. Analysis of variance revealed a phenotypic variance of 29.027 as compared to genotypic variance of 8.546 with environmental variance being 20.48. Based on the estimation of components of variance, the phenotypic and genotypic coefficients of variation were 8.67 and 4.70 per cent, respectively. Heritability (broad sense) was 29.43 per cent and the expected genetic gain as only 5.261 (per cent of the mean).

4.8 Nut yield per tree

Estimation of nut yield (kg/tree) was worked out for different age groups of trees so as to minimize the variation in yield arising due to age of trees, vigour, spread, soil condition and management practices etc. Accordingly, the population in the present study was divided into four age groups on the basis of visual observation like stem diameter, canopy spread, vigour etc, together with the information collected from the owners about approximate age of the tree.

Table-23: Estimation of variability and genetic parameters for nut yield (kg tree⁻¹) in the seedling raised natural walnut (*Juglans regia* L.) population in the Kashmir valley

(Pooled values over 2 years)

Parameters estimated	Yield (kg tree ⁻¹) under different age groups			
	15-30 yr	31-50 yr	51-70 yr	Above 70 yr
Population mean	20.92 ±1.95	31.35 ±2.35	42.02 ±2.67	63.24 ±3.10
Range	10.2-55.5	19.5-68.0	30.3-165.5	37.5-185.0
Phenotypic variance (σ^2_p)	133.66	146.11	171.98	762.05
Genotypic variance (σ^2_g)	65.83	74.87	77.08	396.52
Environmental variance (σ^2_e)	67.83	71.24	94.90	365.53
Phenotypic coefficient of variation (PCV)	55.26 %	38.56 %	31.21 %	43.65 %
Genotypic coefficient of variation (GCV)	38.78 %	27.60 %	20.89 %	31.48 %
Heritability broad sense (h^2)	49.25 %	51.24 %	51.81 %	52.03 %
Expected genetic gain (Per cent of mean) at 5% selection intensity ($k = 2.06$)	56.07	40.69	45.70	46.78

Estimation of variability parameters for the age group of 15-30 years revealed a population mean of 20.9 kg tree⁻¹ and range of 10.2 to 55.5 kg tree⁻¹. The maximum yield was recorded in the walnut selection WS-001 (55.5 kg tree⁻¹) and the minimum yield (10.2 kg tree⁻¹) was recorded in the walnut selection WS-20. Estimation of components of variance revealed a phenotypic variance of 133.66, genotypic variance of 65.83 and environmental variance of 67.83. Estimation of phenotypic and genotypic coefficients of variation were to the extent of 55.26 and 38.78 per cent, respectively, Heritability (broad sense) was 49.25 per cent and the expected genetic gain (per cent of the mean) was to the extent of 56.07.

Estimation of variability parameters for the age group of 31 to 50 years revealed a population mean of 31.35 kg tree⁻¹ with a range of 19.5 to 68.0 kg tree⁻¹. The maximum yield (68kg tree⁻¹) in this group was recorded in the walnut selection WS-13 and the minimum yield (19.5 kg tree⁻¹) was recorded in the walnut selection WS-119. Estimation of components of variance revealed a phenotypic variance of 146.11, genotypic variance of 74.87 and environmental variance 71.24. Estimation of phenotypic and genotypic coefficients of variation were to the extent of 38.56 and 27.60 per cent, respectively. Heritability (broad sense) was 51.24 per cent and expected genetic gain to the extent of 40.69 (per cent of the mean).

Estimation of the variability parameters for the age group of 51-70 years revealed a population mean of 42.02 kg tree⁻¹ with a range of 30.30 to 165.50 kg tree⁻¹. The maximum yield was recorded in the walnut selection WS-139 (165.5 kg tree⁻¹) and the minimum (30.3 kg tree⁻¹) was recorded in the walnut selection WS-008. Estimation of the components of variance revealed a phenotypic variance of 171.98, genotypic variance of 77.08 and the environmental variance of 94.90. Estimation of phenotypic and genotypic coefficients of variation were to the extent of 31.21 and 20.89 per cent, respectively. Heritability (broad sense) was 51.81 per cent and expected genetic gain was 45.70 per cent of the mean.

Estimation of the variability parameters in the age group of above 70 years revealed a population mean of 63.24 kg tree⁻¹ and range of 37.50-180 kg tree⁻¹. The maximum yield (185.0 kg tree⁻¹) was recorded in the walnut selection WS-46 and the minimum (37.5 kg tree⁻¹) was recorded in the walnut selection WS-60. Estimation of the components of variance revealed phenotypic variance of 762.05, genotypic variance of 396.52 and the environmental variance of 365.53. The phenotypic and genotypic coefficients of variation were 43.65 and 31.48 per cent, respectively. Heritability (broad sense) was 52.03 per cent and expected genetic gain (per cent of the mean) was 46.78.

4.8 Overall performance of some elite selections

Based on nut and kernel characteristics (Tables-24 and 25) some of the elite cultivars in the natural seedling raised walnut population in the Kashmir valley are briefly highlighted.

Based on the size of nut (weight in g), the accession WS-147 had the boldest nut (30.0 g) with medium shell texture and intermediate shell strength followed by WS 017 (26.0 g), WS 148 (25.5 g) and WS-028 (24.5 g). The mean nut weight of other accessions viz. WS-012, WS-133, WS 022, WS-150, WS-049, WS-018 and WS-028 ranged between 20.25 to 24.50 g. Shell texture was smooth and medium, and shell strength intermediate except in the case of WS-017, WS-022 and WS-133 which had weak shell strength. The best standard check Hamdaan had a mean nut size 21.50 g with very smooth shell texture and intermediate shell strength.

Among kernel characteristics emphasis was laid on the compactness of the kernels and their per cent recovery from nuts. The recovery per cent of kernels in ten top accessions varied from 50.0 to 70.0 per cent. Rankwise the accession were WS-114 (70.0%), WS-116 (66.66%), WS-004 (65.21%), WS-138 (57.14%), WS-141 (55.0%) and WS-011 (51.61%). The remaining accession viz. WS-118, WS-094, WS-117 and WS-145 had mean kernel recovery of 50.0 per cent. However, only three accessions viz. WS-114,

Table-24: List of top 10 accession numbers identified (over and above international standards) from the natural walnut (*Juglans regia* L.) population as per the nut characteristics

S. No.	Accession No.	Nut weight (g)	Shell texture	Shell colour	Shell seal	Shell strength
1.	WS-147	30.00	Medium	Light	Strong	Intermediate
2.	WS-017	26.00	Medium	Light	Intermediate	Weak
3.	WS-148	25.50	Medium	Light	Strong	Intermediate
4.	WS-028	24.50	Medium	Light	Intermediate	Intermediate
5.	WS-018	24.00	Medium	Light	Strong	Intermediate
6.	WS-049	23.50	Medium	Medium	Intermediate	Intermediate
7.	WS-150	22.50	Smooth	Medium	Weak	Intermediate
8.	WS-022	22.05	Medium	Medium	Weak	Weak
9.	WS-112	22.25	Smooth	Light	Intermediate	Intermediate
10.	WS-133	22.00	Smooth	Light	Intermediate	Weak
<u>Standard Checks</u>						
1.	Hamdan	14.70	Smooth	Light	Weak	Intermediate
2.	Sulaiman	21.50	Very smooth	Light	Intermediate	Intermediate

Table-25: Top ten accessions on the basis of kernel characteristics (over and above international standards) identified from the natural seedling raised walnut (*Juglans regia* L.) population in the present study

S. No.	Accession No.	Nut weight (g)	Kernel weight per nut (g)	Kernel recovery percentage	Kernel colour
1.	WS-114	10.00	7.00	70.00	Extra Light
2.	WS-116	12.00	8.00	66.66	Extra Light
3.	WS-004	11.50	7.50	65.21	Extra Light
4.	WS-138	14.00	8.00	57.14	Extra Light
5.	WS-141	20.00	11.00	55.00	Extra Light
6.	WS-011	15.50	8.00	51.61	Extra Light
7.	WS-118	18.00	9.00	50.00	Extra Light
8.	WS-094	15.00	7.50	50.00	Extra Light
9.	WS-117	14.00	7.00	50.00	Extra Light
10.	WS-145	22.00	11.00	50.00	Extra Light
<u>Standard Checks</u>					
11.	Hamdan	14.70	8.05	57.63	Light
12.	Sulaiman	21.50	11.26	52.37	Light



(a) WS-011



(b) WS-138



(c) WS-004



(d) WS-094

Plate-2 : Some of the elite walnut (*Juglans regia* L.) genotypes identified in the *in situ* seedling raised walnut population of Kashmir Valley

- | | | | |
|-----|--------|---|--|
| (a) | WS-011 | : | Nut weight 15.5 g with 51.6 per cent kernel recovery |
| (b) | WS-138 | : | Nut weight 14.0 g with 57.2 per cent kernel recovery |
| (c) | WS-004 | : | Nut weight 11.5 g with 65.2 per cent kernel recovery |
| (d) | WS-094 | : | Nut weight 15.0 g with 50.0 per cent kernel recovery |



(a) WS-133



(b) WS-017



(c) WS-145



(d) WS-141

Plate-3 : Some of the elite walnut (*Juglans regia* L.) genotypes identified in the *in situ* seedling raised walnut population of Kashmir Valley

- (a) WS-133 : Nut weight 22.0 g with smooth shell texture, light shell colour and intermediate shell seal
- (b) WS-017 : Nut weight 26.0 g with medium shell texture and light shell colour
- (c) WS-145 : Nut weight 22.0 g with 50.0 per cent kernel recovery
- (d) WS-141 : Nut weight 20.0 g with 55.0 per cent kernel recovery



(a) WS-117



(b) WS-018



(c) WS-012



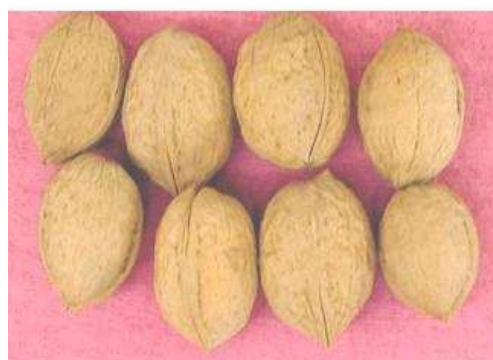
(d) WS-114

Plate-4 : Some of the elite walnut (*Juglans regia* L.) genotypes identified in the *in situ* seedling raised walnut population of Kashmir Valley

- (a) WS-117 : Nut weight 14.0 g with 50.0 per cent kernel recovery**
- (b) WS-018 : Nut weight 24.0 g with medium shell texture and light shell colour**
- (c) WS-012 : Nut weight 22.5 g with smooth shell texture and light shell colour**
- (d) WS-114 : Nut weight 10.0 g with 70.0 per cent kernel recovery**



(a) WS-116



(b) WS-028



(c) WS-147



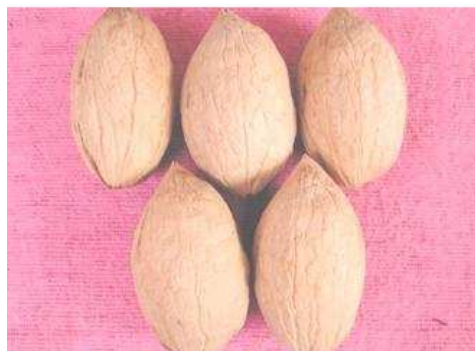
(d) WS-148

Plate-5 : Some of the elite walnut (*Juglans regia* L.) genotypes identified in the *in situ* seedling raised walnut population of Kashmir Valley

- (a) WS-116 : Nut weight 12.0 g with 66.7 per cent kernel recovery**
- (b) WS-028 : Nut weight 24.5 g with medium shell texture and light shell colour**
- (c) WS-147 : Nut weight 30.0 g with medium shell texture and light shell colour**
- (d) WS-148 : Nut weight 25.5 g with medium shell texture and light shell colour**



(a) WS-118



(b) WS-094



(c) WS-049



(d) WS-150

Plate-6 : Some of the elite walnut (*Juglans regia* L.) genotypes identified in the *in situ* seedling raised walnut population of Kashmir Valley

- (a) WS-118 : Nut weight 18.0 g with 50.0 per cent kernel recovery**
- (b) WS-094 : Nut weight 15.0 g with 50.0 per cent kernel recovery and thin shelled**
- (c) WS-049 : Nut weight 23.5 g with medium shell texture and light shell colour**
- (d) WS-150 : Nut weight 22.5 g with medium shell texture and light shell colour**

WS-116 and WS-004 exceeded the performance of the standard check Hamdan that had mean kernel recovery of 57.63 per cent. Kernel colour of the top ten selections was extra light as compared to light colour of both the standard checks.

4.9 *In vitro* shoot morphogenetic studies.

Explants of three elite walnut cultivars viz. SKUA-002, SKUA-008 and SKUA-010 were studied for their response to shoot morphogenesis under micropropagation (tissue culture) techniques. The standard basal medium of Murashige and Skoog (1962) was supplemented with BAP (0.3, 0.6, 0.9 and 1.2 mg l⁻¹) together with a uniform concentration of 0.1 mg l⁻¹ of IBA. The results pertaining to different parameters of *in vitro* studies are briefly presented in the following pages.

4.9.1 Media browning

Effect of growth regulators and cultivars on the per cent media browning (Table-26) revealed that 80.00 to 85.33 per cent culture tubes showed browning in different treatments. The per cent values were transformed to square root and analyzed for significance of treatment effects. Cultivars exhibited no effect on the media browning development where the transformed values (mean values between 82.00 to 82.33%) were non significant. Among growth regulators also only T₂ (BAP 0.6 mg l⁻¹



Plate-7 : Forcing of walnut cuttings to induce sprouting and accelerate explant success during *in vitro* micro-propagation of walnut (*Juglans regia* L.) cultivars

Table-26: Effect of different growth regulator combinations on the per cent media browning in walnut *in vitro* studies using MS medium

Walnut accessions	Growth regulators				Mean
	T ₁	T ₂	T ₃	T ₄	
SKUA-002	8.97* (80.00)	9.19 (84.00)	9.11 (82.66)	9.04 (81.33)	9.08 (82.00)
SKUA-008	8.97 (80.00)	9.26 (85.33)	9.11 (82.66)	9.04 (81.33)	9.10 (82.33)
SKUA-010	9.04 (81.33)	9.19 (84.00)	9.04 (81.33)	9.04 (81.33)	9.08 (82.00)
Mean	8.99 (80.44)	9.21 (84.44)	9.09 (82.22)	9.04 (81.33)	9.08 (82.11)

*Square root transformed value of the mean = ($\sqrt{0.5 + \text{mean value}}$)

Values in parentheses are the mean values of original data.

