TAGGING GENETIC DETERMINANTS FOR NUT WEIGHT AND SHELLING PERCENTAGE IN CASHEW (*ANACARDIUM OCCIDENTALE* L.)

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Thesis

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in

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BANGALORE **APRIL, 2010**

AFFECTIONATELY DEDICATED

TO MY PARENTS, TEACHERS

AND FRIENDS

DEPARTMENT OF BIOTECHNOLOGY
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BANGALORE – 560 065
CERTIFICATE

This is to certify that the thesis entitled, "Tagging genetic determinants for nut weight and shelling percentage in cashew (*Anacardium occidentale* L.)", submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Biotechnology of the University of Agricultural Sciences, GKVK Campus, Bangalore is a bonafide record of research work done by Mr. Sunil Kumar, N., during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

BANGALORE
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"Gratitude is the memory of the heart"

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ABBREVIATIONS

AFLP	: Amplified Fragment Length Polymorphism
bp	: Base pair
BSA	: Bulk Segregant Analysis
CAPS	: Cleaved Amplified Polymorphic Sequence
CTAB	: Cetyl Trimethyl Ammonium Bromide
cm	: Centimeter
^{0}C	: Degree celsius
DNA	: Deoxyribose Nucleic Acid
dNTPs	: Deoxy Nucleotide Triphosphates
EDTA	: Ethylene Diamine Tetra Acetic Acid
g	: Gram (s)
ISSR	: Inter Simple Sequence Repeats
kbp	: Kilo base pair
L	: Ladder
M	: Molar
MAS	: Marker Assisted Selection
Mb	: Mega base pair
MT	: Million tones
m	: meter (s)
min	: Minutes (s)
ml	: Millilitre
mM	: Millimolar
mg	: Milligram
µg	: Microgram
µl	: Microlitre
µM	: Micromolar
ng	: Nanogram
nm	: Nanometer
PCR	: Polymerase Chain Reaction
pg	: Picograms
pmoles	: Picomoles
%	: Percentage
QTL	: Quantitative Trait Loci
RAPD	: Random Amplified Polymorphic DNA
RFLP	: Restriction Fragment Length Polymorphism
RNA	: Ribo Nucleic Acid
RNAse	: Ribonuclease
rpm	: Rotation per minute
STS	: Sequence Tagged Sites
SSLP	: Simple Sequence Length Polymorphism
SCAR	: Sequence Characterized Amplified Region
SSR	: Simple Sequence Repeats
Sec	: Second (s)
TAE	: Tris Acetate EDTA

TE	: Tris EDTA
UV	: Ultra violet
V	: Volts
v/v	: Volume by volume
w/v	: Weight by volume

Introduction

1. INTRODUCTION

Cashew (*Anacardium occidentale* L.) is one of the important plantation crops in India, cultivated in the east and west coasts. It is a member of the family Anacardiaceae and native of Brazil. Cashew was introduced into India in the 16th century by the Portuguese (De Coata, 1578). From India, it was carried eastward to Amboina in Indonesia (Rumphius, 1962). Dispersal of the species to South-East Asia appears to have been carried by birds, bats, monkeys and human agents (Burkill, 1935; Johnson, 1973). Though cashew was originally introduced as a soil binder, in recent years it has assumed a pre-eminent position in Indian economy as the leading foreign exchange earner. During 2007-2008 cashew was grown in 8,68,000 ha in India with an annual production of 665000 MT, with a productivity of 860 kg per hectare. Apart from kernels, by-products of cashew namely, cashew apple and Cashew Nut Shell Liquid (CNSL) are potentially important in local and international markets. Cashew nut shell liquid, a naturally occurring phenol, present in the honeycomb structure (mesocarp) between the outer shell (epicarp) and the inner shell (endocarp) of the cashew nut is used in the manufacture of paints, varnishes, brake lining material etc.

The present production of cashew nuts meets 50 per cent of the requirement of the country's processing units; the remaining quantity is imported from African and South East Asian countries. There is an immediate need to increase the cashew production in India to meet the requirement of the domestic processing industry and the ever-increasing demand of the cashew kernels in the international market. The original introduction of cashew was through the Malabar Coast between the sixteenth and seventeenth centuries (De Castro, 1994) from where it gradually spread to other growing regions. Indian cashew is considered

to have a narrow genetic base owing to the small size of initial introductions (Bhaskara and Swamy, 1994), and hence hybridization among these introduced clones has its own limitations due to the limited genetic diversity that it can generate. Ever since its introduction, cashew has been a self-propagating species of the Malabar Coast and no apparent secondary centers of diversity has arisen (Nayar, 1983). However, over the years some of the raw cashew nuts imported from African countries for processing was used as planting material. These introductions along with the highly cross pollinating nature of the crop and seed propagation methods followed till recently, have led to considerable variation in all the economically important characters (Gunjate and Deshpande, 1994).

An important way to increase the productivity in any crop plant is to first select desirable genotypes from among the existing variations and to use the superior once in breeding programmes. The extent of initial variability or genetic diversity determines the success of a crop improvement programme to a greater extent. Apart from being useful in choosing diverse parents for generating superior hybrids, it can also provide genes/alleles for different characters that can be useful in crop improvement. Hence, in recent years much emphasis is being placed upon enhancement, evaluation and maintenance of genetic diversity of cashew in India as a first step towards genetic improvement.

The perennial nature of the crop, long phase juvenility, environmental influence, heterozygosity and large plant size are the major limitations in conventional breeding of cashew. Molecular markers, have great potential in selection and breeding process in cashew through MAS. The DNA pooling strategy, Bulk Segregant Analysis (BSA) is a technique developed (Michelmore *et al.*, 1991) to identify markers linked to desired traits using a suitable population.

Since the advent of molecular markers two decades ago, they have found various applications in biotechnology for improvement of crops such as diversity analysis, mapping, gene tagging, QTL analysis, paternity analysis, fingerprinting etc. Random Amplified Polymorphic DNA (RAPD) (Welsh and Mc Clelland, 1990) markers are easy to perform when compared with others and have certain advantages over other markers. RAPD markers do not require prior DNA sequence information, require very little amount of DNA, there is no need to use radioactive compound, the technique is quick and less cumbersome, and are seldom influenced by the environment but the results are less reproducible. SSR are co-dominant and results are reproducible hence it provide an excellent tool for marker identification. Associating DNA markers like RAPD, ISSR, SSR, RFLP and AFLP's with economic characters can greatly aid in cashew improvement efforts.

Cashew being one of the most important plantation crops evolving new varieties with enhanced quality traits is highly desired. Being a perennial crop, straightforward application of principles of plant breeding is seldom possible and identification of DNA markers linked to economic traits is even more difficult due to non availability of right segregation populations for target traits. It is in this context that the present investigation entitled "Tagging genetic determinants for nut weight and shelling percentage in cashew" was carried out at the Department of Genetics and Plant Breeding, UAS, GKVK Campus, Bangalore.

The present study was conceived with the following objectives :

1. To identify molecular markers linked to economic characters.
2. To validate molecular markers for use in breeding programmes.

Review of literature

2. REVIEW OF LITERATURE

In this chapter available literature pertaining to the various aspects of the present investigation is reviewed and presented. Literature on the importance, origin and distribution, taxonomy, genetics and molecular markers has been reviewed. Similar research carried out in cashew, perennial horticultural crops and other crops in the areas of gene tagging, phylogenetic relationships, cultivar identification and paternity analysis for crop improvement has been reviewed.

2.1. Importance

Cashew (*Anacardium occidentale* L.) trees are grown for their kernels, which when roasted have a pleasant taste and flavor. Cashew nuts are often used as one of the ingredients in many dishes in the tropics. Roasted nuts are relished in the temperate and tropical parts of the world. Cashew butter is quite similar to peanut butter and is made from broken kernels, while still smaller pieces find their application in confectionery industry. The kernel oil is pale-yellow, sweetish and of excellent quality. However, due to the high price of the kernels and also due to its fatty-acid composition, it hardly offers any prospects for export as edible oil. Apart from kernels, the by-products of cashew namely, cashew apple and Cashew Nut Shell Liquid (CNSL), are potentially important in local and international markets. CNSL, a naturally occurring phenol, present in the honeycomb structure (mesocarp) between the outer shell (epicarp) and the inner shell (endocarp) of the cashew nut is used in the manufacture of paints, varnishes, brake lining material etc.

Another main economic part is the cashew apple, used for making jam, jelly, syrup, juice, alcoholic and non-alcoholic beverages as well as candied fruit. However, the processing of this fruit upto now is only in a very limited scale. Other products of the cashew tree are mainly of local significance. In many South East Asian countries the tender young leaves are used for flavouring rice. The bark is said to have medicinal properties. Because it is termite-resistant, the wood can be used in the construction of houses, fence posts and boats. Charcoal obtained from this wood is of fairly good quality. The gum, closely resembling gum arabic, can be made into mucilage with very good adhesive and insect-repellent properties and used for specific purpose such as book binding.

2.1.1. Origin and distribution

Cashew (*Anacardium occidentale* L.) is indigenous to Brazil. It is the only species of *Anacardium* cultivated in many tropical and sub tropical regions of the world for its nuts. There are as many as 20 species of *Anacardium* all of which are found in Central and South America. The high number of wild species in the North Eastern part of South America suggests that it is the site of origin for the genus *Anacardium* and species *occidentale* L. In this region, different forms of cashew can be found with high variability in the local populations (Nomisma, 1994). Thevet was the first to describe cashew as a wild plant extremely common in Brazil where the cashew apples and their juice was consumed and nuts roasted in fires and kernels consumed (Johnson, 1973). The Tupi Indians also used cashew for centuries and are believed to have been responsible for the dispersion of the species towards the coast of North Eastern Brazil where considerable intraspecific variation exist (Ascenso, 1986). The entire cashew fruit, nut and peduncle will float when mature and this could have also been the cause for coastward dispersal of the species in Brazil by rivers flowing north and east. Fruit

bats may also have been involved in seed movement. Within the Amazon forest fruit bats are the most important agents of seed dispersal of tree species (Johnson, 1973). It is because of man's intervention that cashew is found in other parts of the world.

The Portuguese discovered cashew in Brazil and introduced it first to Mozambique and later to India between sixteenth and seventeenth centuries respectively (De Castro, 1994). In India cashew was first introduced to the Malabar Coast from where it spread to various coastal areas in the country. It is an important cash crop commercially cultivated mainly along the coastal regions of the country. Indian cashew is considered to have a narrow genetic base owing to the small size of initial introductions (Bhaskara and Swamy, 1994), and hence it was surmised that hybridization among these introduced clones has its own limitations owing to the limited genetic diversity that it can generate. Ever since its introduction, cashew has been a self-propagating species of the Malabar Coast and no apparent secondary centers of diversity has arisen (Nayer, 1983). However, over the years some of the raw cashew nuts imported from African countries for processing was used as planting material. These introductions along with the highly cross pollinating nature of the crop and seed propagating followed till recently, have led to considerable variation in all the economically important characters (Gunjate and Deshpande, 1994).

2.1.2. Taxonomy

The cashew tree (*A. occidentale* L.) belongs to the family Anacardiaceae which comprises of about 60 genera and 400 species of trees and shrubs with resinous bark and grows most abundantly in the tropics and sub tropical parts of the world. It is an erect growing perennial evergreen tree with an umbrella shaped canopy. The tree may

grow up to 15m but under less favourable conditions it is much smaller. Branching begins close to the ground, lower branches rest on the ground, a few meters from the trunk, and branches of older trees which have grown without being disturbed, may creep over the ground over a considerable distance, sometimes rooting where they touch the soil. Hence it prevents soil erosion and is often grown as a soil binder (Davis, 1961).