Critical difference at 5%

Accessions (AC)	0.164
Growth regulators (GR)	0.189
Interaction (AC x GR)	NS

T₁ = BAP 0.3 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₂ = BAP 0.6 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₃ = BAP 0.9 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₄ = BAP 1.2 mg l⁻¹ + IBA 01 mg l⁻¹

+ IBA 0.1mg l^{-1}) had significantly higher per cent media browning than T_1 (BAP 0.3 mg l^{-1} + IBA 0.1 mg l^{-1}). Rest of the comparisons had non significant influences on the per cent media browning.

Interaction arising among accessions and growth regulators revealed non significant influence.

4.9.2 Volume of browned media

Volume of browned media (Table-27) in the *in vitro* culture tubes was measured in terms of $\text{mm}^3 \times 1/100$. Significant effect of cultivars on the volume of browned media was observed. The volume of browned media was significantly higher only in the accessions SKUA-002 as compared to SKUA-010 and SKUA-008. Rest of the comparisons were non significant. Among growth regulators, the combinations T_1 (BAP 0.3 mg l^{-1} + IBA 0.1 mg l^{-1}) and T_2 (BAP 0.6 mg l^{-1} + IBA 0.1 mg l^{-1}) significantly increased the volume of browned media than T_3 (0.9 mg l^{-1} + IBA 0.1 mg l^{-1}). The other differences were non significant.

Among interactions the T_1 (BAP 0.3 mg l^{-1} + IBA 0.1 mg l^{-1}) had significantly lower volume of media browning in the cultivar SKUA-010 than the other two cultivars. Similarly, T_2 (BAP 0.6 mg l^{-1} + IBA 0.1 mg l^{-1}) showed significantly lower volume of media browning in SKUA- 008 than other two accessions. Rest of the interaction differences were non significant.

Table-27: Effect of different growth regulator combinations on the volume of browned media in walnut *in vitro* studies using MS medium

(mm³ x 1/100)

Walnut accessions	Growth regulators				Mean
	T ₁	T ₂	T ₃	T ₄	
SKUA-002	9.80	9.59	9.11	9.50	9.50
SKUA-008	9.59	9.33	9.17	9.20	9.32
SKUA-010	9.17	9.40	9.33	9.20	9.27
Mean	9.52	9.44	9.20	9.30	9.36

Critical difference at 5%

Accessions (AC)	0.177
Growth regulators (GR)	0.204
Interaction (AC x GR)	NS

T₁ = BAP 0.3 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₂ = BAP 0.6 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₃ = BAP 0.9 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₄ = BAP 1.2 mg l⁻¹ + IBA 0.1 mg l⁻¹

4.9.3 Explant browning (per cent)

Per cent explant browning in the culture tubes (Table-28) revealed, by and large, higher frequency (77.33 to 85.33%). Analysed data (square root transformed) showed non significant effect among walnut accessions (SKUA-002, SKUA-008 and SKUA-010) on the explant browning frequency. Contrarily, growth regulators significantly increased the explant browning in the treatment combinations T_2 (BAP 0.6 mg l^{-1} + IBA 0.1 mg l^{-1}), T_3 (BAP 0.9 mg l^{-1} + IBA 0.1 mg l^{-1}) and T_4 (BAP 1.2 mg l^{-1} + IBA 0.1 mg l^{-1}) as compared to T_1 (BAP 0.3 mg l^{-1} + IBA 0.1 mg l^{-1}). Non significant differences were found among T_2 , T_3 and T_4 combinations of growth regulators.

Interaction effects among the accessions x growth regulators revealed that T_4 BAP 1.2 mg l^{-1} + IBA 0.1 mg l^{-1} had higher per cent explant browning in SKUA-010 than SKUA-002 and SKUA-008. Similarly, T_1 BAP 0.3 mg l^{-1} + IBA 0.1 mg l^{-1} showed significantly lower explant browning in the accessions SKUA-008 and SKUA-010 than all other interactions except at BAP 1.2 mg l^{-1} + IBA 0.1 mg l^{-1} concentration in the SKUA-008.

4.9.4 Mean browning score explant⁻¹

The extent of explant browning was measured in terms of score with a value of zero (no browning of the explant) to 4 (complete browning of the

Table-28: Effect of different growth regulator combinations on the per cent browning of explants in walnut *in vitro* studies using MS medium

Walnut accessions	Growth regulators				Mean
	T ₁	T ₂	T ₃	T ₄	
SKUA-002	8.97* (80.00)	9.19 (84.00)	9.11 (82.66)	9.04 (81.33)	9.08 (82.00)
SKUA-008	8.82 (77.33)	9.19 (84.00)	9.19 (84.00)	8.89 (78.66)	9.02 (81.00)
SKUA-010	8.82 (77.33)	9.11 (82.66)	9.11 (82.66)	9.26 (85.33)	9.08 (82.00)
Mean	8.87 (78.22)	9.16 (83.55)	9.14 (83.11)	9.06 (81.77)	9.06 (81.66)

*Square root transformed value of the mean = $(\sqrt{0.5 + \text{mean value}})$

Values in parentheses are the mean values of original data.

Critical difference at 5%

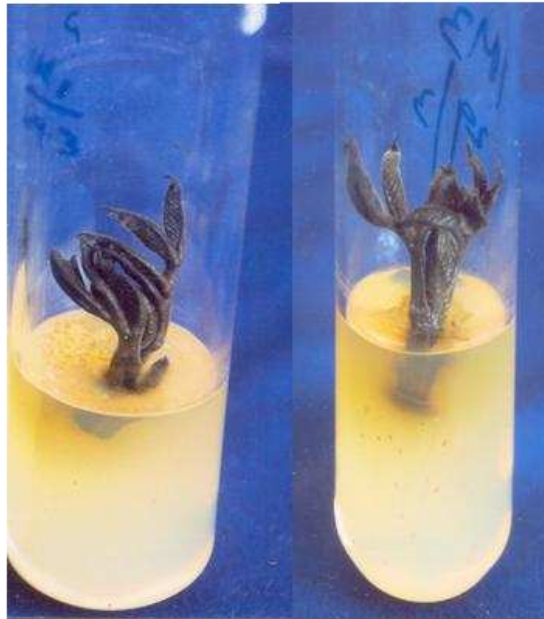
Accessions (AC)	0.138
Growth regulators (GR)	0.159
Interaction (AC x GR)	0.276

T₁ = BAP 0.3 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₂ = BAP 0.6 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₃ = BAP 0.9 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₄ = BAP 1.2 mg l⁻¹ + IBA 0.1 mg l⁻¹



a) Media browning



b) Explant browning

Plate-8 : Browning of media and explants during *in vitro* studies of walnut (*Juglans regia* L.) cultivars

explant tissue). The legend of the score (0,1,2,3 and 4) and mean values of the score of data recorded on the explant tissues in the present study are given in the Table-29. No significant influence of the accessions on the extent of explant tissue browning was observed in the *in vitro* studies. The mean score for browning of the explant tissues ranged from 3.110 to 3.264 (browning upto the middle portion of explant tissues from outer periphery). The influence of growth regulators revealed that T₁ (BAP 0.3mg l⁻¹ + IBA 0.1 mg l⁻¹) and T₂ (BAP 0.6 mg l⁻¹ + IBA 0.1 mg l⁻¹) had significant browning of explant tissue as compared to the other combinations of the growth regulators. The growth regulator combination T₂ (mean score of 3.432) had the maximum mean browning whereas, the combination T₁ (mean score of 2.833) had the minimum mean score browning. The other combinations T₃ and T₄ had almost similar influence (mean score value of 3.234 and 3.228, respectively).

Interaction among accessions and growth regulators revealed that all the accessions had significant influence on the media of browning explant tissues in the growth regulator combinations of T₁ (BAP 0.3 mg l⁻¹ + IBA 0.1 mg l⁻¹) and T₂ (BAP 0.6 mg l⁻¹ + IBA 0.1 mg l⁻¹) at each level of comparison, even the comparison within growth regulator combination of T₂ between SKUA-002 and SKUA-010 was significant. The influence within and between T₃ and T₄ growth regulator combinations were non significant at each level of accessions x growth regulator interaction.

Table-29: Effect of different growth regulator combinations on mean browning score per explant in walnut *in vitro* studies using MS medium

Walnut accessions	Growth regulators				Mean
	T ₁	T ₂	T ₃	T ₄	
SKUA-002	2.966*	3.653*	3.226	3.210	3.264
SKUA-008	2.833	3.433	3.210	3.210	3.171
SKUA-010	2.700	3.210	3.266	3.266	3.110
Mean	2.833	3.432	3.234	3.228	3.182

Critical difference at 5%

Accessions (AC) 0.171

Growth regulators (GR) 0.198

Interaction (AC x GR) 0.343

T₁ = BAP 0.3 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₂ = BAP 0.6 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₃ = BAP 0.9 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₄ = BAP 1.2 mg l⁻¹ + IBA 0.1 mg l⁻¹

* Values represent the mean score with the following legend

0 = No browning,

1 = Browning on excised wound tissue

2 = Partial browning on outer tissue of explant

3 = Complete browning of the outer tissue of explant

4 = Browning of the entire explant tissue

4.9.5 Survival of the shoot apices

Survival of the shoot apices in the *in vitro* studies (Table-30) revealed that the mean value ranged between 13.78 to 24.40 per cent. The values were transformed to square root and data analysed. The mean transformed values (4.30 to 4.45) corresponding to a mean survival of 18.05 to 19.35 per cent revealed non significant influence of the cultivars on the survival success. The influence of growth regulators revealed that the combination T_1 (BAP 0.3mg l^{-1} + IBA 0.1 mg l^{-1}) had significantly higher mean square root value (4.89) corresponding to survival per cent of 23.45 as compared to rest of the growth regulator combinations (T_2 , T_3 and T_4). The mean square root value of 4.05, 4.09 and 4.47 (corresponding to a survival per cent of 15.96, 16.28 and 19.49, respectively) were non significant among each other.

Interaction effects arising from accessions x growth regulators revealed that within individual level of a growth regulator combination the accessions did not influence significantly the survival of shoot apices except in the case of SKUA-010 as compared to both SKUA-002 and SKUA-008 at T_4 (BAP 1.2mg l^{-1} + IBA 0.1 mg l^{-1}). However, at an individual level of a accessions the growth regulator combinations revealed significant influence on the survival per cent at many levels of comparisons. In the accession SKUA-002 the survival of explant shoot

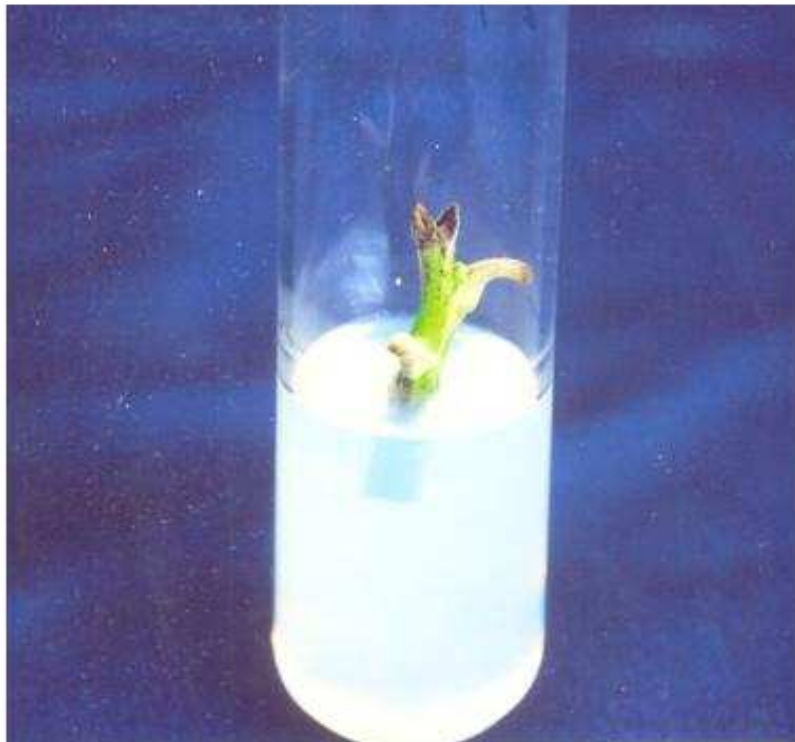


Plate-9 : Micro-propogules showing survival during *in vitro* studies of walnut cultivars

Table-30: Effect of different growth regulator combinations on *in vitro* survival of shoot apices in walnut using MS medium

Walnut accessions	Growth regulators				Mean
	T ₁	T ₂	T ₃	T ₄	
SKUA-002	4.83* (22.88)	3.79 (13.78)	4.25 (17.62)	4.72 (21.80)	4.41 (19.02)
SKUA-008	4.98 (24.40)	4.10 (16.32)	3.93 (14.95)	4.71 (21.74)	4.45 (19.35)
SKUA-010	4.86 (23.12)	4.27 (17.80)	4.10 (16.32)	3.93 (14.95)	4.38 (18.05)
Mean	4.89 (23.45)	4.05 (15.96)	4.09 (16.28)	4.47 (19.49)	4.39 (18.80)

*Square root transformed values of the mean = $(\sqrt{0.5 + \text{mean value}})$

Values in parentheses are the mean values of the original data

Critical difference at 5%

Accessions (AC)	0.377
Growth regulators (GR)	0.436
Interaction (AC x GR)	0.755

T₁ = BAP 0.3 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₂ = BAP 0.6 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₃ = BAP 0.9 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₄ = BAP 1.2 mg l⁻¹ + IBA 0.1 mg l⁻¹



Plate-10 : Surviving explants showing growth during *in vitro* studies of walnut (*Juglans regia* L.) cultivars

apices was significantly higher at T₁ (BAP 0.3mg l⁻¹ + IBA 0.1 mg l⁻¹) and T₄ (BAP 1.2 mg l⁻¹ + IBA 0.1 mg l⁻¹) as compared to T₂ (BAP 0.6 mg l⁻¹ + IBA 0.1 mg l⁻¹). In the case of SKUA-008 the survival of explant of shoot apices was significantly higher at T₁ (BAP 0.3mg l⁻¹ + IBA 0.1 mg l⁻¹) as compared to T₂ (BAP 0.6 mg l⁻¹ + IBA 0.1 mg l⁻¹) and T₃ (BAP 0.9mg l⁻¹ + IBA 0.1 mg l⁻¹) and also in terms of T₄ (BAP 1.2 mg l⁻¹ + IBA 0.1 mg l⁻¹) as compared to T₃ (BAP 0.9mg l⁻¹ + IBA 0.1 mg l⁻¹). . Similarly, the accessions SKUA 010 revealed a significantly higher survival of the explant shoot apices with the application of BAP 0.3mg l⁻¹ + IBA 0.1 mg l⁻¹ as compared to the growth regulators at BAP 0.9 mg l⁻¹ + IBA 0.1 mg l⁻¹ and BAP 1.2 mg l⁻¹ + IBA 0.1mg l⁻¹.

4.9.6 Per cent growing culture

Effect of different growth regulator combinations on the success of growing cultures in the *in vitro* studies involving different cultivars (Table-31) revealed that only 13.33 to 24.00 per cent cultures could grow further involving different combinations. The mean per cent values of the data were transformed to square root for statistical analysis. Accessions, as such, had no significant effect in the success rate of growing cultures. The mean transformed values of 4.11 to 4.22 corresponding to per cent growing cultures of 16.66 to 17.66 were non-significant. Among the growth

Table-31: Effect of different growth regulator combinations on per cent growing cultures in walnut *in vitro* studies using MS medium

Walnut accessions	Growth Regulators				Mean
	T ₁	T ₂	T ₃	T ₄	
SKUA-002	4.37 (18.66)	3.92 (14.92)	4.06 (16.00)	4.21 (17.33)	4.15 (16.73)
SKUA-008	4.94 (24.00)	4.37 (18.66)	3.88 (14.66)	3.71 (13.33)	4.26 (17.66)
SKUA-010	4.80 (22.66)	3.71 (13.33)	4.06 (16.00)	3.88 (14.66)	4.14 (16.66)
Mean	4.71 (21.77)	4.01 (15.63)	4.00 (15.55)	3.95 (15.11)	4.18 (17.01)

Square root transformed values of the mean = ($\sqrt{0.5 + \text{mean value}}$)

Values in parentheses are square root transformation of the original data

Critical difference at 5%

Accessions (AC)	0.287
Growth regulators (GR)	0.331
Interaction (AC x GR)	0.574

T₁ = BAP 0.3 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₂ = BAP 0.6 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₃ = BAP 0.9 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₄ = BAP 1.2 mg l⁻¹ + IBA 0.1 mg l⁻¹

regulator combinations the mean value of 4.70 (corresponding to per cent growing cultures of 21.77) in only T₁ (BAP 0.3 mg l⁻¹ + IBA 0.1 mg l⁻¹) was significantly higher than the other combinations of T₂, T₃ and T₄ that had a mean value of 3.99, 3.99 and 3.93 (corresponding to 15.55, 15.55 and 15.11%), respectively.

Among the interactions at each accessions level, the accessions SKUA- 008 had significantly higher success of growing cultures at T₁ (BAP 0.3mg l⁻¹ + IBA 0.1mg l⁻¹) as compared to other three growth regulator combinations. In this accessions even the growth regulator combination of BAP 0.6mg l⁻¹ + IBA 0.1mg l⁻¹ showed significantly higher success of growing cultures as compared to BAP 1.2mg l⁻¹ + IBA 0.1mg l⁻¹. In the case of SKUA 010 the growth regulator combination BAP 0.3mg l⁻¹ + IBA 0.1mg l⁻¹ had significantly higher success of growing cultures as compared to the other three growth regulator combinations. At individual level of a growth regulator combination with different cultivars, only one significant difference on the success of growing cultures was found between SKUA-008 and SKUA-010 at BAP 0.6mg l⁻¹ + IBA 0.1mg l⁻¹. Rest of the comparisons were non significant.

4.9.7 Growth rate of the explants

Growth rate of explants (Table-32) in the *in vitro* studies involving

three accessions viz, SKUA-002, SKUA-008 and SKUA-010 receiving different combinations of growth regulators was measured in terms of a score with a value of 1, 2 and 3 corresponding to low, medium and high growth rate, respectively. Perusal of the table revealed that explants of all the three accessions had nearly medium growth rate (1.77 to 1.97), the difference was however, non significant. Contrarily, the growth regulator combinations revealed low to medium effect on the growth rate of explants. The maximum growth rate of 2.49 was found in BAP 0.3mg l^{-1} + IBA 0.1mg l^{-1} followed by 2.19 in BAP 1.2mg l^{-1} + IBA 0.1mg l^{-1} as compared to 1.46 and 1.29 in the BAP 0.9 mg l^{-1} + IBA 0.1mg l^{-1} and BAP 0.6 mg l^{-1} + IBA 0.1 mg l^{-1} , respectively. This difference was significant in both T_1 (BAP 0.3mg l^{-1} + IBA 0.1mg l^{-1}) and T_4 (BAP 1.2mg l^{-1} + IBA 0.1mg l^{-1}) as compared to both T_2 (BAP 0.6mg l^{-1} + IBA 0.1mg l^{-1}) and T_3 (BAP 0.9mg l^{-1} + IBA 0.1mg l^{-1}). Rest of the comparison among the growth regulator combinations were non-significant.

The interaction effects arising from accessions x growth regulator combinations revealed that none of the accessions at a particular growth regulator combination had any significant influence on the growth rate of the explants. However, significant effects were found in some growth regulator combination at an individual level of a accession. In case of

Table-32: Effect of different growth regulator combinations on growth rate of explants in walnut *in vitro* studies using MS medium

Walnut accessions	Growth regulators				Mean
	T ₁	T ₂	T ₃	T ₄	
SKUA-002	2.51*	1.41	1.75	2.21	1.97
SKUA-008	2.56	1.16	1.36	2.00	1.77
SKUA-010	2.40	1.23	1.26	2.36	1.81
Mean	2.49	1.27	1.46	2.19	1.85

Critical difference at 5%

Accession (AC)	0.321
Growth regulators (GR)	0.370
Interaction (AC x GR)	0.642

T₁ = BAP 0.3 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₂ = BAP 0.6 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₃ = BAP 0.9 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₄ = BAP 1.2 mg l⁻¹ + IBA 0.1 mg l⁻¹

* Values represent the mean score with the following legend

- 1 = Low growth rate
- 2 = Medium growth rate
- 3 = High growth rate



Plate-11 :A cross-section of walnut (*Juglans regia* L.) media tubes showing explant survival and growth during *in vitro* studies of walnut cultivars

SKUA-002 significantly higher growth rate was observed at BAP 0.3mg l^{-1} + IBA 0.1mg l^{-1} as compared to T₂ (BAP 0.6mg l^{-1} + IBA 0.1mg l^{-1}) and T₃ (BAP 0.9mg l^{-1} + IBA 0.1mg l^{-1}). In this accession the difference was also significant at BAP 1.2mg l^{-1} + IBA 0.1mg l^{-1} as compared to T₂ (BAP 0.6mg l^{-1} + IBA 0.1mg l^{-1}). In case of both SKUA-008 and SKUA-010 significantly higher growth rate was observed at T₁ (BAP 0.3mg l^{-1} + IBA 0.1mg l^{-1}) as compared to T₂ (BAP 0.6mg l^{-1} + IBA 0.1mg l^{-1}) and T₃ (BAP 0.9mg l^{-1} + IBA 0.1mg l^{-1}). In case of SKUA-010 the growth rate was also significant with the application of BAP 1.2mg l^{-1} + IBA 0.1mg l^{-1} as compared to T₂ (BAP 0.6mg l^{-1} + IBA 0.1mg l^{-1}) and T₃ (BAP 0.9mg l^{-1} + IBA 0.1mg l^{-1}), however, in case of SKUA-008 the growth rate at BAP 1.2mg l^{-1} + IBA 0.1mg l^{-1} was significant only as compared to T₂ (BAP 0.6mg l^{-1} + IBA 0.1mg l^{-1}).

4.9.8 Effect on callusing of explants

Mean callusing of the explants from different walnut accessions after the application of different growth regulator combination (Table 33) revealed that in the explants, that survived, the success varied between zero to 63.88 per cent. Data recorded in per cent was transformed to arc sin^{-1} values. Accessions revealed a non significant difference in influencing the callusing of explant tissues after application of the growth regulators. The

Table-33: Effect of different growth regulator combinations on *in vitro* callusing of walnut (*J. regia* L.)

Walnut accessions	Growth regulators				Mean
	T ₁	T ₂	T ₃	T ₄	
SKUA-002	4.055* (0.000)	20.201 (15.000)	36.154 (35.000)	51.923 (61.666)	28.085 (27.910)
SKUA-008	4.055 (0.000)	12.703 (8.333)	34.239 (32.060)	49.998 (58.333)	25.249 (24.680)
SKUA-010	4.055 (0.000)	20.207 (15.000)	36.585 (35.333)	53.244 (63.880)	28.523 (28.55)
Mean	4.055 (0.000)	17.706 (12.770)	35.660 (34.130)	57.722 (61.290)	27.285 (27.060)

Values in parentheses are the arc sin transformation values of the original data

Critical difference at 5%

Accessions (AC)	7.662
Growth regulators (GR)	8.847
Interaction (AC x GR)	15.324

T₁ = BAP 0.3 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₂ = BAP 0.6 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₃ = BAP 0.9 mg l⁻¹ + IBA 0.1 mg l⁻¹

T₄ = BAP 1.2 mg l⁻¹ + IBA 0.1 mg l⁻¹

values for SKUA-002, SKUA-008 and SKUA-010 were 28.08, 25.25 and 28.52 corresponding 27.91, 24.68 and 28.55 per cent callusing, respectively.

Among the growth regulator combinations there was significant increase in the callusing of the explant tissues as the concentration of BAP increased at each individual level of this concentration. The transformed mean values of 4.055, 17.706, 35.660 and 57.722 corresponding to per cent callusing of 0.00, 12.77, 34.13 and 61.90 in respect of T_1 (BAP 0.3 mg l^{-1} + IBA 0.1 mg l^{-1}), T_2 (BAP 0.6 mg l^{-1} + IBA 0.1 mg l^{-1}), T_3 (BAP 0.9 mg l^{-1} + IBA 0.1 mg l^{-1}) and T_4 (BAP 1.2 mg l^{-1} + IBA 0.1 mg l^{-1}) were all significantly different from each other.

The interaction effect on the callusing due to accessions x growth regulators revealed that none of the accessions had significant difference at individual growth regulator combination. However, in each accessions significantly higher callusing was recorded as the concentration of BAP increased from 0.3 mg l^{-1} to 1.2 mg l^{-1} together with a constant application 0.1 mg l^{-1} IBA except in the case of SKUA-008 between T_1 (BAP 0.3 mg l^{-1} + IBA 0.1 mg l^{-1}) and T_2 (BAP 0.6 mg l^{-1} + IBA 0.1 mg l^{-1}).



Plate-12 : An overview of tissue culture laboratory during the course of present investigation

CHAPTER – 5

DISCUSSION

5.1 General

The Northwestern Himalayan region is one of the richest and most diverse gene pool of the cultivated walnuts. China, Iran and India are extremely rich sub centers of walnut germplasm diversity (Bhat *et al.*, 1992).

The success of any breeding programme depends on the presence of sufficient genetic variability to pursue effective selection. It is necessary to assess the relative magnitude of the existing genetic diversity in order to use such information together with other selection parameters for the improvement of fruit yield and other quality parameters of any fruit crop through adoption of an effective breeding approach (Williams, 1964). The extent of genetic variability indicates the potential of exercising selection of a particular genotype, whereas, heritability (h^2) along with genetic advance (per cent of mean) are more useful in predicting the resultant effect of selection of the best genotypes. Knowledge of the extent of genetic variation and diversity for fruit phenology, quality, maturity and yield component traits in walnut populations (seedling origin) and subsequent

identification of adapted superior genotypes/cultivars as potential donors for yield, quality improvement, nut weight, kernel weight, kernel colour, kernel recovery percentage, extension of harvest period, etc. is therefore, essential. Potent variability is the result of prolonged natural and artificial selection, which is heritable and accumulation of the significant magnitude of variability for economic traits leads to the genetic diversity, which is important for creation of new genetic variability through hybridization and reorganization of new gene constellation.

In the valley of Kashmir the walnut cultivation is concentrated in some important pockets. Though, of course, individual seedling raised plants are scattered throughout the length and breadth of the valley in sub-tropical (Uri, Ramban, Karnah etc.) to temperate conditions (valley basin), growing successfully from 1000 to 2000 m a.m.s.l. Being highly cross-pollinated because of unisexual flowers, each seedling raised plant is therefore, a distinct genotype due to their highly heterozygous nature of genotype. The magnitude of cross pollination together with diversity at allelic level for most of the genes results in formation of new gene groups and constellations in the resultant seed. Thus, tremendous genetic variability is created which on the outer play of environmental conditions

produce some excellent genotypes (possessing many desirable traits in a single plant).

Keeping in view the importance of biodiversity of walnut genotypes, it is important to survey these populations and identify superior genotypes for their use in the future breeding programme through *in-situ* conservation and subsequent use in the hybridization.

The study was accordingly taken up to characterize and catalogue the local gene pool and identify some promising genotypes for future improvement of this fruit crop species. In the present study 150 seedling genotypes growing in and adapted to different areas of the Kashmir valley were evaluated for the Phonological, quantitative and qualitative traits and the results obtained are discussed in this chapter.

Mother plants of two checks viz. (Hamdan and Suleiman) growing *in-situ* in their original locality and identified in the previous studies were used for comparison.

The study of each tagged plant was carried over two years and the observation data pooled for interpretation of the results.

5.2 Maturity and harvesting

The pooled data on harvesting time of the genotypes revealed that the harvesting time was spread over from 29th August to 17th September covering about 3 weeks of time. Maturity and harvesting in walnuts is more governed by the topographical conditions, altitude, climatic conditions, weather, etc. and minor role is played by the genetic background in most of the genotypes. This variation is observed in the same genotype over the years. Kester and Asay (1975) are of the opinion that maturity, though characteristic of a particular genotype, is affected greatly by seasonal variations, climatic changes and other weather factors.