The leaves are glabrous, thick and leathery, oblong to obovate, and rounded to emarginated at the apex, 10 to 20 cm long and 5 to 10 cm wide. The cashew tree has an extensive lateral root system and a tap-root, which penetrates deep into the soil. The inflorescence is a panicle, and it may be conical, pyramidal or irregular in shape. According to Copeland (1961), the ultimate cluster of flowers is a typical monochasial cyme and the panicle is a thyrse. Flowers are small, slightly scented, white, cream or light pink. Cashew is andromonoecious *i.e.*, in each panicle perfect flowers and staminate flowers are produced. The kidney-shaped nut is the 'true fruit' of the cashew tree and the swollen fleshy thalamus is the 'false fruit'. The apple is juicy and swollen and often weighs five to ten times more than the nut when ripe. The shell of the nut has a leathery exocarp, a hard and somewhat brittle endocarp, and a spongy mesocarp containing the CNSL, which is rather viscous, oily or balsom-like substance of relatively high volumic. It has a pale-yellow to dark-brownish colour, bitter taste with caustic property and when heated, gives off pungent and choking fumes (Aggarwal, 1954). The kernel has a wrinkled surface and is covered by a reddish brown or pink testa. The kernel itself is white.

2.1.3. Species and varieties

Mango (*Mangifera indica* L.), pistachio nut (*Pistacia vera* L.) and various species of Spondias L., such as Otaheite apple (*S. cytherea* Sonn. or *S. dulcis* Forst), a hog-plum (*S. mombin* L.) and Spanish plum or red mombin (*S. purpurea* L.), belong to the same family as cashew. According to Bailey (1949), the genus *Anacardium* contains eight tropical American species. Parente (1972), citing Machado (1944), names ten species, but Peixoto (1960) names twenty different species, several of which had edible peduncle, such as : 'cajueiro de seis meses' (*A. nanum* St. Hilaire), a very early bearing small shrub, *A. subterraneum* Liasis, a small shrub with its trunk almost completely underground, containing water reserves; *A. microcarpum* Ducke, a small tree form sandy savannas; *A. spruceanum* Bth., a large tree; and the largest species of this genus, *A. giganteum* Hanock, which grows in the Amazon forests.

Valeriano (1972) named five different species, *viz. A. occidentale* L., *A. pumibum* St. Hilarie, *A. giganteum* Hanca, *A. rhinocarpus* and A. *spruceanum* Benth. He suggests that it might be more realistic to distinguish two species only, *A. nanum* and *A. giganteum*, each divided into varieties, characterized by colour (yellow or red) and shape (round, pear-shaped or elongated) of the pseudo-fruit. Each variety could be subdivided into an infinite number of sub-varieties according to intermediate colours or shapes of the pseudo-fruit. In both species, the dwarf and the giant, the same colours and the same shapes of the pseudo-fruit can be found with all the intermediate characteristics, resulting from natural cross-pollination. Valeriano (1972) considers the division into dwarf and giant species taste, size of the pseudo-fruit and size of nut. Apparently he based his hypothesis only on pseudo-fruit

characteristics. But probably there are other, more important characteristics, which determine a species.

From the description presented by Peixoto (1960), it is clear that there are more than two species. It is, however, possible that some types have resulted from interspecific hybridization. From a scientific and breeding point of view, it would appear to be worthwhile to investigate the crossability among the species.

Within the genus there is a wide variety in colour, size and shape of the peduncle, as well as in size and shape of the nuts. There are also differences in leaf-size and leaf-shape, and a detailed study might reveal yet more differences between individual trees.

The 'marking nut' from India (*Semecarpus cassuvium*) is closely related to cashew (Bailey, 1949). Heyne (1950) describes the peduncle or 'apple' as smaller but sweeter than that of the cashew. The nut is almost similar to that of the cashew, but smaller.

Among the related genus and species *A. occidentale* is the only species, which has high commercial importance and is wide spread all over the world. In India, since the introduction of cashew considerable amount of work has been carried out towards the improvement of this crop. Different research station have been set up, which are located at Madakkathara (Kerala), Bapatla (Andhra Pradesh), Ullal and Chintamani (Karnataka), Vengurla (Maharastra), Vridhachalam (Tamil Nadu), Jhargram (West Bengal) and Bhubaneshwar (Orissa). All these research centres work concentrate on mission oriented research whereas at the Directorate of Cashew Research (DCR) in Puttur, both basic and applied aspects are dealt with.

2.1.4. Cytology

The chromosome number of *A. occidentale* L. is reported to be 2n=42 (Darlington and Janaki Ammal, 1945 and Purseglove, 1988). According to Khosla *et al.* (1973), the chromosome count of n=12 from a plantation in Assam differs from the earlier reports of 2n=42 (Darlington and Janaki Ammal, 1945) and 2n=40 (Simmonds, 1954). Such chromosome polymorphism is well known in many domesticated trees (Khosla *et al.*, 1973).

2.2. Polymerase Chain Reaction (PCR)

PCR was discovered by Kary Mullis for which he received Noble prize in Chemistry in 1993. PCR technique produces similar result to DNA cloning (producing several copies) the selective amplification of a DNA sequence. If the process is repeated many times, there is an exponential increase in the number of copies of the starting sequence. Automation of the cycles of the operation is achieved by using thermal cycler. PCR is used to clone specific sequences; with a wide application in amplification of gene of interest, analysis of genetic diversity, classification, genetic fingerprinting and related studies.

2.2.1. Critical factors for successful PCR

2.2.1.1. Denaturing temperature and time

The complementary association of template-primer DNA through hydrogen bonding is referred as 'annealing'. Heating the double stranded DNA to a point above the melting temperature and then flash cooling ensures the denatured or separated strands not to re-anneal. For nucleic acid in buffers of ionic strength lower than 150mM NaCl, the

melting temperature is generally less than 100^0 C. PCR works with the denaturing temperature of 91-97^0 C.

Half-life of *Taq* polymerase is 30 min at 95^0 C, and hence requires control of the denaturation time to keep the maximum activity of the enzyme. Once, the mean length of target DNA is decreased, it is possible to reduce denaturation temperature during amplification. For templates of 300 bp or less, denaturation temperature may be reduced to as low as 80^0 C for 50% G+C content, without much decrease in enzyme efficiency.

Time and temperature is the main cause for denaturation/loss of activity of *Taq* polymerase. Thus, the number of cycles needs to be increased with the decrease in time. Normally the denaturation time is 1min at 94^0 C (Innis and Gelfand, 1990) recommended.

2.2.1.2. Annealing temperature and primer design

Primer length and sequence are most important in primer designing and for successful amplification. The melting temperature (T_m) increases with length and with (G+C) content of DNA (T_m = 4(G+C) + (A+T)^0 C). One should aim at using an annealing temperature about 5^0 C below the lowest T_m of the pair of primers to be used (Innis and Gelfand, 1990). Maintaining temperature 1^0 C above annealing temperature both the specificity of amplification and yield products <1 Kb in length can be increased. Most primers require a minimum annealing time of 30 sec or less.

Set of rules for primer sequence design :

- Primers should be 17-28 bp in length.
- Base composition should be 50-60 % (G+C).
- Primers should end (3^1) in a G or C or CG or GC. This increases the priming efficiency.
- T_m between 55-80^0 C is preferred.
- Runs of 3 or more cycles or nucleotides at the 3^1 ends of primers may promote mis-priming at G or C rich sequences and should be avoided.
- 3^1 ends of the primer should not be complementary, as otherwise primer dimers will be synthesized preferentially to any other product.
- Primers self complementarity (ability to form secondary structure such as hairpins) should be avoided.

2.2.1.3. Primer length

17 mer or longer primers are routinely used for amplification of genomic DNA. Too long primer length may mean that even high annealing temperature is not enough to prevent mismatch and nonspecific priming.

2.2.1.4. Elongation temperature and time

This is normally 70-72^0 C for 0.5 to 3 min. *Taq* polymerase has a high specific activity around 70^0 C and primer extension occurs at up to 100 bases/sec one minute is sufficient for reliable amplification of 2 Kb sequences (Innis and Gelfand, 1990). Longer products require about 3 minute (3 Kb and more) of elongation time especially when product concentration exceeds enzyme concentration and dNTPs and primer are

low. Higher than 50mM KCl or NaCl inhibits *Taq* polymerase, but sometimes it is necessary to facilitate primer annealing.

2.2.1.5. Mg^{2+} concentration

Mg^{2+} affects primer annealing, T_m of template, product and primer-template association, product specificity, enzyme activity and fidelity. *Taq* polymerase requires free Mg^{2+}, hence allowances should be made for dNTPs, primers and template, all of which chelate and sequester the cation. Mg^{2+} should be 0.5-2.5mM greater than dNTPs. This requires the determination of the optimum concentrations of Mg^{2+} for given template – primer/*Taq* polymerase combination. Some enzymes work markedly better in the presence of detergents probably because it prevents the natural tendency of the enzyme to aggregate.

2.2.1.6. Deoxy nucleotide triphosphates (dNTPs)

The stability of dNTPs during repeated cycles of PCR is such that approximately 50 per cent remains as dNTPs after 50 cycles (Innis and Gelfand, 1990). Usually, each dNTP is used at a concentration between 50 µM and 200 µM, which results in the optimal balance among yield, specificity and fidelity. Higher concentrations encourage mis incorporation by the DNA polymerase (Baumforth *et al.,* 1999). The four dNTPs should be used at equivalent concentrations to minimize misincorporation errors. Low dNTP concentrations minimize mispriming at non-target sites and reduce the likelihood of extending misincorporated nucleotides. Therefore, one should decide on the lowest dNTP concentration appropriate for the length and composition of target sequences (Innis *et al.,* 1990). Concentrations of 50 µM and 200 µM of

each dNTP are sufficient to synthesize 6.5 ng and 25 ng of DNA respectively.

2.2.1.7. DNA polymerase

A variety of polymerases could be used for chain reactions. However, the most commonly used DNA polymerase is *Taq* polymerase, which is isolated from a bacterium found in hot springs known as *Thermus aquaticus*. *Taq* polymerase works optimally at 72^0 C and is also heat stable, allowing the enzyme to with stand repeated denaturation cycles (Baumforth *et al.*, 1999). Therefore it need not be added at each cycle, which greatly simplifies the automation of PCR. One drawback of *Taq* polymerase is that it lacks 3^1 to 5^1 exonuclease (proof reading) activity, which can lead to misincorporation of nucleotides (Eckert and Kunkel, 1992). Enzyme activity is also sensitive to the concentrations of magnesium and other monovalent ions.

The recommended concentration range for *Taq* polymerase is between 1 to 2.5 units per 100 µl reaction mix when other parameters are optimum. However, enzyme requirements may vary with respect to individual target templates or primers. When optimizing a PCR, it is recommended, to testing the enzyme concentration from 0.5 to 5 units per 100 µl and assaying the results by gel electrophoresis. If enzyme concentration is too high, nonspecific back ground products may accumulate and if too low an insufficient amount of desired product is made. *Taq* polymerase from different supplier may behave differently because of different formulations, assay conditions and or unit definitions (Innis and Gelfand, 1990).

Recently a new DNA polymerase has been isolated from the Thermophillic bacteria *Thermococcus litoralis*, called vent DNA polymerase. It has 3^1 to 5^1 exonuclease activity and may therefore have lower misincorporation rate. However, the capacity of this to degrade single stranded molecules (like oligonucleotide primers or PCR product prior to primer annealing) will pose problem for PCR amplification (Erlich *et al.*, 1991).

2.2.1.8. Reaction buffer

The buffer most often used in the PCR is 10 mM Tris buffer, with a pH range between 8.5 and 9.0 at 25^0 C. Because the pH of Tris buffers decreases by 0.3 units for each 10^0 C rise in temperature, a buffer made to pH 8.8 at 25^0 C is only pH 7.4 at 72^0 C. This value is optimal for the activity of *Taq* polymerase since *Taq* appears to have a pH optimum of 7.0-7.5 at 72^0 C (Baumforth *et al.*, 1999).