The harvesting of mature nuts of walnut genotype was within the range of 20 days i.e. w.e.f. 29th August to 17th September. Mature nuts were first harvested from district Budgam followed by district Baramulla, Kupwara and lastly in Pulwama and Shopian. Labvithicu and Polito (1985) suggested that 19 to 20 weeks after full bloom of flowers was approximate time for walnut harvesting. In the present investigation also harvesting of all the genotypes was completed within 19-22 weeks after full bloom of pistillate flowers. Sharma and Chauhan (1980) evaluated 14 seedling raised plants which came to maturity of nuts from 2nd week of August and continued to last week of September. The variation in harvesting dates

could be because of inherent maturity background of the materials or because of the locations/climatic conditions. The findings were in agreement with most of the findings reported earlier.

5.3 Morphological Characters

Morphological characters were recorded as per the Standard Descriptor of Walnut recommended by IBPGR.

5.3.1 Tree growth habit

The genotypes in the study exhibited distinct and varied tree growth habits, tree vigour, leaf shape and nut bearing thereby, exhibiting variability for these characters. In the present study 43 genotypes (28.30%) had low tree vigour, 45 genotypes (29.60%) intermediate and 63 genotypes (42.10%) had high tree vigour. In the present study 54 genotypes (35.52%) were erect, another 54 genotypes (35.52%) semi-erect and the remaining 44 genotypes (28.96%) were spreading. This variation observed in the tree vigour and growth habit could be due to age, soil fertility and environmental conditions.

Cultivars of walnut studied by other workers were found to vary tremendously in size and growth habit from erect, upright to spreading and tree vigour ranged from low to high. Similar observations were made by Godneau and Botumihai (1997) when they evaluated 16 walnut genotypes

from Oltenia (Romania) on the basis of their agro-productive characteristics. Among these genotypes two genotypes were found to be vigorous, six genotypes had intermediate vigour and eight genotypes were found to produce high vigour trees. Solar and Stamper (2006) reported that ‘Ramsau’ variety had most vigorous growth habit with dense branching habit and majority of genotypes studied over a period of 3 years exhibited semi-erect to semi-spreading growth habit. The exceptions were “Z-62” with markedly erect habit and “C-6/7” with spreading branches..

5.3.2 Branching habit

Diversity was observed for branching habit in the seedling raised natural population in the present study. Thirty genotypes (19.73%) had sparse, 57 genotypes (37.50%) had intermediate and 65 genotypes (42.77%) had dense branching habit. Ferhatoglu (1989) reported great genetic diversity in the walnuts of Turkey where some trees were upright spreading with normal branching habit and others had upright spreading trees with dense branching.

5.3.3 Leaf characters

5.3.3.1 Leaflet shape and margin

The leaflet shape in the population studied varied from broad to narrow elliptic. Sixty seven genotype (44.09%) had broad elliptic; 46

genotypes (30.26%) had narrow elliptic and 39 genotypes (25.65%) had elliptic leaflet shape. The leaf margin was entire in all the genotypes studied with no serration.

Gilman and Weston (2006) observed that most of the walnut genotypes studied had ovate to obovate leaf shape. Similar findings were reported for the Turkish walnuts where majority of the genotypes exhibited ovate leaf shape with cordate leaf base (Anonymous, 2007).

Yoshida and Yamaishi (1988) reported the apricot leaves vary from elongated to rounded and round oblate, leaves are serrate, ovate or elliptic, very often cordate and have long reddish petioles with 1-5 glands (Mehdizadeh, 1990). The hazelnut (*Corylus* spp.) are perennial deciduous shrubs, rarely trees, generally ovate and serrate and more or less pubescent and cylindrical pendulous.

5.3.3.2 Leaf colour

In the population studied the score for the leaf colour varied from 3 to 7 (light to dark green). Majority of genotypes (75.0%) had green leaf colour followed by 35 genotypes (23.03%) having light green colour and 3 genotypes (1.97%) had dark green colour.

5.3.4 Shoot colour

Variation in the shoot colour of the walnut plants raised from heterozygous nuts has been reported by Szentivary (1990). Shoot colour variation in the present population studied varied from green, brown to black with a frequency of 8.56 per cent (13 genotypes), 75.66 per cent (115 genotypes) and 15.78 per cent (24 genotypes), respectively.

5.4 Nut characters

Among the nut characters the descriptor was used for recording the variation for nut shape, nut colour, nut texture, shell seal, shell strength and shell integrity.

5.4.1 Nut shape

Shape of the nuts in natural population studied varied from round to cordate. Round nut shape was more frequent (36.85%) followed by ovate (23.69%), broad elliptic (10.52%), elliptic (8.55%), broad ovate (6.58%), triangular (5.27%), short trapezoid (4.60%) and long trapezoid and cordate (1.97% each)

5.4.2 Nut colour

Majority of genotypes had light coloured nut shell (61.18%) followed by medium shell colour (27.63%) and very light colour (9.22%).

The undesirable dark shell colour was found in only 1.97 per cent of the population studied.

5.4.3 Nut texture

While 5.27 per cent of the population studied had the very desirable shell texture of very smooth nature, the majority of the population exhibited either smooth (49.34%) or medium (34.21) shell texture. Rough shell texture was found in only 11.18 per cent and very smooth in 5.27 per cent.

5.4.4 Shell seal

The nature of the shell seal viz. weak or strong was having almost equal distribution in the population studied (nearly 30.0 per cent). However, intermediate type of shell seal was more frequent (nearly 40.0 per cent).

5.4.5 Shell Strength

As per the International Standard Descriptor of walnut the score for shell strength in the population studied revealed only 4 types viz. papery, weak, intermediate or strong. Majority of the walnut genotypes (50.0%) exhibited intermediate shell strength followed by weak, shell strength (26.98%), strong (16.44%) and papery (6.58%).

5.4.6 Shell integrity

Shell integrity was scored as incomplete, intermediate or complete. Majority of the genotypes (62.50%) exhibited intermediate shell integrity followed by complete type (33.55%). Incomplete shell integrity was recorded in only 3.95 per cent population.

In walnut the consumer preferences for whole nut is governed largely by different parameters of nut viz. shape, colour, texture and shell strength and accordingly the price structure increases if more of these desirable traits are found in a single genotype. Shell seal is influenced by the temperature variation near maturity stage.

Nuts are formed only in pistillate (female) flowers which usually arise at the end of a shoot from a dormant bud of the previous seasons growth. Contrarily, the staminate (male) unisexual flowers called catkin arise from most of the buds on the branches (Szentivary, 1990). Bhat *et al.* (2002) reported that round shape of the nuts was most common in the seedling raised walnut populations of Kashmir valley.

5.5 Kernel characters

Kernel characters were studied with respect to kernel shrivel, kernel fill, ease of kernel removal and kernel colour.

5.5.1 Kernel shrivel and fill

The extent of the kernel shrivel was scored as tip shrivel, medium shrivel (<50.0%) and complete shrivel ($\geq$ 50.0%). In the present population the extent of kernel shrivel was intermediate (57.33 %) followed by tip shrivel (31.57%) and complete shrivel (11.18%).

Similarly, the kernel fill was scored as poor, moderate and well. The frequency of poor and well fill kernels in the walnuts of the genotypes of present study was almost equal (nearly 17.0%). Nuts with moderate kernel fill represented nearly 65.0 per cent population.

5.5.2 Ease of kernel removal from nuts

Kernel removal from nuts was scored as very easy, easy, moderate and difficult. The proportion of these groups in the present population was to the extent of 17.11, 53.29, 25.66 and 3.94 per cent, respectively.

5.5.3 Kernel colour

Colour of the kernels was scored as extra light, light, light amber and amber. Majority of the population studied (65.79%) revealed light kernel colour followed by extra light colour (15.79%), light amber (11.85%) and amber (6.57%).

Kernel colour, wholeness of the kernels and bold kernels are most

important feature to determine the export competition and market price both at the national and international level. Most preferred kernel colour is light to extra light.

In the present study a wide range of variation was observed in nut and kernel characters of the genotypes. Variation for these characters have also been recorded by various workers in different walnut cultivars and seedlings trees (Pandey and Sinha, 1984; Thakur, 1993; Sharma and Sharma, 1998). The tendency of genotypes to occur in clusters, cutting across geographical boundaries, demonstrates that geographical isolation is not the only factor causing genetic diversity. Similar observation have also been made by Garg and Gautam (1988) and Walia and Garg (1996).

The seedling populations in all the walnut growing states in India have been surveyed and superior genotypes have been identified. Presently the entire walnut gene pool in the country represents trees of seedling origin. These seedling raised trees are highly heterozygous in the genetic makeup. Because of cross pollination and subsequent release of residual genetic variability hidden in the polygenic blocks during chromatid recombination (crossing over), there has been creation of tremendous genetic diversity especially for nut and kernel characteristics. Substantial genetic variability has been reported in the natural seedling raised walnut

population (Chauhan and Sharma, 1979, Qureshi and Dalal, 1985).

Ke (1990) evaluated 36 promising clones of walnuts. He observed that some clones were having good sized nuts with soft smooth shell texture. Shell colour being important and good for consumer acceptability was recorded in the walnut cultivars by Radicati *et al.* (1990) and the best cultivars were MB24, Elit, G-139 and “Mayettee”. Sofi *et al.* (1992) observed that shell seal ranged from papery to very strong in some indigenous types and exotic cultivars of almond. They were of the opinion that shell seal was influenced by high temperature at the time of maturity. Bhat *et al.* (1999) reported that shell strength constituted an important component of nut character as it determines the crack out quality of walnut and its market price. Considering the consumer acceptability the inshell walnut, weak shell seal and weak or papery shell strength have good market potential (Agabbie *et al.*, 1984).

Pandey and Sinha (1984) surveyed Jounser Bhabhar area of U.P hills and identified fifteen promising walnut seedlings. ‘Chakrata number 14’ was the best due to good filling and shell integrity.

Longoriet *et al.* (2001) assessed the existing diversity within the Moroccan walnut germplasm and identified some superior genotypes. They

observed that smaller fruits had more shrivelled kernels and lower fruiting potential.

Rathore (1983b) evaluated twenty two genotypes of walnut and reported a wide genetic diversity and made ten promising selections on the basis of their various morphological and pomological traits.

Bhat *et al.* (2002) reported that dominant kernel colour of walnut was light. Light kernel colour was observed in the cultivars MB-24, Elit, G-139 and Moyettee by Radicati *et al.* (1990). The kernel colour solely determined the consumer acceptability (Bhat *et al.*, 1999; Sofi *et al.*, 1992). So extra light to light kernel colour is considered to be the best for consumer acceptability in walnuts.

5.6 Estimation of the genetic variability and other components of genetic variation

Genetic variability (genotype and phenotype), coefficients of variation (genotypic and phenotypic), heritability (broad sense) and expected genetic gain (per cent of the mean) were estimated from the quantitative observational data recorded for different morphological yield and yield components and quality parameters in the seedling raised *in situ* natural population. Samples of walnuts growing in different agro-ecological

pockets of the Kashmir valley over a period of two years. The data analysed and findings estimated are discussed herein.

5.6.1 Morphological traits

5.6.1.1 Stem girth and canopy spread

The mean stem girth of the population studied was 1.37 ± 0.58 m with a range of 0.3-3.6 m. The magnitude of phenotypic and genotypic variance was 0.687 and 0.452, corresponding to phenotypic and genotypic coefficients of variation as 60.40 and 49.07 per cent, respectively. Broad sense heritability was 65.79 per cent. Similarly, the canopy spread had a population mean of 6.25 ± 1.37 m with a range of 1.20 to 12.50 m. Estimation of the coefficients of variation from phenotypic (5.656) and genotype (2.852) variance were 37.98 and 26.97 per cent respectively. Heritability in broad sense was 50.42 per cent. Expected genetic gain (per cent of mean) was 82.18 and 39.47 for stem girth and canopy spread, respectively.

Ozkan and Cellepe (2001) reported variation in the growth of trees of similar age groups over years due to environmental and location specific effects. In the population studied a range of 3.60-8.80 m was recorded in the canopy spread. The stem trunk girth also varied from 28-90cm.

5.6.1.2 Leaf length and breadth

Mean leaf length and breadth was 12.156 ± 1.909 cm and 6.490 ± 1.466 cm in the population studied with a range of 5.25-24.50 cm and 2.55-13.50 cm, respectively. Phenotypic variance was 19.502 and 8.433; and genotypic variance 12.163 and 4.102 in respect of leaf length and width, respectively. Accordingly the coefficients of phenotypic and genotypic variation for leaf length was 36.32 and 28.69 per cent, and for leaf width 44.74 and 31.20 per cent, respectively. The heritability (broad sense) for the two traits was 62.30 and 48.06 per cent, respectively. Expected genetic gain (per cent of the mean) for the two traits was 46.67 and 44.84 respectively.

5.6.2 Yield and yield components

5.6.2.1 Nut length, weight and size

Nut length, weight and diameter (both at suture and cheek) are important features to increase or decrease market price. In the population studied the nut length varied from 30.0-60.0 mm with a population mean of 55.62 ± 3.28 mm. The phenotypic and genotypic variance was 605.14 and 427.56, corresponding to their coefficients of variation to the extent of 44.25 and 37.17 per cent, respectively. Heritability (broad sense) and expected gain (per cent of the mean) was 70.65 per cent and 64.36, respectively.

Nut weight revealed a population mean of 14.48 ± 2.54 g with a range of 8.50-30.0 g. The phenotypic (18.785) and genotypic (12.760) variance when translated into their respective coefficients of variation gave the estimate of 27.98 and 23.02 per cent, respectively. Broad sense heritability was 67.9 per cent and expected genetic gain (per cent of the mean) as 39.15.

The size of the nut was measured in terms of its diameter at the suture line and cheek. The mean values for different variability parameters for these two traits were nearly similar. Population mean was 33.73 ± 2.82 and 34.63 ± 2.92 mm, respectively. Range of variability as 24.5- 50.5 and 25.5-50.0 mm, respectively. Phenotypic variance as 18.544 and 19.457 and genotypic variance as 10.421 and 12.338, respectively. Phenotypic coefficient of variation as 12.76 per cent in both the traits, while genotypic coefficient of variation was 9.53 and 10.13 per cent, respectively. Heritability (broad sense) was 56.2 and 63.1 per cent, respectively. The expected genetic gain (per cent of the mean) was 14.77 and 16.59, respectively.