2.2.1.9. Cycle number

The number of amplification cycles necessary to produce a band visible on a gel depends largely on the concentration of the target DNA (Innis and Gelfand, 1990). The cycle commences with the denaturation step, which ensures complete template DNA strand separation. Typically denaturation conditions are 95^0 C for 30 second or 97^0 C for 15 sec. and are quite enough to denature G+C rich templates. However, it has been observed that incomplete denaturation allows the DNA strands to 'snap back' and thus, reduce the product yield. The denaturation steps that are too high and/or too long lead to unnecessary loss of enzyme activity. Therefore, it is better to perform PCR with temperature range of 94-97^0 C for denaturation in order to avoid mis-priming, and incorporation of incorrect nucleotides (Innis and Gelfand, 1990).

2.2.2. Helix destabilizers and additives in PCR

With nucleic acids of high G+C content it may be necessary to use

- Dimethyl Sulphoxide (DMSO)
- Dimethyl Formamide (DMF)
- Urea
- Formamide

In the reaction mixture these additives are presumed to reduce the T_m of the target nucleic acid. DMSO at 10 % and higher concentration is known to decrease the activity of *Taq* polymerase up to 50 % (Innis and Gelfand, 1990).

Additives may also be necessary in the amplification of long target sequences. Formamide can apparently dramatically improve the specificity of PCR. Glycerol improves the amplification of templates with high G+C content. Poly Ethylene Glycol (PEG) is a useful additive when DNA template concentration is very low. It promotes macromolecular association by solvent exclusion, providing more access for the polymerase to the template DNA.

2.2.3. Migration medium of nucleic acids

The choice between agarose and acrylamide as a medium for migration of nucleic acids depends on several factors. Practically, agarose gels are easier to make and use, than acrylamide gels. The choice of migration medium depends mainly on level of resolution required. Usually 6% acrylamide allows us to distinguish between fragments that differ only by few base pairs. This resolution is mainly

exploited in microsatellites as well as for genetic fingerprinting techniques. Separation on agarose gel (around 1 %) enables us to separate DNA fragments ranging from 300 to 15,000 bp. Agarose gel is well suited for RFLP analysis and makes it possible to transfer significant quantities of DNA into the nitrocellulose membranes during hybridization studies.

2.3. Different types of markers

2.3.1. Morphological markers

Morphological markers are the oldest and widely used descriptive markers and are very informative in germplasm and cultivar management where the cultivars can be identified by morphological traits. They can be used to assess the distances between the accessions and incorporate morphologically distant once into the germplasm. Disadvantages with this strategy are in the time and expense involved in evaluating morphological traits. Reproductive traits take a long time to express especially in perennial crops, are highly influenced by environment conditions and are subjected to personal bias. In order to get a meaningful assessment of the genetic diversity a large number of polymorphic markers are required; this limits the use of morphological markers which are relatively few in number in many perennial crops. In some perennial crops the actual identity of some cultivars is still in question, because similar cultivars grown in different areas often have various names because of the differences in the manifestation of morphological traits (Lakshminarayana, 1980). The prime advantages of the use of morphological traits is that they are simpler, fast and inexpensive (when available), even from herbarium specimens and other dead tissues.

Since the establishment of Directorate of Cashew Research (DCR) in 1986 at Puttur, efforts have been made to collect, grow and evaluate various cashew accessions from all over India. A National Cashew Gene Bank (NCGB) has been established with more than 1000 accessions procured from different parts of India and abroad. Cashew descriptors have been developed by IBPGR (presently IPGRI) for the evaluation of cashew. By adopting these descriptors 433 clonal accessions of cashew conserved in NCGB at the DCR, Puttur, Karnataka, India, have been characterized and catalogued (Swamy *et al.*, 1997; 1998 and 2000).

2.3.1.1. Components of variability, genetic advance and heritability

Detection and estimation of genetic variability in working collections is a prerequisite in crop improvement programmes. The variability observed in any population is due to genetic and environmental factors. The relative contribution of these factors for total variability dictates genetic gain possible through selection.

Cashew being cross pollinated crop exhibit wide genetic variability for morphological, qualitative and yield characters. A brief review of literature on genetic variability has been categorically presented below.

Sena *et al*. (1994) studied the nature and magnitude of genetic variability and their inter-relationship for nut yield and its eleven component traits in 17 genotypes of cashew (*Anacardium occidentale* L.). High estimates of genotypic coefficients of variation, heritability and genetic advance was observed for sex ratio, fruit set, number of fruits and apple weight, indicating their reliability for effecting selections for high nut yield. Fruit set per panicle and single nut weight was the best contributors to nut yield per plant and further, the nut weight and apple

weight showed high positive association with nut yield both at genotypic and phenotypic levels. Hence, the studies revealed the importance of nut weight, fruit set and apple weight as selection criteria for improvement of nut yield in cashew.

Singh (2002) studied during 1997-99 with 36 clones of selected mango (*Mangifera indica* L.) for early and regular bearing after survey of different islands in Andaman and Nicobar islands during flowering and fruiting period. The genetic and phenotypic coefficient of variance, heritability, genetic advance, coefficient of correlation was estimated for weight of fruit, length and breadth of fruit, total soluble solids (TSS), acidity, stone and pulp weight. Variability was observed among clones for the characters studied which exhibited highest estimate of broad sense heritability and low to moderate genetic advance. Length (98.4%) and breadth of fruit (99.5%), weight of fruit (99.4%) and pulp (90.0%) had higher estimate for broad sense of heritability as well as higher expected genetic advance. Fruit weight, length and breadth was found to be effective selective index. Four selected clones grafted on local rootstock was evaluated in field for flowering and fruiting. Clone No.6-1-3a and 6-3-1-2 was found to bear regularly and early, *i.e.*, before onset of monsoon and gave maximum yield (90 kg and 42 kg/tree) with highest value for TSS (11.2 and 11.0° brix), vitamin C (30.5 and 28.5 mg/100g) and vitamin A (421.0 and 694.25 ug/100g) respectively.

Attri *et al*, (1999) studied fourteen collections of mango for their genetic variability among various fruit characters. The genetic and phenotypic coefficients of variance, heritability and genetic advance was estimated for 15 fruit characters which included length, breadth, peel, pulp, stone. TSS, sugars, ascorbic acid, carotenoids and overall quality.

A remarkable variability was observed among collections for these characters. All the characters showed higher estimates of broad-sense heritability, whereas genetic advance was recorded very high in carotenoids, fruit weight, volume of fruit and ascorbic acid. Fruit weight, fruit length, fruit breadth, fruit pulp and overall quality was found to be effective selection indices.

2.3.1.2. Correlation and path coefficient analysis

Aliyu (2006) reported the relationships between cashew nut yield and nine agronomic traits comprising seven reproductive (nut and floral) and two vegetative characters was studied in 59 selected cashew genotypes over three production seasons. Phenotypic correlation analysis showed that nuts per panicle (r = 0.844), number of nuts per tree (r = 0.988) and number of hermaphrodite flowers per panicle (r = 0.863) was positively correlated with nut yield and could be used as primary components for improving yield. Although correlation analysis showed insignificant association between nut weight and nut yield, path analysis revealed that the trait had significant positive direct effect (0.317) on nut yield. The subtle indirect effects of nut weight and leaf size on nut yield was more important than their direct effects and could be classified as secondary components. Both the direct and indirect effects of weight of the whole fruit and tree canopy on nut yield was negative and appeared detrimental.

Azevedo *et al.*, (1998) estimated genetic and phenotypic correlations for five traits in 27 progenies of cashew trees. Data was obtained from a trial conducted in 1992 at Pacajus, Ceara, experimental station of Embrapa Agroindústria Tropical. The characters studied was plant height (PH), North-South and East-West canopy spreads (NSS,

EWS), and primary and secondary branch numbers (PBN, SBN). All genetic and phenotypic correlations presented positive and significant values. Selection to increase or decrease the average of any one of the five characteristics of cashew plants in the progenies studied affected the average of the others. The 16 month old canopy spread can be predicted from NSS or EWS since correlations between them were high. Correlations between PH and SBN were low, indicating that there is a good possibility of obtaining smaller plants without causing drastic reductions in SBN.

2.3.1.3. Genetic divergence studies

Chipojola *et al.* (2009) reported genetic diversity in cashew would assist in planning for future selection of good high yield germplasm that will produce nuts of high quality and fetch high prices on the market. The genetic diversity and relationship among 40 accessions of cashew collected from 4 populations (Liwonde, Nkope, Kaputu and Chikwawa) was characterized using quantitative and qualitative traits. The study results have revealed similarity values between 35 to 66%. Analyses of genetic similarity based on unweighted pair group method of arithmetic averages grouped the 40 accessions into 4 clusters with 14 sub-clusters and the principal component analysis revealed that apple length, apple nut ratio, nut weight, kernel weight, out turn percent and flower sex ratio accounted for most of the variation. The variation could be attributed to genetic history, ecogeographic origin and selection for desired agronomic traits by farmers. Accessions LW41, NE2, NE4, CH18 and PAL26 showed potential for selection in nut and kernel weight and out turn percent suggesting that this could be a valuable source of variation for tree improvement programme in cashew nuts. The findings suggest availability of broad genetic base that could be exploited for future cashew selection and breeding in Malawi.

Lingaiah *et al.* (1998) studied the genetic divergence estimation by using 20 yield components and yield related characters in one season on 25 cashew genotypes from the Agricultural Research Station, Chintamani, Karnataka. A Dendrogram was constructed which revealed the grouping among the 25 genotypes. The first vector that contributed maximum in discriminating the entries comprised of the developmental morphological traits *viz.*, plant height, stem girth, canopy spread, flowering shoots/m^2 and per cent flowering shoots/m^2. The principal component analysis revealed that Vengurla-3, M 44/3, Vengurla-5, Hybrid 2/16 and Ullal-1 was most divergent. The dendrogram had 4 clusters, with the maximum number of genotypes in the first cluster 9 followed by 6 genotypes in the fourth cluster and 5 each in the other two clusters. The genotypes from the first cluster was late flowering types, and lower yielders (6.13 kg/tree) while second cluster had early flowering types, higher flowering shoots/m^2 (58.22), least panicle length (11.01 cm) and breadth (12.9 cm), less fruits per panicle (3.21) and higher shelling per cent (28.84). The genotypes in cluster III recorded higher nut yield (7.87 kg/tree), number of fruits per panicle but with low kernel weight, nut and apple weight. Cluster IV had the highest values for mean plant height, stem girth, leaf area, panicle length and breadth, apple weight, nut and kernel weight, and least shelling per cent. It was also evident that there was no relationship between genetic diversity and geographical diversity. This was attributed to the movement of germplasm among the various cashew research stations under the AICRP (All India Co-ordinate Research Project) for cashew.

Rajan *et al.* (2009) reported Indian subcontinent is well known for rich genetic diversity in mango. Forty two important cultivars with

potential use in breeding programme was studied for heritability and divergence in fruit characters. Data on 14 quantitative fruit traits was subjected to analysis of genotypic and phenotypic coefficient of variation, heritability, genetic advance and clustering using D^2 with group constellation following Tocher's method. Weight of pulp, fruit, stone and peel, pulp: stone ratio, length of fruit and stone had high genotypic coefficient of variation and heritability accompanied with greater genetic advance as percent of mean indicating important role of these traits for selecting parents in hybridization programme. Using group constellation, cultivars was grouped into three distinct clusters. Cultivar with higher mean for weight of fruit and peel, fruit length, width and thickness of fruit and stone, fruit length and width ratio and pulp: stone ratio was grouped in cluster III and high pulp weight and percent, TSS, weight, length and width of stone were grouped in cluster II. Highest intra-cluster distance was observed in cluster I, while highest inter-cluster distance was between cluster I and II. Pulp weight contributed maximum towards the genetic divergence (34.03%) followed by peel weight (22.65%), TSS (10.22%) stone weight (7.90%) and width (5.46%). These fruit traits may be considered for selecting promising parents.

2.3.2. Molecular markers

The discovery of molecular markers in recent years has greatly enhanced the scope for detailed genetic analysis and approaches to improvement of crop plants. The recently developed DNA-based markers like Restriction Fragment Length Polymorphism (RFLP) and Random Amplified Polymorphic DNA (RAPD) provides excellent tools to study the genetic diversity, eliminate duplicates in germplasm, study genetic relationships, gene tagging, genome mapping etc. These markers

measure diversity at DNA level and are seldom influenced by environmental conditions as compared to morphological markers.

2.3.2.1. Protein markers

Enzyme polymorphisms have been used successfully to identify cultivars in various fruit crops, where analysis is carried out based on the proteins extracted. Enzyme polymorphisms have been used successfully to identify cultivars in various fruit species, including avocado (Goldring *et al.*, 1985), apple (Weeden and Lamb, 1985), loquat (Degani and Blumenfeld, 1986), cherimoya (Ellstrand and Lee, 1987) and pineapple (De Wald *et al.*, 1988). Enzyme polymorphisms have also been used to distinguish hybrids from selfs (Degani and Gazit, 1984; Degani *et al.*, 1989; Goldring *et al.*, 1987) and zygotic from nucellar seedlings in citrus (Roose and Raught, 1988). Isozymes, as genetic markers, have been proven to be reliable, consistent and essentially unaffected by environmental conditions (Bailey, 1983; Torres and Bergh, 1980). However, isozymes can be affected by stages of development and tissue used for extraction (Feret and Bergmann, 1976). Mango leaf isozymes of esterases, aspertate aminotransferase, acid phosphatases and alkaline phosphatases was used to detect possible genetic variation among individuals of so called clones (Gan *et al.*, 1981). However, enzyme polymorphism in mango has not been examined systematically.

Aliyu *et al.* (2007) studied the pattern of diversity among fifty-nine cashew accessions of three breeding populations conserved at the Cocoa Research Institute of Nigeria, Ibadan, Nigeria, assessed using protein-isozyme marker technique. The accessions grouped into six clusters on the dendrogram of Ward's method of squared euclidean distance, indicating "moderate" diversity among Nigerian cashew collections.

Clustering pattern reflects the eco-geographical origin of the accessions. Closer genetic affinity observed between Indian and Local clonal populations. The importance of electrophoresis in genetic diversity study also elucidated.

Gan *et al*. (1981), Degani *et al*. 1990 and Schnell and Knight (1992) have demonstrated the feasibility of using isozymes as biochemical markers in mango. Isozyme variation among mango trees of the same putative cultivar was described by Gan *et al*. (1981) thereby indicating that somatic mutation may occur frequently in certain mango clones. Enzyme polymorphisms was used by Degani *et al*. (1990) to differentiate among mango cultivars and to identify parentage of certain modern mango cultivars. Schnell and Knight (1992) have been able to differentiate zygotic from nucellar seedlings on the basis of enzyme polymorphisms.

Although isozyme markers provide the basis for a relatively simple tool for genetic analysis and linkage studies, it is unlikely that a sufficient number of isozymes will be found to saturate the genome completely and uniformly (Tanksley, 1983). The enzyme extracted and subjected to electrophoresis are a tiny and probably non-representative sample of the total array of proteins present in them. Besides for the mapping purpose, marker loci are useful only if different alleles are segregating in the population of interest.

2.3.2.2. DNA based markers

Molecular marker (DNA marker) is any measurable chemical or molecular characteristic that is inherited in a simple Mendelian fashion (Waltson, 1993). In recent years, molecular biology has provided tools such as DNA markers which can detect differences in genetic information

carried by two or more individuals. Such information is of tremendous importance in forensic studies, paternity testing, identifying genes responsible for disease resistance, evolutionary, linkage mapping, map based cloning, genetic diversity etc. DNA based markers are superior to other markers since they are more in number, highly polymorphic and seldom influenced by the environment.

There are different types of DNA markers and many more are being discovered and find application in various aspects of crop improvement. A broad classification is a) hybridization based markers and b) PCR (Polymerase Chain Reaction) based markers. PCR based markers are relatively easier, cheaper and more widely used, for example RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), SSR etc. The various types of markers and their application in agriculture particularly perennial trees has been reviewed and presented in the following sections.

2.3.2.2.1. Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphism is the original DNA marker, and was developed in the late 1970s (Botstein *et al.,* 1980). The development of this technique was facilitated by the discovery of restriction enzymes. Substitutions occurring in DNA can result in sequence difference within a particular recognition sequence leading to either loss or gain of a particular restriction site and a length difference in the fragment produced. Alternatively, insertions or deletions of DNA segments between two restriction sites may occur changing the length of a particular fragment. RFLP process allows the detection of these length polymorphisms in particular restriction fragments following hybridization with labeled probes.

RFLP markers was first used by Grodzicker *et al.* (1974) in retrovirus and later found various applications in many crop improvement programmes. It involves the restriction of the genomic DNA with endonucleases, fractionating the restricted DNA in a gel, transferring it to a nylon membrane then preferentially visualizing fragments containing particular homologous sequences by hybridizing them to specific DNA probes. RFLPs are codominate *i.e.*, can distinguish between homozygous and heterozygous individuals and can detect large number of polymorphisms in individuals hence ideal for mapping studies. RFLPs have thus been used to construct and anchor linkage maps in many important crops, maize (Helentjaris *et al.*, 1986), tomato (Tanksley *et al.*, 1988), rice (Mc Couch *et al.*, 1988), arabidopsis (Chang *et al.*, 1988), potato (Gebhardt *et al.*, 1989), barley (Huen *et al.*, 1993) and sorghum (Whitkus *et al.*, 1992).

The disadvantages of RFLPs are that they are expensive, laborious, time consuming, involve the use of hazardous radioactive isotopes and need large amount of highly pure DNA. Therefore, presently RFLPs are being replaced by other codominant markers that generate as much information as RFLPs and are easier and cheaper to develop.

2.3.2.2.2. Random Amplified Polymorphic DNA (RAPD)

With the invention of the PCR (Polymerase Chain Reaction), thermocycler machine a new type of DNA based marker revolutionized the field of molecular biology. RAPD markers was developed separately in two laboratories (Welsh and Mc Clelland, 1990; Williams *et al.*, 1990). Single stranded DNA of known sequences (primers) generally 10 base pair long are used to prime a reaction with *Taq* polymerase, deoxy nucleotide triphosphates (dNTP's) in a PCR machine to generate RAPDs.

In case of RAPDs, each amplified product is derived from a region of the genome that contains two short segments which share sequence similarity to the primer and which are on opposite strands and sufficiently close together for amplification to occur (100 bp to 3000 bp). These amplified products are resolved in an agarose gel and visualized under ultraviolet light after staining with ethidium bromide.

RAPDs are easier markers to work with and require very low amount of DNA (5-40 nano grams is sufficient for each reaction). They are also quick, no prior sequence information of the target genome is necessary, detects good number of polymorphisms. RAPDs have been proposed as an alternative to RFLP (Williams *et al.*, 1990), do not require Southern blotting, radioactive labeling and are relatively quick to assay. It is inherited in a Mendelian fashion and can be generated without any prior knowledge of the target DNA sequence (Welsh *et al.*, 1991). It is inherited as a dominant markers, where the presence of a particular band is dominant, and its absence is recessive (Tingley and Tufo 1993).

Co-dominant RAPD markers are comparatively rare. They have been identified when manifested as two bands of different gel mobilities that exhibit complete repulsion-phase linkage in a segregation population (Schulz *et al.*, 1994). RAPD analysis has found applications in population studies (Welsh *et al.*, 1991), biosystematics (Stiles *et al.*, 1993), gene tagging (Nagvi and Chattoo, 1995) and fingerprinting (Mackill, 1996; Virk *et al.*, 1995).

Michelmore (1991) provided new opportunities for accessing the great diversity of disease resistance genes in various crop plants by RAPD marker. Zhang *et al.*, (1977) identified six RAPD markers

associated with the nuclear fertility-restoring gene *Rf*-3. Three of these OPK 5_{800}, OPV 10_{1100} and OPW 1_{350} was mapped on chromosome 1.

2.3.2.2.3. Amplified Fragment Length Polymorphism (AFLP)

AFLP markers was discovered by Vos *et al.* (1995). It involves the restriction of the genomic DNA with two endonucleases, a rare and a frequent cutter, the ligation of an adaptor to the restricted fragment and then amplification in a PCR machine. The 5^1 end of the adaptors is complementary to the restriction sites and the 3^1 end has different nucleotides. The primers correspond to the adaptors and restriction site and the other end corresponding to selective bases. Primers are labeled with p^{32} and the fragments are resolved in polyacrylamide gels. Radioactive labeling is now substituted by following silver staining. AFLPs are also dominant and can detect large number of polymorphisms. They are slightly expensive and laborious when compared to RAPDs but more informative. Like RAPDs the quantity of DNA required for each reaction is low (30 ng).

2.3.2.2.4. Simple Sequence Repeats (SSRs)

Cavalcanti and Wilkinson (2007) reported cashew is a widespread tropical tree crop that is grown primarily for its nuts. Here, they produce an F_1 mapping population of 85 individuals from a cross between CP 1001 (dwarf commercial clone) and CP 96 (giant genotype), and use it to generate two linkage genetic maps comprising of 205 genetic markers (194 AFLP and 11 SSR markers). The female map (CP 1001) contains 122 markers over 19 linkage groups and the male map (CP 96) comprises 120 markers assembled over 23 linkage groups. The total map distance of the female map is 1050.7 cM representing around 68% genome coverage, whereas the male map spans 944.7 cM (64% coverage). The average map

distance between markers is 8.6 cM in the female map and 7.9 cM in the male map. Homology between the two maps was established between 13 linkage groups of the female map and 14 of the male map using 46 bridging markers that include 11 SSR markers. These maps represent a platform from which to identify loci controlling economically important traits in this crop.

The DNA content in higher plants is highly variable. Arumuganathan and Earle (1991) estimated the DNA content in over 100 important crop species. DNA content varied from 0.30 picogram (pg) per 145 million base pairs (mbp) in *Arabidopsis* to over 50 pg or 24,255 mbp in leek. Higher plants have a considerable portion of DNA as repetitive non-coding DNA that is not transcribed. Species with larger genomes normally have more repeated DNA to single copy DNA (Tanksley and Pichersky, 1988). Thus, only a small fraction of the total genetic variation at a DNA nucleotide changes will not affect the amino acid sequence of the protein produced. Thus, the great bulk of genetic variation at the nucleotide level may not have any detectable expression at phenotypic level. SSRs are used to detect polymorphisms in these tandem repeats. They can be hybridization based, or PCR based where the primer(s) used for flank-repeated sequences. SSRs are codominant and relatively easy to perform and are extensively used in marker studies.

2.3.2.2.5. Sequence Characterized Amplified Region (SCAR)

A SCAR is a genomic DNA fragment at a single genetically cloned locus that is identified by PCR amplified using a pair of specific oligonucleotide primers (Williams *et al.*, 1991). Mispriming error amounted in replication studies due to frequently observed problems with reproducibility over all RAPD profiles and specific bands, to

overcome this problem, Paran and Michelmore (1993) converted RAPD fragments to simple and robust PCR markers, termed Sequenced Characterized Amplified Region (SCAR). It is similar to the RAPD method but uses longer primers, which are generally composed of between 18 and 24 bases. By increasing the specifically of the primers, the result become more reproducible and more specific (Hernandez *et al.*, 1999).