Nut size especially diameter at suture line is an important attribute for commercial grading standard and should not be less than 3.11 mm (Serr

and Forde, 1956). Four selections P-3, P-123, V-I and Wosan-3, had a nut diameter ranging between 3.41 and 3.70 cm (Bhat *et al.*, 1992).

Beyham and Permir (2006) evaluated 13 promising walnut genotypes. In the selected genotypes the nut length ranged from 34.48-60.42 mm, nut width from 30.38-45.24 mm, nut diameter at the cheek from 32.54-47.96 mm, nut size at the suture from 33.97-50.80 mm and nut weight from 11.84-24.19 g.

Akca and Sen (2001) reported that the genetic variability in nut weight of the seedling raised population of walnut varied from 9.36-18.60g. Sharma and Sharma (2001) observed that for nut characters the magnitude of variation was 6.40-20.55 g (nut weight), 27.05-47.15mm (nut length), 0.59-1.09 (index of nut roundness) and 2.30-6.95mm (pad width). Guzken and Weiching (1990) reported that nut weight could serve as a good parameter for selection in walnuts. Lal *et al.* (1993) recorded a nut weight of 10.97 to 11.55g.

5.6.2.2 Kernel weight and per cent recovery

Weight of kernels nut^{-1} in the population studied revealed a population mean of 7.75 ± 1.54 g with the magnitude of variability from 3.50-15.0 g. The phenotypic and genotypic variance was 6.149 and 3.329 resulting in the corresponding coefficients of variation of 31.99 and 14.87

per cent, respectively. Heritability (broad sense) was 54.14 per cent and expected genetic gain (per cent of mean) as 35.68.

Similarly, the kernel recovery percentage revealed a population mean of 50.04 ± 5.10 with a range of 31.03-70.00 per cent. The phenotypic (54.280) and genotypic (31.917) variances gave coefficient of variation of 41.72 and 11.29 per cent, respectively. Broad sense heritability was 58.88 per cent and the expected genetic gain (per cent of the mean) as 17.85.

Qureshi and Dalal (1985) found shelling percentage as an index of kernel percentage recovery. Nuts with higher shelling percentage assume importance as these can yield higher kernel tonnage per unit area. Lal *et al.* (1993) recorded kernel weight of 4.71-4.82 g per nut with a kernel recovery of 43.93 to 46.58 per cent.

Sharma *et al.* (2006) studied the variability in nut characters of 134 walnut trees growing in the area of district Sirmour and Bharmar area of district Chamba in H.P. Among the 47 seedling trees that were selected for the study, the kernel weight varied between 1.02 to 6.91 g nut⁻¹ and kernel percentage recovery from 20.74-53.51.

5.6.2.3 Nut yield (kg tree⁻¹)

In order to reduce the variation in the nut yield the entire population of the seedling raised walnut trees in the present study were put into

different age groups viz. 15-30, 31-50, 51-70 and above 70 years age on the basis of information from the owner and visual estimate (tree girth and canopy).

Mean dried nut yield of 20.92 ± 1.95 ; 31.35 ± 2.35 ; 42.02 ± 2.67 and 63.24 ± 3.10 kg tree⁻¹ was recorded for 15-30, 31-50, 51-70 and above 70 year age groups, respectively. The magnitude of variability recorded was 10.2- 55.5; 19.5-68.0, 30.3-165.5 and 37.5-185.0 kg tree⁻¹, respectively.

Phenotypic variance estimated was 133.66, 146.11, 171.98 and 762.05, while the genotypic variance stood at 65.83, 74.87, 77.08 and 396.52, respectively. Phenotypic coefficient of variation was 55.26, 38.56, 31.21 and 43.65 per cent, whereas, genotypic coefficient of variation was 38.78, 27.60, 20.89 and 31.48 per cent, respectively.

Heritability (broad sense) estimates were 49.25, 51.24, 51.81 and 52.03 per cent for different age groups, respectively. Similarly, the expected genetic gain (per cent of mean) was 56.07, 40.69, 45.70 and 46.78 for age groups 15-30, 31-50, 51-70 and above 70 years, respectively.

Lal *et al.* (1993) evaluated different cultivars of walnuts and found highest yield of 654 kg tree⁻¹. Ozkan and Cellepe (2001) evaluated some walnut varieties for nut yield over several years. The average yield per year ranged from 40.8 to 129.0 kg tree⁻¹. A positive correlation was found

between the yield and vegetative growth. Similar findings regarding the positive correlation between nut yield and age, canopy, vigour etc have been reported (Olez, 1971; Germain, 1990; Ozkan, 1993; Cellepe, 1996). Kaushal and Sharma (2004) found similar type of variation in Pecan nut yield tree⁻¹ due to age, canopy and vigour of the tree and this yield ranged from 0.20 to 22 kg tree⁻¹. Sharma (1991) reported that filberts vary in yield greatly according to the age and vigour of the tree, planting distance, soil cultural practices and many other factors. However, Wertheim (1997) reported that the walnut yield in mature orchards fluctuates from year to year even from the same tree.

5.4.3 Qualitative traits

In the qualitative traits the protein and oil content of the kernels was estimated for each genotype in both the years and data was pooled.

Protein and oil content of the kernels had a population mean 17.967 ± 1.075 and 62.102 ± 3.989 with the magnitude of variability ranging from 14.145 -24.25 and 50.22-70.00 per cent, respectively.

Phenotypic variance was estimated as 4.315 and 29.027 whileas, the genotypic variance was 1.985 and 8.456 for protein and oil content, respectively. Similarly, the phenotypic coefficient of variation was 11.56

and 8.67, whereas the genotypic coefficient of variation was 7.84 and 4.70 per cent respectively.

Heritability (broad sense) was 46.02 and 47.43 per cent, whileas the genetic gain (per cent of the mean) was estimated as 10.96 and 5.26 for the two qualitative traits, respectively.

Average kernel protein content of 13.2 per cent was recorded by Furuchi *et al.* (1981) and about 15.0 per cent by Easayan and Bessegean (1984) in the walnuts. However, Bugaria *et al.* (1985) reported a range of 10.0-19.5 per cent.

Bhat and Mir (1992) reported a range of 51.5 to 59.0 per cent oil content in the kernels of some exotic walnut genotypes. Similarly, Bugaria and Orgus (1985) reported a range of 61.0 to 72.5 per cent kernel oil content in 48 specimens of walnuts.

5.7 *In vitro* system

5.7.1 Browning in relation to *in vitro* culture

In the present study the magnitude of media browning was recorded between 80.0 to 85.3 per cent in the culture tubes. Accessions played no significant role in increasing or decreasing the extent of the media browning. Application of growth regulators at the concentration of BAP

0.3mg l^{-1} + IBA 0.1mg l^{-1} significantly reduced the media browning extent as compared to BAP 0.6mg l^{-1} + IBA 0.1mg l^{-1} . Other comparisons were non significant. None of the interaction effects arising between any individual cultivar and growth regulator combination was significant.

Presence of phenolic compounds has been reported in the vacuoles of many tissues (Grab and Mantile, 1979) and these have been recorded in tissue culture systems of more than 70 species (Guren *et al.*, 1987). Several reports dealing with tissue culture of perennial woody species have revealed biosynthesis of tanins in the coniferous species (Chafe and Durzan, 1973).

5.7.2 Volume of browned media

Significantly lower volume of the browned media was recorded in the accessions SKUA-008 and SKUA-010 as compared to SKUA-002. Similarly, significantly lower volume of browned media was observed in the treatment BAP 0.9 mg l^{-1} + 0.1 mg l^{-1} as compared to BAP 0.3 mg l^{-1} + 0.1 mg l^{-1} and BAP 0.6 mg l^{-1} + 0.1 mg l^{-1} . Other comparisons of interaction were non significant.

The use of antioxidants is one method that has been used to control media browning. The soaking of explants in water as pre-treatment reduced the browning in walnuts (Rodriguez, 1982) and chestnut (Chevere *et al.*, 1983).

Modification of media composition by reducing salts has been an effective means of reducing media browning. Anderson (1975) observed that browning problems of *Rhododendron* could be overcome by reducing by half, the concentration of nitrogen containing compounds in the Murashige and Skoog's formula.

5.7.3 Explant browning

Higher proportion of explant browning was observed in the *in-vitro* studies in all the three accessions and the effect of growth regulators also did not have sizeable impact. The explant browning in all the treatment combinations ranged from 77.33 to 85.33 per cent. Walnut accessions had almost similar and non significant impact on explant browning. However, application of BAP 0.3 mg l^{-1} + IBA 0.1 mg l^{-1} significantly reduced the explant browning as compared to higher concentration of BAP ($0.6, 0.9$ and 1.2 mg l^{-1}) + IBA 0.1 mg l^{-1} .

Application of BAP 0.3 mg l^{-1} + IBA 0.1 mg l^{-1} revealed significantly lower explant browning in the cultivars SKUA-002 and SKUA-008 as compared to most of other interaction (cultivars x growth regulator) effects.

Ichihashi and Kako (1977) reported that the browning of cattleya shoot tips was most effectively controlled by the addition of antioxidants (potassium cyanide, ascorbic acid, cysteine, indolebutyric acid) into

stationary liquid medium. Monaco *et al.* (1977) suggested that coffee explants should be pretreated with an antioxidant (e.g. cysteine, HCl, ascorbic acid) to reduce browning.

Tissues are injured during explant excision from stock plants. This often causes the release of various compounds that are oxidised (Robinson, 1983) by peroxidases (Loomis and Battaile, 1966; Vaugh and Duke, 1984) or polyphenol oxidases (Hu and Wang, 1983; Mayer and Harel, 1979) and turn brown or black resulting in darkening of both tissue and culture medium. Polyphenol oxidases, including catechol oxidases, are confined to particles in healthy and non-senescing tissues (Vaugh and Duke, 1984) when explant is injured in course of explant preparation. Phenolic compounds that are largely localized in vacuole are mixed with the contents of plastids or other organelles resulting in formation of dark or brown pigmentation associated with oxidised polyphenolic compounds (Compton and Preece, 1986). The oxidative browning caused by the oxidation products of phenols inhibit enzyme activity (Hu and Wang, 1983) and may result in the decline or lethal darkening of the explants. The build up of toxic brown or black substance on wounded surface of cultured tissues was listed as one of the basic problems of micropropagation by Pierik (1988).

5.7.4 Mean browning score explant⁻¹

The extent of explant browning was scored for efficacy (no browning to complete browning of tissues). Accessions had no inherent mechanism to increase or decrease this extent and accordingly revealed a non significant effect. Application of BAP 0.3 mg l⁻¹ + IBA 0.1 mg l⁻¹ and BAP 0.6 mg l⁻¹ + IBA 0.1 mg l⁻¹ had significantly lower and higher extent of explant browning, respectively as compared to all other growth regulator combinations. Accessions and growth regulators showed significant interaction as far as BAP concentration of 0.3 and 0.6 mg l⁻¹ was involved. Both the accessions (SKUA 002 and SKUA 010) exhibited significant difference in both the growth regulator concentrations of BAP.

Presence of certain hormonal substance in the nutrient media might bring about browning in cultured tissues, either directly or indirectly (Zaid, 1987). Treutter *et al.* (1985) reported that addition of benzylaminopurine (BAP) to the culture media of *prunus* tissues resulted in an increased synthesis of eriodictol-7-glycosides and particularly prunin. While working with walnut cotyledons, Rodriguez (1982) attributed the failure of organogenesis in the callus induced by 2,4-D and kinetin for production of phenolic compounds.

5.7.5 Survival of shoots apices

A lower survival per cent of the explants was observed in all the treatment combinations (13.33-24.00). Accessions exhibited non significant impact on the survival rate of explants. Significantly higher survival was observed in the explants receiving BAP concentration of 0.3 and 1.2mg l^{-1} as compared to other concentrations of 0.6 and 0.9 mg l^{-1} along with IBA 0.1 mg l^{-1} . Many growth regulator x accessions interaction effects were significant.

Long shoot tips for mass propagation were considered as ideal material. Morel (1980) was the first to publish the procedure of using excised stem tips to render orchids free of virus. Since then shoot tip and lateral bud cultures have been tried by several workers in pistacio (Barghchi and Anderson, 1985), almond (Mehra and Mehra, 1974). Shoot tip explants as small as 0.1 to 1.0 mm apices were used (Chee and Pool, 1982). Walkey (1972) reported that establishment of apple shoot tips was facilitated by a few days culture in flask of liquid medium on their sides and rotating in vertical plane. Shoot tip culture of actively growing seedling was tried in *J. regia* by Somers *et al.* (1982). They observed that the presence of BAP 0.1 mg l^{-1} and IBA 0.1 mg l^{-1} concentration stimulated out growth of axillary shoot bud development.

5.7.6 Per cent growing cultures

The explants showing survival were further examined for the growth. Only 13.33 to 24.00 per cent explants exhibited growth of apices. Accessions had no significant impact on per cent growing cultivars. Significantly higher percentage of growing cultures was recorded with the application of BAP 0.3 mg l^{-1} + IBA 0.1 mg l^{-1} as compared to other concentration ($0.6, 0.9$ and 1.2 mg l^{-1} of BAP + IBA 0.1 mg l^{-1}). Interaction effects arising from accessions x growth regulator combinations were significant in some treatments. Driver and Kuniyuki (1984), using shoot segment of paradox actively growing seedling, reported an excellent (five shoot per media) after 56 days of culture with passage of every 7 days.

Explant is cultured in establishment medium containing different growth regulators. Constitution of media varies from species to species. Apple meristem tips were established on a medium including Lapoure macronutrients (Quirin *et al.*, 1977), MS micronutrients (Murashige and Skoog, 1962).

5.7.7 Growth rate

All the three accessions exhibited medium growth rate of explant tissues and no significant difference was observed among the accessions. Application of growth regulators revealed low to medium impact on growth

rate. Significantly higher growth rate was observed with the application of BAP 0.3 mg l^{-1} and 1.2 mg l^{-1} as compared to BAP concentration of 0.6 and 0.9 mg l^{-1} along with IBA 0.1 mg l^{-1} in all the concentrations of BAP. Several interaction effects arising from cultivars and growth regulators were significant.