Evans *et al.* (2003) reported resistance to powdery mildew is an important objective for cultivar improvement programmes of apple and several different major genes for resistance to mildew are available. Molecular markers linked to such key traits can be used to screen progenies for resistant individuals. A progeny derived from the crab apple 'White Angel' (the source of *Pl-w*) was screened for resistance to mildew for two seasons in the glasshouse and four seasons in the field. DNA bulks of resistant and susceptible seedlings was screened with 176 AFLP primer combinations. Seven AFLP markers was identified that differentiated the bulks, and two of these markers was developed into SCARs, EM M01 and EM M02, mapping at 4.6 and 6.4 recombination units from *Pl-w*.

Kasai *et al.* (2000) developed SCARs based on nucleotide differences within resistant gene like fragments isolated from a potato plant carrying the *Ryadg* gene, which confers extreme resistance to potato Y potyvirus (PVY). It originates from *Solanum tuberosum* subsp. andigena, a susceptible potato plant. The SCAR marker RYSC3 was generated only in genotypes carrying *Ryadg* gene.

Sugita *et al.* (2004) reported RAPD markers linked to the L^3 locus by applying the BSA method to two double haploid (DH) populations in *Capsicum*. These RAPD markers E 18_{272} and E 18_{286} was converted into

SCAR markers by molecular cloning and nucleotide sequencing and they mapped at a distance of 4.0 cM from the L^3 locus.

Potato virus Y (PVY) is the only potyvirus infecting *Capsicum annuum* L., a monogenic dominant gene *Pvr4* confers resistance to PVY pathogen. Andres *et al.*, (2004) conducted BSA to search for RAPD markers linked to the *Pvr4* gene using segregating progenies obtained by crossing a homozygous resistant 'Serrano Criolle de Morelos-334' with a homozygous susceptible 'Yolo Wonder' cultivar. Eight hundred decamer primers was screened to identify one RAPD marker UBC 19_{1432} linked in repulsion phase to *Pvr4*. This marker was converted into a SCAR marker SCUBC 19_{1423}.

Quirin *et al.* (2005) reported SCAR primers for the detection of *phyto*.5.2 a major QTL for resistance to *Phytophthora capsici* Leon. in pepper. They screened genotypes of *C. annuum* and *C. chinese* with a series of RAPD primers. One primer, OPDo4, amplified a single band only in those *C. annuum* and *C. chinese* genotypes showing the highest level of resistance and amplified product was cloned, sequenced and converted to a SCAR marker. These primers was observed to define locus on *Capsicum* chromosome 5 which was tightly linked to *phyto.5.2*.

Techawongstein *et al.* (2006) studied the inheritance of pungency in *Capsicum* by SCAR marker and they crossed the highly pungent variety. "YuYi" (YY) from China. They conducted bulked segregant analysis and reported capsaicinoid content, broad sense heritability (h_b^2) and DNA finger print with UBC 20 SCAR primer. They compared results among F_2 populations and their parents. The SCAR primer for UBC 20 could amplify a common fragment of 1700 bp in both low and high pungency chilli plants of all generation.

Julio *et al.* (2006) reported that SCAR markers linked to three disease resistances *viz.*, black root, blue-mold and potato virus Y (PVY) was reported in a *Nicotiana tabaccum* L. accession by AFLP assay. Tomato spotted wilt virus (TSMV) is a serious disease in tobacco (*Nicotiana tabaccum* L.). The breeding line "Polalta" contains a single dominant gene conferring resistance to TSMV that was introgressed from *N. alata* Link and Otto. The DNA bulks from susceptible and resistant double haploid lines derived from a cross between susceptible cultivar "K326" and "Polalta" was analysed to identify resistance gene by AFLP technology and bulked segregant analysis. Four AFLP fragment was successfully converted to SCAR marker (Moon and Nicholson, 2007).

Kim *et al.* (2008) developed molecular marker linked to the locus conferring resistance to tobamovirus pathotype in pepper plants, they performed AFLP with 512 primer combinations for susceptible 'S pool' and resistant 'R pool'. A total of 19 primer pairs produced scorable bands in the R pool. Further screening with these primers pairs was done on DNA bulks from T102, a $BC_{10}F_2$ generation for the L4 locus. AFLP markers was finally selected and designated L4-a, L4-b and L4-c, whereas no recombination for L4-b was seen in 20 individuals of each DNA bulk. L4-b was successfully converted into a simple 340 bp SCAR marker designated L4SC340, which mapped 1.8 cM from the L4 locus in T102 and 0.9 cM in another $BC_{10}F_2$ population.

2.3.2.2.6. Expressed sequence tags (EST)

EST markers have been developed in several laboratories. These markers are developed based on extensive sequence data of regions of the genome that are expressed. However, once developed they provide

high quality and highly consistent results. These markers are directly associated with functional genes. EST markers are likely to be less polymorphic than SSR markers.

2.3.2.2.7. Single Nucleotide Polymorphism (SNP)

The vast majority of differences between individuals are single nucleotide polymorphisms due to point mutations. As such, there are a vast number of potential SNP markers in all species. Considerable amounts of sequence data are required from parental genotypes to develop SNP markers, however, their great advantage lies in the potential to screen those using methods such as microarrays, which do not involve electrophoresis.

2.3.2.3. Other types of markers

Many types of DNA based markers are being developed and find a myrid of application in crop improvement. Polymorphism in chloroplast DNA (cpDNA) is detected by first amplifying the chloroplast DNA and then restricting it. Since the chloroplast is maternally inherited, such polymorphisms are useful for evolutionary studies. More sophisticated markers like Single Nucleotide Polymorphism (SNPs), Expressed Sequence Tags (ESTs) are now being used.

2.4.1. Application of DNA based markers in the improvement of cashew

Croxford *et al.* (2005) reported cashew (*Anacardium occidentale* L.) is the most economically important tropical nut crop in the world, and yet there are no sequence tagged sites (STS) markers available for its study. They used an automated, high-throughput system to isolate

cashew microsatellite from a non-enriched genomic library blotted onto membrane at high density for screening. Sixty-five sequences contained a microsatellite array, of which 21 proved polymorphic among a closely related seed garden population of 49 genotypes. Twelve markers was suitable for multiplex analysis. Of these, 10 amplified in all three related tropical tree species tested: *Anacardium microcarpum*, *Anacardium pumilum*, *Anacardium nanum*.

Dhanaraj *et al*. (2002) reported Random Amplified Polymorphic DNA (RAPD) markers used to estimate the diversity among 90 cashew accessions from the National Cashew Gene Bank. A dendrogram constructed using Ward's method, squared euclidean distance which confirmed that the diversity of Indian cashew collections can be considered to be "moderate" to "high". A core collection identified based on the study which represents the same diversity as the entire population. This could be the first step towards more efficient germplasm management of cashew in India.

Samal *et al*. (2003) studied the genetic relationships of twenty varieties of cashew on the basis of morphological characters and RAPD markers. Results obtained for the phenotypic characters based on similarity coefficient was divided into four clusters with 70% similarity. By means of similarity coefficients, cluster I was found to consist of twelve varieties. Cluster II consisted of a single variety, NRCC-1, cluster III consisted of six varieties and cluster IV had only one variety, Vridhachalam-2. The analysis started by using RAPD markers that allowed us to distinguish 20 varieties. A total of 80 distinct DNA fragments ranging from 0.2 to 3.0 kb was amplified by using 11 selected random 10-mer primers. Genetic similarity analysis was conducted for the presence or absence of bands in the RAPD profile. Cluster analysis clearly showed that 20 varieties of cashew grouped into two major

clusters based on similarity indices. The first major cluster comprised one minor cluster. The other major cluster was divided into two sub-minor clusters, one sub-minor cluster having three varieties and the other sub-minor cluster was represented by 15 varieties. Among the 20 varieties, Ullal-3 and Dhana (H-1608) showed the highest similarity indices (87%). It was noted that Vengurla-2 and Vengurla-3 was not grouped into a single cluster but Vengurla-4 has 82% similarity to Vengurla-3. The variety Vengurla-2 has very close similarity (85%) with variety Vridhachalam-3 (M-26/2). The analysis of genetic relationships in cashew using morphological traits and RAPD banding data can be useful for plant improvement, descriptions of new varieties and also for assessment of varietal purity in plant certification programmes.

RADP markers was used to study the genetic differences among twenty Tanzanian cashew accessions and between individual cashew genotypes from Tanzania, Mozambique, Guinea-Bissau, Brazilian dwarf genotypes and a Brazilian genotype. Out of six Operon 10 mer primers studied, three of them *viz.*, OPF 2, OPF 3 and OPF 5 revealed maximum polymorphism. The results revealed that was considerable similarity among the twenty Tanzanian genotypes and differences between the genotypes from different geographical regions. The importance of broadening the present narrow genetic base of Tanzanian cashew by including exotic germplasm emphasized (Mneney *et al.*, 1997). In another study, RAPD markers was used to study the genetic relatedness among accessions from Ceylon, India, Mozambique, Tanzania, Brazil and Tanzania and a unique fragment was identified from the accessions from Cook Island (Mneney *et al.*, 2001).

Archak *et al.* (2003) reported Indian cashew breeding programme has produced 24 selections and 11 hybrids with increased yield and excellent nut characters. Molecular profiles of these varieties was

developed using a combination of five RAPD and four ISSR primers pre-selected for maximum discrimination. A total of 94 markers was generated which discriminated all the varieties. There was no correlation between the relationships based on molecular data and the pedigree of the varieties. Narrow range of average similarity values among major cashew breeding centres with only 3.6% of molecular variance partitioned between them was attributed to the exchange of genetic material in developing varieties. Difference in the average similarity coefficients between selections and hybrids was low indicating the need and scope for identification of more parental lines in enhancing the effectiveness of hybridisation programme.

Eiadthong *et al.* (1998) studied the similarity among some *Mangifera* species and cashew by using chloroplast DNA. Total DNA was extracted and chloroplast DNA was amplified using the primer OPF 106 which is responsible for amplifying the conserve sequence of rbcL. The amplified product was then restricted by 20 different endonucleases and similarity was estimated among the cultivars studies based on the number of restricted products obtained. Cashew and some species of *Mangifera* (both belonging to family Anacardiaceae) was found to have about 72 per cent similar DNA indicating the possibility of common ancestry.

Thirty four released cashew varieties and hybrids from India and a clone resistant to tea mosquito have been fingerprinted by using RAPD markers. Ten Operon 10mer primers was found suitable to distinguish all the 35 cultivars and the genetic distances was estimated among them. The results revealed that the genetic base of the cashew cultivars is not narrow as reported earlier, but is moderate. The clustering of the cultivars was more or less similar to their geographical origin. The

cultivars from Kerala was the most diverse when compared to the cultivars from other geographical regions (Rao, 1999).

Karihaloo *et al.* (2000) reported the use of RAPD, AFLP and ISSR in fingerprinting cashew accessions. A total of 564 markers was amplified when 50 decamer primers was produced on 19 cashew accessions. The average number of bands produced by a primer was 11.2 and the frequency of polymorphic markers was 87.9 per cent. One primer (S-11) was adequate to distinguish all 19 accessions. Twelve accessions was analyzed with AFLP markers, where 94 markers was produced which exhibited a high level of polymorphism and differentiated all the accessions. Seven microsatellite primers, $(GATA)_4$, $(GACA)_4$, $(ACTG)_4$, $(CCAT)_4$, $(GGAT)_4$, $(GAAGTGGG)_2$ and $(CA)_8$ was used to detect inter microsatellite polymorphism and for fingerprinting. None of them was useful in detecting polymorphism or fingerprinting.

2.4.2. Application of DNA based markers in the improvement of other members of Anacardiaceae

Mnejja *et al.* (2005) reported 47 new simple sequence repeats (SSRs) obtained from CT/AG enriched genomic library of almond cv. Texas (syn. Mission). Forty-two of them was polymorphic in a sample of eight almond cultivars and 31 of these was single-locus. The average values of the number of alleles per locus (6.6), and mean observed (65%) and expected (76%) heterozygosities for these 31 SSRs indicated a high level of variability. All cultivars studied could be individually identified using any one of the five SSRs. Transportability to other *Prunus* species (Peach, Sweet cherry, Japanese plum and Apricot) was also high (83-100%).

Ahmed *et al.* (2005) reported Simple Sequence Repeat (SSR) and Sequence-Related Amplified Polymorphism (SRAP) molecular marker systems was used to analyse four commercially important pistachio rootstocks two species of *Pistacia atlantica* (cv. 'Standard Atlantica'). *P. integerrima* (cv. 'Pioneer Gold') and two interspecific hybrids of the same, 'Pioneer Gold II' ('PGII') and 'University of California at Berkeley 1' ('UCB-1'). A total of 35 putative alleles was detected by 12 SSR primer pairs with an average of 2.9 alleles per locus. The number of putative alleles ranged from 2 to 5 in the pistachio rootstocks tested. The number of bands produced by the SRAP protocol was highly variable, ranged from 11 to 38, with an average of 25.2 per primer combination. Eight primer combinations resulted in 104 (51%) polymorphic markers in these samples. SSR and SRAP markers successfully identified all pistachio rootstocks tested from their unique fingerprints. Both SSR and SRAP molecular markers confirmed that the observed variation in 'UCB-1' rootstock is genetic. Thus, there will always be variation among 'UCB-1' hybrid seedling progeny due to the segregation of alleles when propagated by seed. They also found evidence of contaminating pollen other than from *P. integerrima* in some hybrid 'UCB-1' rootstock progeny produced by closed pollination. Only alleles from the cultivar 'Standard Atlantica' was observed in abnormal 'UCB-1' rootstock in the nursery. They found that the poor performance of the scion cv. 'Kerman' on 'UCB-1' rootstock was not due to 'UCB-1' rootstocks displaying abnormal behavior in the nursery. They have successfully developed two efficient marker systems for genome analyses in pistachio, which can be used for identification and management in pistachio rootstock production.

Schnell *et al.* (2005) reported that development and characterization of 15 microsatellite loci isolated from *Mangifera indica* L. These markers was evaluated using 59 Florida cultivars and four related

species from the USDA germplasm collection for mango. Two loci was monomorphic and 13 polymorphic, with two to seven alleles per locus. Four loci departed significantly from Hardy-Weinberg equilibrium and have significant heterozygote deficiency. Nine loci exhibited significant linkage disequilibrium. Cross-species amplification was successful in four related species. These loci are being used to investigate patterns of genetic variation within *M. indica* and between closely related species.

Kashkush *et al.* (2001) reported Amplified Fragment Length Polymorphism (AFLP) information was used for identification of mango (*Mangifera indica L.*) cultivars, for studying the genetic relationship among 16 mango cultivars and seven mango rootstocks and for the construction of a genetic linkage map. Six AFLP primer combinations produced 204 clear bands and on the average 34 bands for each combination. The average band-sharing between cultivars and rootstocks was 83% and 80%, respectively. The average band-sharing for mango is 81%. A preliminary genetic linkage map of the mango genome was constructed, based on the progeny of a cross between 'Keitt' and 'Tommy-Atkins'. This linkage map consists of 13 linkage groups and covers 161.5 cM defined by 34 AFLP markers.

Yamanaka *et al.* (2006) used AFLP analysis to explore the genetic relationship and diversity between and within 4 *Mangifera* species. They analyzed 35 accessions comprising 8 cultivars and 3 landraces of *M. indica* L., 11 landraces of *M. odorata* Griff., 7 landraces of *M. foetida* Lour., and 6 landraces of *M. caesia* Jack. Using 8 primer combinations produced a total of 518 bands, 499 (96.3%) of which was polymorphic among the 35 accessions. Clustering analysis showed that all 35 accessions was basically classified into 4 groups corresponding to the 4 *Mangifera* species. Our results indicate that the genetic relationship of

these 4 *Mangifera* species based on AFLP analysis is in good agreement with their classification by classic methods. In addition, it was clearly revealed the genetic diversity between and within 4 *Mangifera* species. The findings obtained in this study are useful for the breeding in *Mangifera* species.

Viruel *et al.* (2005) report the sequence and variability parameters of 16 microsatellite primer pairs obtained from two mango (*Mangifera indica* L.) genomic libraries after digestion of DNA of the cultivar Tommy Atkins with *Hae*III and *Rsa*I and enrichment in CT repeats. Although no significant differences was recorded between the two libraries in the informativeness of the markers obtained, the RsaI library was shown to be more useful than the HaeIII taking into account the efficiency of the library and the feasibility of clone sequencing. The polymorphism revealed by those microsatellites was evaluated in a collection of 28 mango cultivars of different origins. A total of 88 fragments was detected with the 16 simple sequence repeats (SSRs) with an average of 5.5 bands/SSR. Two primer pairs amplified more than a single locus. The mean expected and observed heterozygosities over the 14 single-locus SSRs averaged 0.65 and 0.69 respectively. The SSRs studied allowed the unambiguous identification of all the mango genotypes studied and this discrimination can be carried out with just three selected microsatellites. UPGMA cluster analysis and principal coordinates analysis group the genotypes according to their origin and their classification as monoembryonic or polyembryonic types reflecting the pedigree of the cultivars and the movement of mango germplasm. The results demonstrate the usefulness of microsatellites for studies on identification, variability, germplasm conservation, domestication and movement of germplasm in mango.

Adato *et al.* (1995) used ten different minisatellite probes to fingerprint 20 mango cultivars. DNA was extracted and restricted with *Hind* III or *Dra* I and then hybridized with minisatellite probes. Jeffery's minisatellite probe 33.6 was found to be most effective to show many polymorphic loci.

The genetic affinity among 15 mango cultivars was estimated by using RAPD markers. One hundred and nine amplified products was produced by using 13 selected 10 mer primers. From the dendrogram it was evident that 'Manila' and 'Carabao' was the most similar and the clustering was based on their geographical origins. Bulked segregant analysis was carried out on the monoembryonic and polyembryonic cultivars and a specific RAPD band was identified for polyembryony (Lopez-Valenzuela *et al.*, 1997).

Seven SSR-anchored primers was used in an attempt to fingerprint and estimate the genetic relatedness and diversity of 22 commercial mango cultivars from Thailand (Eiadthong *et al.*, 1999). Forty primers was screened on 5 cultivars and only 7 was selected, depending on their ability to distinguish the cultivars. The seven primers was not sufficient to distinguish 22 cultivars. Fifty six polymorphic bands was obtained which was used to construct a dendrogram. In the dendrogram, the Thai cultivars formed three groups. The four varieties from Florida formed 2 separate clusters, 'Adam' and 'Tommy Atkins' grouped with the Indian varieties 'Alphonso' and 'Neelum' while the other 2 was placed with the Thai varieties.

Ravishankar *et al.* (2000) assessed the genetic relatedness among 18 commercial mango cultivar from India with RAPD markers. Out of 30 primers screened 19 of them produced a total of 178 bands (130

polymorphic and 48 monomorphic) which was used to study the genetic relatedness. The cultivars from western, northern and eastern India clustered together while the south Indian cultivars clustered separately.

Fifty commercial cultivars of mango from different parts of India was fingerprinted by using 139 RAPD markers generated by 10 decamer primer and the genetic distance among the cultivars was found to be moderate to high. The mango hybrids with a common parent clustered together while the regular and irregular bearers clustered separately. The variety 'Mulgoa' was found to be very distant from all the cultivars studied and the south Indian cultivars was more diverse than the other cultivars (Kumar *et al.*, 2001).

Pistacia is an important dioecious nut tree belonging to Anacardiaceae. Bulked Segregant Analysis (BSA) was used to identify a RAPD marker (OPO 08_{945} bp) associated with the female *Pistacia* trees. Seven hundred different decamer primers was screened in the two bulks of 7 individuals of both the sexes and only one primer OPO 08 was found useful. Once the sex-linked marker was identified, its usefulness was tested in a population of 94 seedlings. Forty of them tested positive and fifty four negative, the results was tested using Chi square test which revealed that the ratio did not differ significantly from 1:1 male: female ratio (Hormaza *et al.*, 1994).

BSA was used to identify RAPD markers with sex in wild *Pistacia* species *viz.*, *P. atlantica, P. terebinthus and P. euryarpa* which are extensively used for rootstocks for *P. vera* in Turkey (Kafkas *et al.*, 2001). The DNA of 10 male and 10 female was bulked and 472 primers was screened. Primers BC 156 and BC 360 produced female specific bands

in *P. eurycarpa*. Primer OPAK 09 amplified a female specific band in *P. atlantica*.

Parfitt and Badeness (1997) performed a phylogenetic study on 10 *Pistacia* species based on chloroplast DNA analysis. *P. khinjuk* and *P. vera* could not be discriminated suggesting the possibilities of them being one species. Two wild species *P. atlantica* and *P. terebinthus* was also closely related to the domesticated species *P. vera*. Based on the DNA profiles they subdivided the genus into *Terebinthus* and *Lentiscus*.

Kafkas and Perl-Treves (2001) studied the taxonomic relationships and genetic variation between *Pistacia vera* and wild species, *P. atlantica*, *P. terebinthus*, *P. eurycarpa* and *P. Khinjuk* using RAPD markers. Forty wild *Pistacia* genotypes and two *P. vera* varieties generated 138 bands with 10 arbitrary primers. The dendrogram revealed that *P. terebinthus* was the most divergent species and clustered separately, *P. vera* and *P. eurycarpa* was closely related. Species specific bands was identified for each of the 4 species. From the results they suggested that *P. eurycarpa* may be a hybrid between *P. khinjuki* and *P. atlantica*, and the close relationship of *P. vera* and *P. khinjuk* confirmed the research findings of the earlier workers who suggested that they are one species based on the analysis of chloroplast DNA.

2.5. Other applications of DNA markers in the improvement of horticultural crops

2.5.1. Phylogenetic analysis

Nicolosi *et al*. (2000) investigated the phylogenetic relationship among 36 *Citrus* accessions and one accessions in *Poncirus*. *Fortunella*,

Microcitrus and *Erimorcitrus* with 262 RAPDs, 14 SCARs and cpDNA. The results was more or less similar to that of the previous workers. *Fortunella* was found to be phylogenetically close to *Citrus* and within *Citrus* the two sub-genera was separated *viz*., *Citrus* and *Papeda*. *C. celebica* and *C. indica* was quite distinct. *Archicitrus* and *Metacitrus* was also separate while the lemon and Palestine sweet lime clustered with Pummelo.

Kumar *et al*. (1999) evaluated the phylogenetic relationship between 3 species of *Paulownia* and an interspecific hybrid. *Paulownia* is a perennial fast growing tree in SE Asia for its wood. Two hundred and two RAPD markers was generated by 5 decamer primers. In the dendrogram, *P. fortune* and the hybrid grouped separately while *P. tomentosa* and *P. kawakamii* clustered separately. The results strongly suggested that *P. fortune* could be one of the parents of the hybrid.

Digitalis is a member of Scrophulariaceae consisting of 19 species grown in the mediterranean regions for its therapeutic use, source of cardiac glycosides and the shrubs have ornamental value. RAPD markers was used to study the phylogenetic relationship among 42 accessions that belonged to 7 species of Digitalis. The four 10-mer produced 91 amplified products sized 400 to 3000 bp out of which 90 was polymorphic and only one monomorphic. The dendrogram constructed showed 7 distinct clusters and each cluster was specific to one species (Nebauer *et al*., 2000).

The phylogenetic relationship was estimated between 5 species *Caladium*, 3 species of *Xanthosom*, one *Hapaline*, *Alocasia* and *Protacrum* species all belonging to Araceae with AFLP markers. Although 235 polymorphic bands was obtained by using 3 primer combinations. A

dendrogram was constructed which clustered the 5 species of *Calcadium* besides each other. The 3 *Xanthosom* species formed a separate cluster and the *Hapaline*, *Alocasia* and *Protocrum* species clustered individually (Loh *et al.*, 2000).