Rodriguez and Fernandes (1982) reported that elongation was successfully achieved by the application of both IBA and BAP at a lower concentration of 0.1 mg l^{-1} each and for maintaining a desirable growth rate.

5.7.8 Callusing of explants

Formation of callus in the explants showing growth ranged between zero to 63.88 per cent. Accessions as such had no impact on the callusing of explants. Increase in the concentration of BAP (from 0.3 to 1.2 mg l^{-1}) along with IBA (0.1 mg l^{-1}) significantly increased the callusing behaviour of the growing explants. The increase was from zero at 0.3 mg l^{-1} BAP concentration to 12.77, 34.13 and 61.29 per cent at $0.6, 0.9$ and 1.2 mg l^{-1} of BAP, respectively. Interaction effects were also significant in most of the cases.

Somers *et al.* (1982) reported that callus formation has been frequently found when embryogenic explants were cultured that also revealed other morphogenetic response. Mature embryos cultured on MS or WP media revealed increase in the callusing as the concentration of BAP was increased.

CHAPTER – 6

SUMMARY AND CONCLUSION

The present investigation entitled “studies on intraspecific biodiversity of horticultural traits and *in vitro* shoot morphogenetic response in walnut (*Juglans regia* L) germplasm” formed a part of the overall population improvement of walnut crop in the state of Jammu & Kashmir. The investigation was carried out to characterize morphological, phenological, maturity, yield and yield component and qualitative traits in this crop species. The study was carried over a period of 2 years on earmarked *in situ* natural seedling raised trees in different pockets and agro-ecological conditions of Kashmir valley. International Standard Descriptor of IBGPR on walnut was used as guide for characterization of characters, besides recording of quantitative data for estimation of variability and different genetic parameters.

In vitro studies on shoot morphogenesis viz-a-viz, media and explant browning and explant survival, growth and callusing were also carried in brief.

Maturity and harvesting of the nuts was completed in about 20-22 weeks after full bloom of pistillate flowers and this period extended from

29th August to 17th September over both the years. The variation in maturity resulted from topographical conditions, attitude, climatic conditions, etc.

The *in situ* earmarked trees revealed low, intermediate and high tree vigour with majority (72.0%) having intermediate to high tree vigour and nearly 70.0 per cent trees also had erect to semi-erect growth habit. Nearly 80.0 per cent trees revealed intermediate to dense branching habit. Leaflet shape was broad to narrow elliptic in about 75.0 per cent trees with entire margin and negligible serration. Green colour of leaves was most predominant (75.0%) followed by light green colour (23.0%). Shoot colour was dominated by brown in nearly 75.0 per cent trees, however, dark and green coloured shoots were also formed in about 16.0 and 8.0 per cent trees, respectively.

Among the nut characters the nut shape was round in about 37.0 per cent trees followed by ovate (24.0%). Other shapes like broad elliptic, elliptic, broad ovate, triangular, trapezoid, etc had less frequency. Majority of the nuts (88.0%) had light to medium shell colour. Similarly, shell texture was smooth to medium in about 85.0 per cent with very smooth in only about 5.0 per cent population. Shell seal was intermediate in nearly 40.0 per cent and both weak and strong each in about 30.0 per cent population. Shell strength was intermediate in about 50.0 per cent

population followed by weak (27.0%), strong (16.0 %) and papery (7.0%). Likewise shell integrity was intermediate in about 62.0 per cent population followed by complete shell in about 34.0 per cent population.

Kernel characters were estimated as kernel fill, tip shrivel, easiness in their removal and colour. The study revealed that nearly 65.0 per cent population had moderate kernel fill with poor fill and well fill in about same frequency of 17.0 per cent each. Similarly, kernel shrivel was intermediate in about 57.0 per cent population followed by tip shrivel in 32.0 per cent and complete shrivel in about 11.0 per cent. Removal of kernels from nuts was easy to moderate in about 79.0 per cent population, with easy removal in 17.0 per cent and difficult in 4 per cent. Kernel colour was light (66.0%), extra light (16.0 %) and amber to light amber (18.0 %)

Estimation of the genetic variability and other genetic parameters for different quantitative and qualitative traits revealed that stem girth ranged from 0.3 to 3.6m with a population mean of 1.37m in the fruit bearing trees depending on their age, topography, soil and climatic factors, etc. Similarly, canopy spread ranged from 1.2 to 12.5m around a population mean of 6.25m. Phenotypic and genotypic coefficients of variation were 60.4 and 49.1 per cent for stem girth, and 38.0 and 27.0 per cent for canopy spread, respectively. Broad sense heritability was 65.8 and 50.4 per cent,

respectively. Leaf length and breadth revealed a mean size of 12.16 and 6.49 cm with a magnitude of variability of 5.3-24.5 and 2.5-13.5 cm, respectively. Phenotypic and genotypic coefficients of variation were 36.3 and 28.7 per cent for leaf length, and 44.7 and 31.2 per cent for leaf breadth, respectively. Heritability (broad sense) was 62.3 and 48.1 per cent, respectively.

Among the yield component traits the nut length varied from 30.0-60.0 mm around the population mean of 55.6mm. Phenotypic and genotypic coefficients of variation were 44.3 and 37.2 per cent, respectively and broad sense heritability of 70.7 per cent. Nut weight ranged from 8.5 to 35.0g around a population mean of 14.5g and exhibited phenotypic and genotypic coefficients of variation to the extent of 28.0 and 23.0 per cent, respectively with a heritability (broad sense) of 68.0 per cent. Nut size measured at suture and cheek revealed a range of nearly 25.0-50.0 mm with a population mean of nearly 34.0 mm in each case. Similarly, phenotypic and genotypic coefficients of variation were nearly equal to 12.8 and 10.0 per cent in both traits, respectively. Heritability (broad sense) was 56.2 and 63.1 per cent for nut suture and cheek, respectively.

Kernel weight nut^{-1} and their percentage recovery revealed a range of 3.5-15.0 g and 31.0-71.0 per cent around their population mean of 7.75g

and 50.0 per cent, respectively. Phenotypic coefficient of variation was 32.0 and 41.7, genotypic coefficient of variation was 14.9 and 11.3; and broad sense heritability was 54.1 and 58.9 per cent, respectively.

Nut yield (kg tree^{-1}) was estimated on trees clubbed into different age groups viz. 15-30, 31-50, 51-70 and >70 years of age. Mean dried nut yield tree^{-1} was 20.9, 31.4, 42.0 and 63.2 kg in the above age groups, with a range of 10.2-55.5, 19.5-68.0, 30.3-165.5 and 37.5-185.0 kg, respectively. Phenotypic coefficient of variation was nearly 55.3, 38.6, 31.2 and 43.7 per cent, genotypic coefficient of variation was nearly 38.8, 27.6, 20.9 and 31.5 per cent and heritability (broad sense) was nearly 49.3, 51.2, 51.8 and 52.0 per cent, respectively.

Two quality traits viz. protein and oil content exhibited a mean of 17.97 and 62.10 per cent with a magnitude of variability as 14.15-24.3 and 50.2-70.0 per cent, respectively. The phenotypic coefficient of variation was nearly 11.6 and 8.7, genotypic coefficient of variation nearly 4.8 and 4.7, and heritability (broad sense) was 46.0 and 47.4 per cent, respectively.

In vitro morphogenetic studies were carried in three walnut accessions viz. SKUA-002, SKUA-008 and SKUA-010 on MS media supplemented with different concentrations of BAP (0.3, 0.6, 0.9 and 1.2 mg l^{-1}) and a constant concentration of 0.1 mg l^{-1} of IBA with each BAP

concentration. The study was confined to survival of explants, their growth, callusing behaviour and browning (media and explants).

Explants revealed a very low success of survival in all the three accessions and with application of growth regulator combinations. Accessions exercised no influence on survival of explants, but application of BAP (0.3 and 1.2mg l⁻¹) significantly increased the survival to about 23.4 and 19.0 per cent, respectively as compared to overall mean of 16.8 per cent. Among the explants cultured only about 13.3 - 24.0 per cent revealed growth. Accessions did not influence significantly the growth of explants but application of BAP at 0.3mg l⁻¹ concentration increased it significantly as compared to other concentration of BAP. The growing explants revealed a medium growth with no influence from accessions but significant impact on growth with application of BAP (0.3 and 1.2mg l⁻¹) as compared to 0.6 and 0.9mg l⁻¹ concentration. Callusing in growing explants was not affected significantly by the accessions. However, there was significant increase from zero per cent with application of BAP 0.3mg l⁻¹ + IBA 0.1mg l⁻¹ to nearly 12.8, 34.1 and 61.3 per cent as the concentration of BAP increased to 0.6, 0.9 and 1.2mg l⁻¹ along with IBA 0.1mg l⁻¹ with each BAP concentration, respectively.

Browning in the present *in vitro* studies was measured both on

explants and nutrient media. The media browning was observed in 80.0 to 85.0 per cent culture tubes. Accessions had no significant effect on media browning, but application of BAP 0.3mg l^{-1} + IBA 0.1mg l^{-1} significantly reduced media browning as compared to other BAP concentrations. Similarly, explant browning was not influenced by the accessions which ranged from 77.3-85.3 per cent in the treatments, but BAP 0.3mg l^{-1} + IBA 0.1mg l^{-1} significantly reduced it as compared to other BAP concentrations. Lower volume of browned media was recorded in the accessions SKUA-008 and SKUA-010. Application of BAP at 0.9mg l^{-1} along with IBA 0.1mg l^{-1} significantly reduced volume of media browning as compared to BAP at 0.3 and 0.6mg l^{-1} , and also in BAP at 1.2mg l^{-1} as compared to 0.3mg l^{-1} . The extent of explant browning was not affected by the accessions. Application of BAP at 0.3mg l^{-1} significantly decreased whileas BAP at 0.6mg l^{-1} significantly increased the explant browning score as compared to other two concentrations (0.9 and 1.2mg l^{-1}) of BAP.

Based on the findings of the present investigation the following broad line conclusions are drawn:

- i. *In situ* seedling raised walnut populations in different agro-climatic conditions of the Kashmir valley revealed that maturity of nuts is governed by the topography, climatic conditions and altitude.

- ii. Characteristics of the cultivars as per the International Standard Descriptor of walnuts (IBPGR) revealed that majority of population studied has intermediate to high tree vigour, erect to semi –erect growth habit and intermediate to dense branching.
- iii. Nuts in majority of the population had round to ovate shape; light to medium in shell colour; smooth to medium shell texture; and shell strength and its integrity as medium.
- iv. Kernel fill was moderate in majority of nuts with easy to moderate removal of kernels. Majority of the kernels had light to extra light colour.
- v. Estimates of genetic variability were good (>30.0 - 50.0%) for nut length and nut yield tree⁻¹ (15-30 and above 70 year age groups), moderate (≥ 20.0 to 30.0%) for nut weight, kernel weight nut⁻¹ and nut yield tree⁻¹ (31-50 and 51-70 year age groups), and low ($<20.0\%$) for nut diameter, kernel recovery percentage and quality traits (protein and oil content).
- vi. Heritability (broad sense) estimates were high ($>60.0\%$) for nut weight, nut length and nut diameter (cheek); moderate (≥ 40.0 - 60.0%) for nut diameter (suture line), kernel weight, kernel recovery percentage and quality traits (protein and oil content).

vii. *In vitro* studies revealed very low survival, growth and callusing of explants. The development of browning on explants, media and its volume was significantly high. Accessions played no role in the *in vitro* studies, but growth regulator BAP at lower concentration significantly increased survival and growth of explants and reduced browning. Callusing increased in the growing cultures with increase in BAP concentration from 0.6 to 1.2 mg l⁻¹.

Overall suggestions that can be made from the present investigation are:

- Extensive survey, characterization and evaluation of the existing germplasm diversity in the seedling raised natural population need to be carried out on regular basis and elite genotypes identified for commercial purposes and future breeding programme.
- Detailed *in vitro* studies on walnut need to be taken up to standardize appropriate nutrient media, most effective combination of growth hormones, micro and macro-nutrients composition and techniques to reduce media/explant browning.

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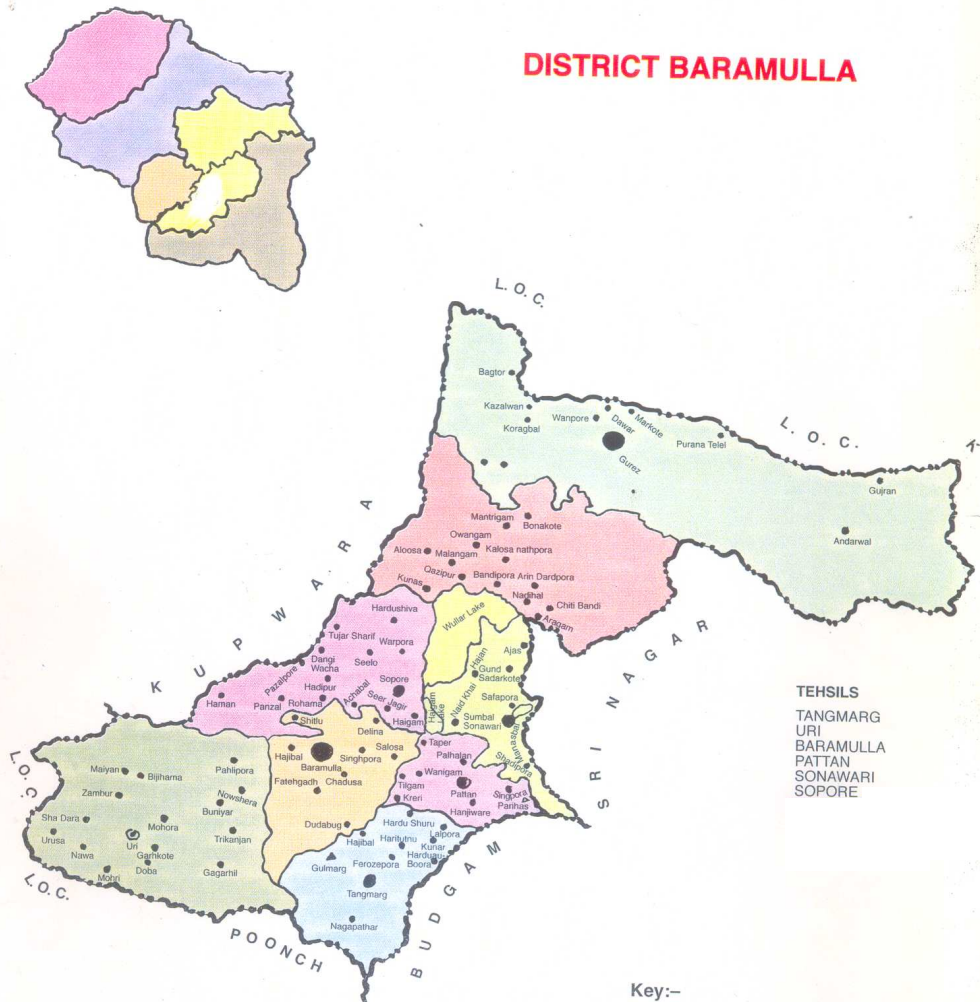
APPENDIX – I