2.5.2. Cultivar identification

Castanea sativa Mill. (Chestnut) is a monoecius crop grown for its nuts and wood in mediterranean region. RAPD markers was used to identify some of the clones especially the brown types which are identified based on the geographical region and not morphological traits. Most of the primers used amplified fragments in all the brown types. Six of the clonal variants was distinguished from the other clones by the primers U3, U1 and U19 or U10, U12, U13 and U15. These clones was distinct from all the other clones. (Galderisi *et al.*, 1999).

'Albarino' is one of the most important grapevine (*Vitris vinifera*) grown in Spain for its wine. However, ampelographic data often fails to identify 'Albarino' from the other closely related clones. The clone also appears different when cultivated in different geographical regions, hence overlapping of varietal names and identification has been a problem. Sixteen accessions of 'Albarino' collected from different parts of Spain and some related cultivars (which was thought to be 'Albarino') was analyzed with RAPD and microsatellite markers. Both techniques revealed that authenticity of the 16 'Albarino' clones and separated them from the other clones. No polymorphism was observed between the 16 clones of 'Albarino' but was observed between the other accessions. (Loureiro *et al.*, 1998).

Starman *et al.* (1999) used DAF (DNA Amplification Fingerprinting) and ASAP to estimate the genetic differences among 11 poinsettia cultivars. The DAF technique involves the use of 8 mer primers and mini hair pin primer. ASAP is said to have a higher resolution power and can detect differences that DAF technique fails to detect. Out of the 11 poinsettia varieties used in this study there is a problem in differentiating the varieties, 'Nutcraker Red' and 'Peterstar Red' because of their similar morphology. DAF technique failed to differentiate these two varieties, so the ASAP technique was employed. Here the DNA of both the varieties was first amplified with an octamer primer and then reamplification was carried out with either HpB52 or HpD4 (hair pin primer). Complex polymorphic bands was produced that was able to differentiate the two varieties.

Caladiums are popular ornamentals and identification solely on morphology is often difficult. Loh *et al.* (1999) used 173 AFLP markers generated by 17 primer combinations to distinguish 7 cultivars. Unique markers was identified in all the cultivars that can be further used for cultivar identification and varietal rights.

2.5.3. Paternity analysis

In crops, paternity analysis is useful to determine the parentage of a superior seedling which could be further used in breeding programmes.

'Braedurn' is the most important apple cultivar of New Zealand whose parentage in not known. RAPD, RFLP and Isozymes was used to identify the parents of this cultivar. First a set of 186 trees was selected based on the origin of 'Braeburn'. Then the allele PGM-3 was detected to be heterozygous in 'Braeburn', hence a subset of 15 cultivars was selected from 186 that had the PGM-3 loci. Two hundred and sixty eight

RFLP fragments was obtained from 41 probe enzyme combinations. Bands was studied for their presence in 'Braeburn' and the possible parents. Thirty nine primers was used to produce 487 RAPD bands. The RAPD markers was used to estimate the genetic diversity among the 16 cultivars. Based on the results generated by the three marker systems, it was reported that 'Lady Hamilton' is probably one of the parents of 'Braeburn' (Gardiner *et al.*, 1996).

Eucalyptus graniticola was discovered only in 1987 and was thought either to be an underscribed species or a rare hybrid. The morphological similarities of certain other species in the vicinity of *Eucalyptus graniticola* suggest it is a hybrid. Rosesetto *et al.* (1997) used RAPD technology to analyze its parentage. Ninety-six markers was detected in the 3 samples using 9 primers (9-mers). The results revealed that there was an additive inheritance of RAPD markers from *E. rudis* and *E. drummondii*, 40 and 35 per cent respectively and 25 per cent of the markers from *E. graniticola* was shared by both the parents, strongly suggesting that *E. graniticola* is a rare hybrid between *E. rudis* and E. *drummondii*.

Heinkel *et al.* (2000) performed a parental analysis on the plum cultivar 'Cacaks Beauty', 'Cacaks Best', 'Cacaks Early' and 'Cacaks Fruitful' with RAPD markers. It has been earlier reported that cultivars 'Cacaks Beauty', 'Cacaks Best' and 'Cacaks Early' are from 'Wangenheim' X 'Pozegaca' and 'Cacaks Fruitful' from 'Stanley' X 'Pozegaca'. Twenty six selected primers amplified 158, 144 and 160 fragments in 'Wangenheim' 'Pozegaca' and 'Stanley', respectively. Out of these 102 was monomorphic and 18 specific to 'Wangenheim', 12 in 'Pozegaca' and 37 in 'Stanley'. On studying the inheritance of these markers it was evident that 'Stanley' and 'Wangenheim' showed high conformity with 'Cacaks

Beauty', 'Cacaks Best' and 'Cacaks Early' contrary to the earlier report on their origin. From the RAPD data for the variety 'Cacaks Fruitful' it was in accordance with the earlier report where 'Pozegaca' and 'Stanley' are the parents.

2.5.4. Identification of somaclonal variants and somatic hybrids

Recently developed five somatic hybrids was tested by RAPD markers to identify their forms. Five 10 mer primers was used to 'fingerprint' the fusion parents and parent specific bands was observed ranging from 100 to 1800 bp. The somatic hybrids had a combination of the parental profiles, whereas one of the parents had a similar banding profile of the parent (Xu *et al.*, 1993).

Japanese pine (*Pinus thunbergii*. Parl.) is being propagated *in vitro* for the past decade. RAPD analysis was carried out on 36 and 10 year old micropropagated shoots in order to test their genetic stability over the years (Goto *et al.*, 1998). The number of amplified products varied with the primers, but all the primers produced monomorphic bands indicating similar genetic constitution. Some of the micropropagated shoots had differences in needle morphology (short, medium and long), but differences was not observed in the amplified products. This difference was an effect of the physiological state and not somaclonal variation.

Tarras *et al*. (1999) reported the application of RAPD analysis in detecting somaclonal variants in a perennial ornamental plant. *Ananas comosus variegatum*. From an earlier tissue culture experiment many phenotypic different shoots was identified. Six 10-mer primers was used for the RAPD analysis which produced many amplified products that easily distinguished the variants.

2.5.5. Markers and sex identification

In many dioecious plants gender influences economic value, breeding schemes, and opportunities for commercial use of genetically transformed materials. Detecting the sex of the plants at the seedlings or early stage is thus very useful.

RAPD markers was used to identify a marker associated with sex in *Pistacia vera* using Bulked Segregant Analysis. Two crosses was used in this study and the DNA of the parents was bulked and screened with 700 decamer primers. One primer OPO8 produced a 945 bp fragment that was present in the female parents of both the crosses and in the female F_1s. To test the usefulness of this marker it was tried on a population of 94 seedlings and the marker was consistently segregated in the expected ratio of 1:1 (Hormaza *et al.*, 1994).

Sondur *et al.* (1996) constructed a genetic linkage map in papaya using a F_2 population and RAPD markers. The cross was between UH 356 and 'Sunrise solo'. Out of 596 decamer primers that was screened, 96 was polymorphic and mapped to 11 linkage groups. The sex locus 'SEX1' was located in the first linkage group and was flanked by T1C and T12 markers. These markers are useful in identifying the sex of papaya seedlings.

Actinidia is an economically important dioecious fruit in South East Asia and China. Harvey *et al.* (1997) used Bulked Segregant Analysis in the progeny in one family to identify a marker with either sex. Five hundred 10 mer primers was screened and one marker 800 bp identified as SmY was found in all the male siblings and the male parent.

Another marker, SmY was an 850 bp fragment that appeared in the female bulk, all in 18 of the 20 female progenies, and the female parent. These markers was analyzed in 11 other families where their segregation ratio was 1:1. Markers was thus identified was useful to sex the plants at an early stage of development.

Rapaport *et al.* (1998) used Bulked Segregant Analysis to identify a RAPD marker $UBC354_{560}$ to be associated with femaleness in *Salix viminalis* L. Three hundred and eighty decamer primers was screened which produced 1080 bands out of which 984 was monomorphic and only the 560 bp fragment produced by UBC354 was present in the female parent and in all the female F_1s.

A 400 bp RAPD marker generated by OPA 8 was found to be associated with male sex phenotype in *Cannabis sativa* L (Mandolino *et al.*, 2000). This sex specific marker was identified in 14 male plants and was screened on 167 plants. All the male plants and 3 female and 20 monoecious plants had the band. This sex specific band was eluted out of the gel, sequenced and primers was developed converting it to a SCAR marker. One of the 3 female plants in which the 400 bp male-associated band was present also had this SCAR marker. Though this marker amplified the product in one female plant it proved very useful in identifying male plants in the entire population.

2.6. Tagging of genes with molecular markers

2.6.1. Tagging major genes with RAPD markers

The process of locating genes of interest via linkage of markers is referred as “gene tagging” (Chawla, 2002). RAPD markers are suitable

for tagging major gene controlling disease resistance, pest resistance etc. In rice, gene conferring resistance to blast was tagged using RAPD markers (Zhu *et al.*, 1993). Wang *et al.* 1995 tagged major genes for thermo sensitive genetic male sterility in rice by RAPD markers.

Martin *et al.* (1991) surveyed polymorphism for bacterial wilt against Pseudomonas in the Near Isogenic Lines (NILs) of tomato through RAPD and identified three markers. These markers was identified to be linked to '*Pto'* gene conferring resistance to the bacterial wilt.

Mohan *et al.* (1994) and Nair *et al.* (1995) used BSA and tagged RAPD markers OPF 8 and OPF 10 to the rice *Gm2* gene that confers resistance to biotype 1 of gall midge in rice. The *Gm4t* gene for gall midge resistance was also tagged by RAPD markers E 20_{570} and E 20_{583} which was then converted into sequenced tagged sites.

Bulk segregant analysis on F_2 populations of Nipponbare x Kasalath cross was used to determine RAPD markers in a specific interval in the middle of the chromosome 6 of rice for tagging the photoperiod sensitivity gene. Fourteen markers tightly linked to the photoperiod sensitivity gene was identified. They was converted into STS by cloning and sequencing the polymorphic fragments (Monna *et al.*, 1995).

Nagvi and Chattoo (1995) reported the identification of two RAPD markers OPF 6_{2700} and OPH 18_{2400} linked to *Pi*-10 blast resistance gene in rice. These markers was converted into SCARs and based on the polymorphisms appearing as differences in the length of the SCARs alternate was considered for the indirect selection.

RAPD analysis was conducted with the combined use of near isogenic lines and BSA to tag the bacterial blight resistance genes *Xa*-21, *Xa*-3, *Xa*-4 and *Xa*-5 in rice (Yoshimura *et al.*, 1995; Zhang *et al.*, 1994). From the survey of 260 decamer primer OPAC 5 was detected to amplify specifically a 0.9 Kb fragments from the DNA of bacterial blight susceptible plants. The distance between the RAPD marker OPAC 5_{900} and *Xa*-13 was estimated to be 5.3 cM (Zhang *et al.*, 1996).

Procunier *et al.*, (1997) tagged the R-gene T_{10} with SCAR marker linked to loose smut resistance gene which caused by *Ustilago segatium tritici* in wheat. Chunwongse *et al.*, (1997) developed high resolution genetic map in tomato and tagged *Lv* resistance locus, which confers powdery mildew resistance by RAPD marker. Tai *et al.* (1999) studied expression of the *Bs2* pepper gene which confers resistance to bacterial spot disease in tomato.

Shen *et al.* 1998 surveyed two F_2 populations and one BC_1 population have used to confirm the linkage of the markers for fertility restorer gene *Rf*-3 gene and they tagged with RAPD markers OPB 18_{1000} at a distance of 5.3 cM in chromosome 1.