Average yearly temperature, location and attitude of different districts of Kashmir Valley surveyed for walnut variability

District	Average altitude (m) a.m.s.l.	Latitude	Temperature (°C)		Annual rainfall (mm)
			Maximum	Minimum	
Baramulla	1580.0	34°-34N	35.0	-8.0	1270
Budgam	1600.0	34°-20N	36.0	-5.0	785
Kupwara	1610.0	34°-40N	32.0	-7.0	1160
Pulwama	1760.0	34°-15N	34.0	-6.0	980
Shopian	2000.0	34°N	31.0	-9.0	1200

[Source : JK online now bulletin]

DISTRICT BARAMULLA

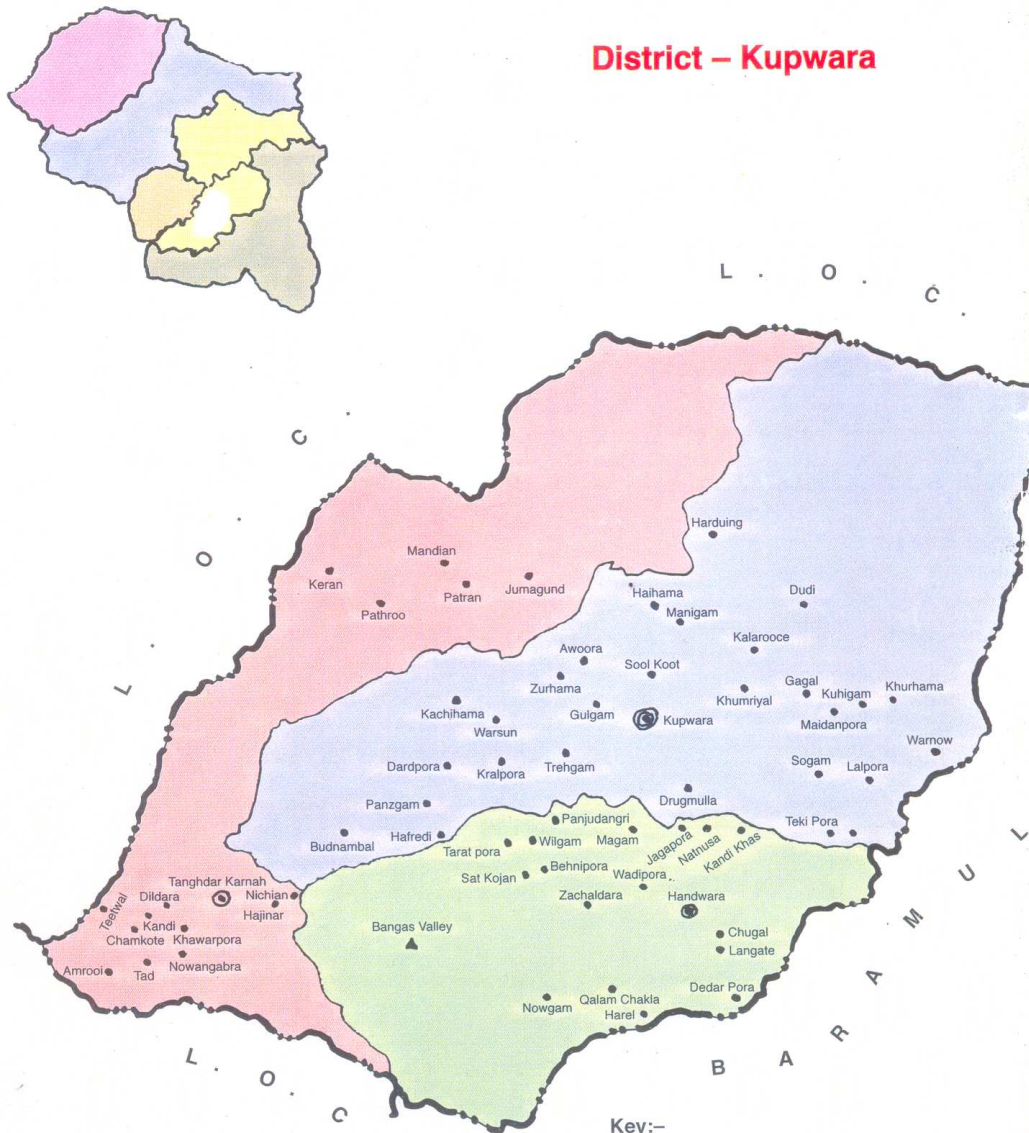


Source:-
1. Survey of India Maps
2. Census of India Maps

Key:-

1. District Boundry:-
2. Tehsil Boundry:-
3. Dist. Head Quarter:-
4. Tehsil Head Quarter:-
5. Places of Tourist Interest:-
6. Places of Religious Importance:-
7. Places of Historical Importance:-
8. Main Villages:-
9. Water Bodies:-

District – Kupwara



Source:-
 1. Survey of India Maps
 2. Census of India Maps

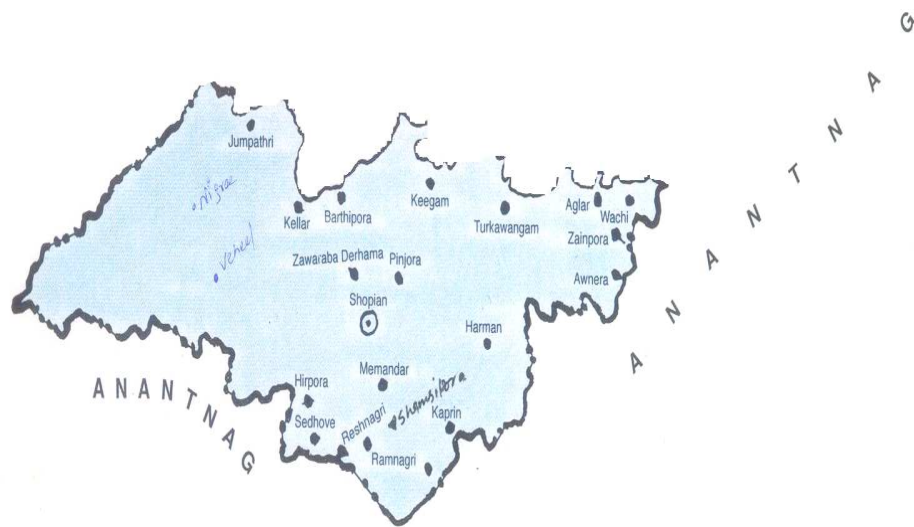
Key:-

1. District Boundry:-
2. Tehsil Boundry:-
3. Distt. Head Quarter:-
4. Tehsil Head Quarter:-
5. Places of Tourist Interest:-
6. Places of Religious Importance:-
7. Places of Historical Importance:-
8. Main Villages:-
9. Water Bodies:-

District – Budgam



DISTRICT SHOPIAN

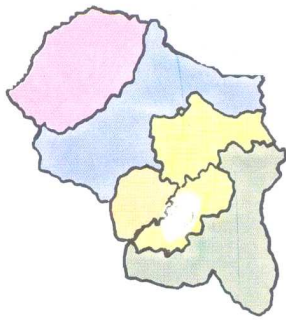


Source:-

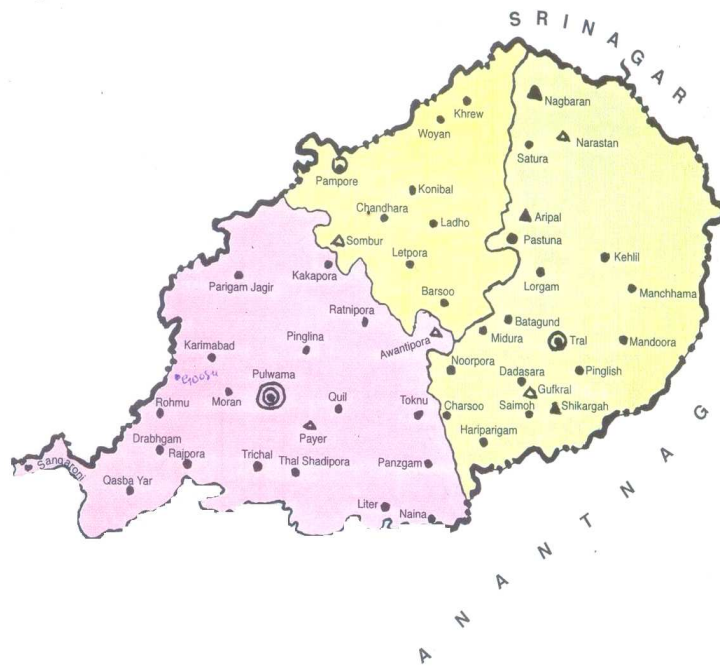
1. Survey of India
2. Census of India Maps

Key:-

1. District Boundry:-
2. Tehsil Boundry:-
3. Distt. Head Quarter:-
4. Tehsil Head Quarter:-
5. Places of Tourist Interest:-
6. Places of Religious Importance:-
7. Places of Historical Importance:-
8. Main Villages:-
9. Water Bodies:-



DISTRICT PULWAMA



Key:-

1. District Boundry:-
2. Tehsil Boundry:-
3. Distt. Head Quarter:-
4. Tehsil Head Quarter:-
5. Places of Tourist Interest:-
6. Places of Religious Importance:-
7. Places of Historical Importance:-
8. Main Villages:-
9. Water Bodies:-

Source:-

1. Survey of India Maps
2. Census of India Maps

S. No.	Name of the Walnut Selections	Address of the Orchardist
1.	WS-001	Bashir Ahmad Khan S/o Ab. Rehman Khan R/o Cheeradari Baramulla
2.	WS-002	Ashiq Hussain War S/o Ab. Ramzan War R/o Cheeradari Baramulla
3.	WS-003	Mohammad Ramzan Sofi S/o Ab. Wahab Sofi R/o Cheeradari Baramulla
4.	WS-004	Bashir Ahmad Sofi S/o Wali SOfi R/o Cheeradari Baramulla
5.	WS-005	Ab. Rashid Khan S/o Latief Khan R/o Chinar Bagh Baramulla
6.	WS-006	Shamusdin Peer S/o Haji Rehman R/o Chinar Bagh Baramulla
7.	WS-007	Mohd. Sultan Mir S/o Ab. Jabbar Mir R/o Gantamulla Baramulla
8.	WS-008	Bashir Ahmad Peer S/o Haji Ahad Peer R/o Gantamulla Baramulla
9.	WS-009	Nowab Hassan Shah S/o Anwar Shah R/o Gantamulla Baramulla
10.	WS-010	Gh. Nabi Mir S/o Bashir Ahmad Mir R/o Gantamulla Baramulla
11.	WS-011	Mohd. Yousuf Peer S/o Jabbar Peer R/o Gantamulla Baramulla
12.	WS-012	Dilawar Ahmad Khan S/o Yousuf Khan R/o Gantamulla Baramulla

13.	WS-013	Ab. Razak Ganaie S/o Habibullah Ganaie R/o Gantamulla Baramulla
14.	WS-014	Ab. Rehman Khan S/o Ahmad Khan R/o Gantamulla Baramulla
15.	WS-015	Gh. Hassan Chahan S/o Hassan Chahan R/o Daken Uri
16.	WS-016	Sonaullah Khan S/o Wali Khan R/o Daken Uri
17.	WS-017	Abdul Khaliq Chopan S/o Abdul Gaffar Chopan R/o Daken Uri
18.	WS-018	Mohd. Sultan Khan S/o Haji Akbar Khan R/o Daken Uri
19.	WS-019	Inderjeet Singh S/o Balwant Singh R/o Gohan Baramulla
20.	WS-020	Jageer Singh S/o Deewan Singh R/o Gohan Baramulla
21.	WS-021	Malik Farooq S/o Ab. Gaffar Malik R/o Gohan Baramulla
22.	WS-022	Ab. Aziz Sheikh S/o Wajahat Sheikh R/o Gohan Baramulla
23.	WS-023	Ab. Gani Shah S/o Subhan Shah R/o Chandoosa Baramulla
24.	WS-024	Bashir Ahmad Beigh S/o Hashmat Beigh R/o Chandoosa Baramulla

25.	WS-025	Ab. Khaliq Mir S/o Gh. Ahmad Mir R/o Chandoosa Baramulla
26.	WS-026	Ab. Ahad Khan S/o Basharat Khan R/o Chandoosa Baramulla
27.	WS-027	Fayaz Ahmad Baba S/o Khazir Mohd Baba R/o Muqaam Baramulla
28.	WS-028	Ab. Gaffar Baba S/o Razak Baba R/o Muqaam Baramulla
29.	WS-029	Ab. Jabar Dar S/o Bashir Ahmad Dar R/o Muqaam Baramulla
30.	WS-030	Ab. Rashid Lone S/o Mohd Akbar Lone R/o Latiefabad Baramulla
31.	WS-031	Munieer Ahmad Lone S/o Ab. Ahad Lone R/o Latiefabad Baramulla
32.	WS-032	Nazir Ahmad Kar S/o Ab. Rashid Kar R/o Latiefabad Baramulla
33.	WS-033	Mir Mushtaq S/o Muzzar Ahmad Mir R/o Latiefabad Baramulla
34.	WS-034	Ab. Qadir Gojree S/o Latief Gojree R/o Shamlaren Baramulla
35.	WS-035	Bashir Gojree S/o Sikander Gojree R/o Shamlaren Baramulla

36.	WS-036	Nazir Gojree S/o Hikmat Gojree R/o Shamlaren Baramulla
37.	WS-037	Ramzaan Gojree S/o Samander Gojree R/o Shamlaren Baramulla
38.	WS-038	Gh. Mohi-ud-din Gojree S/o Akbar Gojree R/o Shamlaren Baramulla
39.	WS-039	Wazir Mohammad Chopan S/o Ahad Chopan R/o Nambla Uri
40.	WS-040	Ali Mohammad Pathan S/o Sonaullah Pathan R/o Nambla Uri
41.	WS-041	Mohammad Hamid Pathan S/o Gh. Rasool Pathan R/o Nambla Uri
42.	WS-042	Ab. Hamid Khan S/o Gh. Rasool Khan R/o Nambla Uri
43.	WS-043	Fayaz Ahmad Dar S/o Wali Mohammad Dar R/o Kharee Pattan Baramulla
44.	WS-044	Mohammad Maqbool Dar S/o Liyaqat Dar R/o Kharee Pattan Baramulla
45.	WS-045	Farooq Ahmad Ahanger S/o Gh. Ahmad Ahanger R/o Bomai Baramulla
46.	WS-046	Khazir Mohammad Dar S/o Ab. Gani Dar R/o Sagipora Baramulla

47.	WS-047	Gh. Qadir Najar S/o Gh. Ahmad Najar R/o Sagipora Baramulla
48.	WS-048	Gh. Mohammad Mir S/o Ab. Gani Mir R/o Zalloora Baramulla
49.	WS-049	Basharat Hassan S/o Liyaqat Hassan R/o Zalloora Baramulla
50.	WS-050	Ab. Khaliq Mir S/o Ab. Rashid Mir R/o Gulgam Kupwara
51.	WS-051	Ab. Gaffar Sheikh S/o Ab. Rehman Sheikh R/o Gulgam Kupwara
52.	WS-052	Fayaz Ahmad Khan S/o Liyaqat Khan R/o Gulgam Kupwara
53.	WS-053	Nisar Ahmad Khan S/o Basharat Khan R/o Gulgam Kupwara
54.	WS-054	Sonaullah Khan S/o Shah Wali Khan R/o Maidanpora Lolab Kupwara
55.	WS-055	Shabir Ahmad Bhat S/o Mohammad Ramzan Bhat R/o Maidanpora Lolab Kupwara
56.	WS-056	Mohammad Yousuf Sheikh S/o Abdul Jabbar Sheikh R/o Khumjalpora Lolab Kupwara
57.	WS-057	Wajahat Ahmad Sheikh S/o Himayat Ali R/o Lolab Kupwara

58.	WS-058	Mast Ab. Rasheed Parray S/o Gh. Mohi-ud-din R/o Lolab Kupwara
59.	WS-059	Sumander Sheikh S/o Wali Sheikh R/o Lolab Kupwara
60.	WS-060	Ab. Rehman Zargar S/o Ab. Gaffar Zargar R/o Lolab Kupwara
61.	WS-061	Mohammad Shafi Zargar S/o Ab. Samad Zargar R/o Lolab Kupwara
62.	WS-062	Mohammad Abdullah Khan S/o Nisar Ahmad Khan R/o Malik Mohalla Tiker Kupwara
63.	WS-063	Ab. Aziz Malik S/o Ab. Jabar Malik R/o Malik Mohalla Tiker Kupwara
64.	WS-064	Ab.Gani Pandith S/o Mohamad Maqbool R/o Malik Mohalla Tiker Kupwara
65.	WS-065	Zaffar Ahmad Shah S/o Abdul Karim Shah R/o Malik Mohalla Tiker Kupwara
66.	WS-066	Manzoor Ahmad Shah S/o Bashir Ahmad Shah R/o Malik Mohalla Tiker Kupwara
67.	WS-067	Gh. Rasool Ganaie S/o Wali Mohammad Ganaie R/o Malik Mohalla Tiker Kupwara
68.	WS-068	Mohd. Subhan Shah S/o Ab. Gani Shah R/o Jagarpora Handwara, Distt. Kupwara

69.	WS-069	Ab. Satar War S/o Mohamad Sultan War R/o Jagarpora Handwara, Distt. Kupwara
70.	WS-070	Bashir Ahmad Bhat S/o Ab. Jabbar Bhat R/o Nigree Handwara, Distt. Kupwara
71.	WS-071	Ahsan Ahmad Bhat S/o Ab. Aziz Bhat R/o Nigree Handwara, Distt. Kupwara
72.	WS-072	Gulzar Ahmad Sofi S/o Ab. Samad Sofi R/o Nigree Handwara, Distt. Kupwara
73.	WS-073	Ab. Jabbar Dar S/o Wali Mohamad Dar R/o Tuli Grund Handwara, Distt. Kupwara
74.	WS-074	Maseer Ahmad Dar S/o Gh. Ahmad Dar R/o Tuli Grund Handwara, Distt. Kupwara
75.	WS-075	Gul Sofi S/o Ahad Sofi R/o Tuli Grund Handwara, Distt. Kupwara
76.	WS-076	Ali Mohammad Rather S/o Razaq Ahmad Rather R/o Volen, Distt. Budgam
77.	WS-077	Gh. Mohammad Najar S/o Ab. Rehman Najar R/o Volen, Distt. Budgam
78.	WS-078	Ab. Wahab Bhat S/o Sonaullah Bhat R/o Ichchat, Distt. Budgam

79.	WS-079	Shabnum Ahad Cheecha S/o Hafizullah Cheecha R/o Ichchat, Distt. Budgam
80.	WS-080	Gh. Hassan Khan S/o Zameer Khan R/o Monapapey, Distt. Budgam
81.	WS-081	Gh. Qadir Ganie S/o Himmat Ganie R/o Monapapey, Distt. Budgam
82.	WS-082	Ab. Salam Najar S/o Ahmadullah Najar R/o Monapapey, Distt. Budgam
83.	WS-083	Gh. Rasool Parray S/o Aziz Parray R/o Mongdal, Distt. Budgam
84.	WS-084	Ab. Ahad Tantray S/o Jabbar Tantray R/o Bapora, Distt. Budgam
85.	WS-085	Gh. Nabi Bhat S/o Saifullah Bhat R/o Bapora, Distt. Budgam
86.	WS-086	Gh. Rasool Bhat S/o Zaffar Bhat R/o Bapora, Distt. Budgam
87.	WS-087	Ab. Hamid Bhat S/o Basharat Ahmad Bhat R/o Shamsabad, Distt. Budgam
88.	WS-088	Ali Mohamad Dar S/o Feroz Ahmad Dar R/o Shamsabad, Distt. Budgam
89.	WS-089	Habib Kuchhay S/o Ramzan Kuchhay R/o Shamsabad, Distt. Budgam

90.	WS-090	Safeer Kuchhay S/o Ramzan Kuchhay R/o Shamsabad, Distt. Budgam
91.	WS-091	Ali Mohamad Mir S/o Ab. Rasheed Mir R/o Shamsabad, Distt. Budgam
92.	WS-092	Wazir Ahmad Mir S/o Mohammad Sultan Mir R/o Khan Sahib, Distt. Budgam
93.	WS-093	Tariq Ahmad Shah S/o Basharat Ahmad Shah R/o Khan Sahib, Distt. Budgam
94.	WS-094	Mohamad Abdullah Khan S/o Hafiz Khan R/o Khan Sahib, Distt. Budgam
95.	WS-095	Khalid Mohamad S/o Ab. Rasheed Shah R/o Khan Sahib, Distt. Budgam
96.	WS-096	Zaffar Khan S/o Dilawra Khan R/o Belomnar, Distt. Budgam
97.	WS-097	Gh. Hassan Khan S/o Araf Khan R/o Belomnar, Distt. Budgam
98.	WS-098	Tariq Ahmad Bhat S/o Suliaman Bhat R/o C.B. Wath, Pulwama
99.	WS-099	Bashir Ahmad Bhat S/o Mohammad Sidiq Bhat R/o C.B. Wath, Pulwama
100.	WS-100	Ab. Gani Najar S/o Mohammad Ismail Najar R/o C.B. Wath, Pulwama

101.	WS-101	Mohammad Rajab Kumar S/o Ab. Ahad Kumar R/o C.B. Wath, Pulwama
102.	WS-102	Fayaz Ahmad Simnani S/o Wali Mohammad Simnani R/o Drabgam, Pulwama
103.	WS-103	Mohammad Shafee Shah S/o Mohammad Abdullah Shah R/o Drabgam, Pulwama
104.	WS-104	Abdul Majeed Ganaie S/o Mohammad Sultan Ganaie R/o Drabgam, Pulwama
105.	WS-105	Abdul Rasheed Sheikh S/o Ab. Gaffar Sheikh R/o Drabgam, Pulwama
106.	WS-106	Abdul Gani Shah S/o Mohammad Sultan Shah R/o Drabgam, Pulwama
107.	WS-107	Bashir Ahmad Bhat S/o Sheikh Mohammad Subhan R/o Drabgam, Pulwama
108.	WS-108	Gh. Rasool Najar S/o Mohammad Sultan Ganai R/o Kakapora, Pulwama
109.	WS-109	Mohammad Abdullah Khan S/o Wazir Khan R/o Kakapora, Pulwama
110.	WS-110	Mohammad Yousuf Sheikh S/o Sonaulah Sheikh R/o Kakapora, Pulwama
111.	WS-111	Wali Mohammad Wani S/o Ab. Rasheed Wani R/o Kakapora, Pulwama

112.	WS-112	Bashir Ahmad Khan S/o Mohammad Khan R/o Kakapora, Pulwama
113.	WS-113	Abdul Aziz Lone S/o Mohammad Abdullah Lone R/o Kakapora, Pulwama
114.	WS-114	Mehraj Ahmad Mir S/o Mohammad Sharief Mir R/o Kakapora, Pulwama
115.	WS-115	Hashmatullah Khan S/o Zaliy Mohammad Khan R/o Kakapora, Pulwama
116.	WS-116	Ab. Hamid Khan S/o Mohammad Fareed Khan R/o Kakapora, Pulwama
117.	WS-117	Bashir Ahmad Dar S/o Ab. Aziz Dar R/o Kakapora, Pulwama
118.	WS-118	Gh. Qadir Dar S/o Ab. Gani Dar R/o Tukroo Keegam, Shopian
119.	WS-119	Ab. Qayoom Reshi S/o Ramzaan Reshi R/o Pohroo, Shopian
120.	WS-120	Ab. Razaaq Reshi S/o Farooq Reshi R/o Pohroo, Shopian
121.	WS-121	Mushtaq Ahmad Sheikh S/o Mohammad Karim Sheikh R/o Trapora, Shopian
122.	WS-122	Ab. Majeed Sheikh S/o Bashir Ahmad Sheikh R/o Trapora, Shopian

123.	WS-123	Mohammad Akram Sheikh S/o Ab. Razeer Sheikh R/o Trapora, Shopian
124.	WS-124	Gh. Mohammad Dar S/o Ali Mohammad Dar R/o Trapora, Shopian
125.	WS-125	Khursheed Ahmad Malik S/o Haji Mohammad Ismaiel Malik R/o Choudergund, Shopian
126.	WS-126	Abdul Rasheed Lone S/o Ab. Gain Lone R/o Veheel, Shopian
127.	WS-127	Gh. Nabi Mantoo S/o Ab. Karim Mantoo R/o Veheel, Shopian
128.	WS-128	Gh. Mohammad Rather S/o Ali Mohammad Rather R/o Veheel, Shopian
129.	WS-129	Gh. Hassan Dar S/o Ab. Qadir Dar R/o Ramnagar, Shopian
130.	WS-130	Atmard Ahmad Dar S/o Gh. Hassan Dar R/o Ramnagar, Shopian
131.	WS-131	Mohammad Shafi Dar S/o Ahad Dar R/o Manipora, Shopian
132.	WS-132	Bilal Ahmad Khan S/o Mohammad Zaffar Khan R/o Veehil, Shopian
133.	WS-133	Gh. Hassan Rather S/o Mohammad Sultan Rather R/o Veehil, Shopian

134.	WS-134	Sheraz Ahmad Najar S/o Altaf Najar R/o Veehil, Shopian
135.	WS-135	Gh. Rasool Bhat S/o Assadullah Bhat R/o Navidgam, Shopian
136.	WS-136	D. N. Khan S/o M. N. Khan R/o Navidgam, Shopian
137.	WS-137	Shivit Khan S/o F. N. Khan R/o Navidgam, Shopian
138.	WS-138	Wali Mohammad Sheikh S/o Ab. Razaak Sheikh R/o Navidgam, Shopian
139.	WS-139	Mohammad Amin Dar S/o Ab. Hamid Dar R/o Kutpora, Shopian
140.	WS-140	Gh. Rasool Ganaie S/o Ab. Salaam Wagay R/o Arishpora, Shopian
141.	WS-141	Farooq Ahmad Lone S/o Gh. Nabi Lone R/o Arishpora, Shopian
142.	WS-142	Farooq Ahmad Lone S/o Gh. Nabi Lone R/o Arishpora, Shopian
143.	WS-143	Omkarnath S/o Nath Kaul R/o Buch Kuchan, Shopian
144.	WS-144	Sonaullah Bhat S/o Ab. Ramzaan Bhat R/o Buch Kuchan, Shopian

145.	WS-145	Pathvinath S/o Sarwander Nath R/o Buch Kuchan, Shopian
146.	WS-146	Gh. Mohi-ud-din Naikoo S/o Gh. Rasheed Naikoo R/o Buch Kuchan, Shopian
147.	WS-147	Ama Bhat S/o Habibulla Bhat R/o Waterpora, Shopian
148.	WS-148	Mohammad Saleem Pal S/o Zahinger Pal R/o Waterpora, Shopian
149.	WS-149	Gh. Hassan Thikoor S/o Gh. Mohd. Thikoor R/o Saidpora Payeen, Shopian
150.	WS-150	Mohd. Assan Bhat S/o HABibullah Bhat R/o Saidpora Payeen, Shopian

Sher-e-Kashmir
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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Dr. Dinesh Kumar, Principal Scientist, CITH, Srinagar during Viva-Voce examination held on December 15, 2010 have been incorporated in the manuscript entitled **“Studies on Intraspecific Biodiversity of Horticultural Traits and *in vitro* Shoot Morphogenetic Response in Walnut (*Juglans regia* L.) Germplasm”** submitted by **Mr. Imtiyaz Ahmad Lone (Regd. No. 2007-182-D).**

(Dr. F.A. Misgar)
Chairman
Advisory Committee