Jeon *et al.* (1999) used BSA and tagged RAPD marker OPD 7 to the rice *bph* 1 gene conferring resistance to biotype 1 of BPH. OPD 7 yielded a 700 bp fragment which was present in resistant parent and resistant F_2 plants but absent in susceptible parent and susceptible F_2 plants. Chromosomal regions surrounding the *Bph*-1 was examined with additional RFLP and microsatellite markers on chromosome 12 to define the location of the RAPD marker and *Bph*-1.

Sandhu *et al.* (2002) carried out RAPD analysis of herbicide-resistant Brazilian rice lines produced via mutagenesis. Among eighty random primers tested, 10 was selected for a detected study of RAPD markers that could tag herbicide resistance genes. Resistant and susceptible lines produced variation in the RAPD patterns and certain bands was found only in certain lines. Twenty lines resistant to herbicide was selected as a result of this study.

2.6.2. QTLs tagged with RAPD markers

Extending the molecular technique from mapping the major genes to the QTL, resulted in the identification of both major and minor genes conferring resistance to pathogens and insect pest. Genetic dissection of several quantitative traits in to single Mendelian factors in tomato and many QTLs have been classified using DNA markers in various crop plants such as in maize (Edwards *et al.*, 1992) and tomato (Chague *et al.*, 1996; Bernacchi *et al.*, 1998).

Nishi *et al.* (2003) found QTL linked to bacterial wilt resistance in tobacco. The susceptible variety 'Michinoku 1' and the resistant variety 'W6' was screened for AFLP polymorphisms with 3072 primer combinations. They identified 117 polymorphism markers and these markers was analyzed in 125 doubled haploid lines for analyzing the association between the markers and bacterial wilt resistance and they drawn a linkage map consisting of 10 linkage group. One QTL for bacterial wilt resistance was identified on a 32 cM linkage group consisting of 15 markers.

2.6.3. Tagging of gene with other markers

Dondini *et al*. (2007) studied the linkage maps of the apricot accessions 'Lito' and 'BO 81604311' was constructed using a total of 185 simple sequence repeat (SSR) markers sampled from those isolated in peach, almond, apricot and cherry, 74 was derived from a new apricot genomic library enriched for AG/CT microsatellite repeats (UDAp series), and in total, 98 had never been mapped in Prunus before. Eight linkage groups putatively corresponding to the eight haploid apricot chromosomes was identified for each parent. The two maps was 504 and 620 cM long, respectively, with 96 anchor markers showing a complete co-linearity between the two genomes. As few as three gaps larger than 15 cM was present in 'Lito' and six in the male parent, the maps align well with all the available SSR-based Prunus maps through the many common anchor loci. Only occasionally inverted positions between adjacent markers was found, and this can be explained by the small size of cross populations analysed in these Prunus maps. The newly developed apricot SSRs will help saturating the existing Prunus maps and will extend the choice of markers in the development of genetic maps for new breeding populations.

In case of white backed plant hopper (WBPH), NILs having individual resistance genes viz., *Wbph1, Wbph2, Wbph3* and *Wbph4* have been developed with 'IR 36' background. RFLP analysis has been carried out to identify the chromosomal segments introgressed from the resistant donor into respective NILs. Segregating population for each gene has been proposed by making cross between susceptible IR 36 and NILs for each gene. Using this NILs he mapped *Wbph* 1 gene from chromosome 7 with an RFLP marker RG146 (McCouch, 1990).

Wongse *et al.* (1994) reported the tagging of a powdery mildew resistance gene in tomato using RFLP markers. Hittalmani *et al.* (1995) developed a PCR-based marker using RFLP marker RG64, which is tightly linked to blast resistant gene *Pi-2* and generated three PCR products known as sequence tagged sites (STS). The amplified products was cleaved with restriction enzymes *Hae II* and produced specific amplicon.

Singh (2002) reported molecular tagging of rice blast resistance gene Pikh using PCR based markers and AFLP. The causal pathogen is *Magnaporthe grisea*.

2.6.4. Bulk Segregant Analysis (BSA)

Bulk Segregant Analysis is a rapid procedure for identifying markers in specific regions of the genome. This method involves using two pooled DNA samples of individuals from a segregating population originating from a single cross. Within each pool, or bulk, the individuals are identified for the traits or gene of interest but are arbitrary for all other gene (Michelmore *et al.*, 1991). BSA removes the need for screening the entire mapping population with every primer. Poulson *et al.* (1995) suggested that when bulks are constructed from enough individuals, the BSA is sufficiently robust to cope with the low level of phenotypic misclassification. BSA is generally used to tag genes controlling simple traits, but the method also be used to identify markers linked to major QTLs (Wang & Paterson, 1994).

Wang *et al.* (1995) used RAPD markers and BSA to identify molecular markers linked to *Pi-ta* gene in rice which resides on chromosome 12 and flanking between SP4B9 and SP9F3 RAPD markers.

Three RAPD markers OPK 17_{1400}, OPA 7_{550} and OPB 10_{450} was reported to be co-segregated with resistance phenotype of neck blast in Gumei 2. Resistance gene was located between OPK 17_{1400} and OPA 7_{550} having genetic distance of 2.4 cM to OPK 17_{1400} and 7.5 cM to OPA 7_{550}.

Nirmal *et al.* (2001) used RAPD markers and BSA to identify molecular markers linked to (*Magnaporthe grisea* Cav.) disease resistance in rice. RAPD analysis and BSA was followed to identify four phenotype specific markers for yellow stem borer resistance in rice. The markers CI_{320} and K_{695} was linked with resistance phenotype whereas markers AH 5_{660} and C 4_{1300} was linked with susceptible phenotype (Selvi *et al.*, 2002).

Milla *et al.* (2005) identified RAPD and SCAR markers linked to the blue mold resistant tobacco cultivars which is caused by fungal pathogen *Peronospora tabaciana*. BSA was used to screen for polymorphisms between DNA bulk from susceptible and resistance cultivar using 1216 RAPD primers. Fifteen RAPD markers was identified as being linked to the major resistance locus to blue mold and two RAPD markers flanking the most likely QTL position was converted to SCAR markers.

Minamiyama *et al.* (2005) studied the character of pungency in pepper (*Capsicum annuum* L.) which is controlled by a single recessive gene (c) and developed a molecular marker linked to the c-locus using two segregating F_2 population (TM2 and TF2) derived from crosses between pungent and non-pungent peppers in *C. annuum*. Using the RAPD technique in combination with a bulked segregant analysis, two RAPD markers, OPD 20_{800} and OPY 9_{800} was obtained.

Materials and methods

3. MATERIAL AND METHODS

The materials used and methodology followed in the investigation in line with the objectives set namely 1) To identify molecular markers linked to economic characters 2) To validate molecular markers for use in breeding programmes, are presented in this chapter. All the laboratory investigations was carried out at the Department of Genetics and Plant Breeding, College of Agriculture, University of Agricultural Sciences, GKVK Campus, Bangalore – 560 065 during the period 2007-2009. Present study consisted of morphological evaluation and tagging of markers for nut weight and shelling percentage in cashew.

3.1. Plant material

The plant material comprised of 104 cashew germplasm source planted and maintained at the All India Co-ordinated Research Project (AICRP), Agricultural Research Station (ARS), Chintamani (Plate 1). The list of germplasm source along with their accession number is presented in Table 1.

Plate 1. A field view of the cashew germplasm source maintained at AICRP, ARS, Chintamani and used in the present study

Table 1 : List of cashew germplasm source and their accession number maintained at ARS, Chintamani and used in the present study

Sl. No.	Germplasm source	Accession no. / Tree no.
1	9/2 Ullal	ARSC 1/1
2	3/108 Gubbi	ARSC 2/1
3	5/23 Kundapura	ARSC 3/1
4	1/26 Neeleshwar	ARSC 4/1
5	8/46 Taliparamba (Chintamani)	ARSC 5/1
6	9/66 Chirala	ARSC 6/1
7	2/77 Tuni	ARSC 7/1
8	2/97 Kottarakara	ARSC 8/5
9	1/11 Ullal	ARSC 9/4
10	6/21 Mudbidri	ARSC 10/3
11	4/43 Wyanadu	ARSC 11/3
12	1/61 Aalangudi	ARSC 12/3
13	4/62 Aalangudi	ARSC 13/3
14	1/63 Chrompet	ARSC 14/1
15	1/64 Madhuranthakam	ARSC 15/2
16	5/11 Ullal	ARSC 16/1
17	3/3 Madhuranthakam	ARSC 16(a)/6
18	6/91 Kanhargad	ARSC 17(a)/6
19	2/61 Aalangudi	ARSC 17/4
20	5/61 Aalangudi	ARSC 18
21	8/61 Aalangudi	ARSC 18(a)/7
22	3/67 Chrompet	ARSC 19/2
23	4/63 Guntur	ARSC 19(a)/9
24	T-56	ARSC 20/2
25	3/3 Simhachalam	ARSC 20(a)/8

Sl. No.	Germplasm source	Accession no. / Tree no.
26	Hyb-2/11	ARSC 21/1
27	9/8 EPM	ARSC 21(a)/6
28	Hyb-2/10	ARSC 22/1
29	Tree No.-1	ARSC 22(a)/8
30	H-3-17	ARSC 23/4
31	NDR-2-1	ARSC 23(a)/7
32	BCA-139-1	ARSC 24/4
33	H-3-13	ARSC 24(a)/6
34	K-25-2	ARSC 25/4
35	BCA-273-1	ARSC 25(a)/7
36	T-56 Vittal	ARSC 26/2
37	M-44/2	ARSC 26(a)/6
38	Vetori-56	ARSC 27/1
39	Ansuri-1	ARSC 27(a)/8
40	M-6/1	ARSC 28/3
41	A-18-4	ARSC 28(a)/8
42	13/5 Kodur	ARSC 29/2
43	M-16/4	ARSC 29(a)/7
44	2/9 Dicherla	ARSC 30/2
45	2/4 Baruva	ARSC 30(a)/7
46	1/11 Dicherla	ARSC 31(a)/6
47	Tree No. 121	ARSC 31/4
48	8/1 Kodur	ARSC 32/3
49	Tree No. 274	ARSC 32(a)/7
50	M-54/4	ARSC 33/3
51	H-2/3	ARSC 33(a)/6
52	8/7 Sompet	ARSC 34/3
53	ME-5/3	ARSC 34(a)/1

Sl. No.	Germplasm source	Accession no. / Tree no.
54	ME-4/4 (Chintamani 2)	ARSC 35/2
55	ME-6/1	ARSC 35(a)/10
56	M-1/3	ARSC 36/2
57	M-6/1	ARSC 36(a)/8
58	Tree No. 40	ARSC 37/1
59	Tree No. 129	ARSC 37(a)/7
60	2/15	ARSC 38/4
61	2/16	ARSC 38(a)/9
62	9/88	ARSC 39/2
63	1/40	ARSC 39(a)/8
64	6/86	ARSC 40/1
65	4/48	ARSC 40(a)/7
66	5/37 Manjery	ARSC 41/3
67	1/84	ARSC 41(a)/6
68	V-1	ARSC 42/2
69	V-2	ARSC 42(a)/6
70	V-3	ARSC 43/3
71	V-4	ARSC 43(a)/6
72	V-5	ARSC 44/4
73	H-19	ARSC 44(a)/8
74	Veng-1	ARSC 45/3
75	Veng-2	ARSC 45(a)/7
76	Veng-3	ARSC 46/3
77	Veng-4	ARSC 46(a)/7
78	Veng-5	ARSC 47/3
79	BPP-1	ARSC 47(a)/6
80	BPP-2	ARSC 48/3
81	BPP-3	ARSC 48(a)/7

Sl. No.	Germplasm source	Accession no. / Tree no.
82	BPP-4	ARSC 49/2
83	BPP-5	ARSC 49(a)/7
84	BPP-6	ARSC 50/2
85	VRI-1	ARSC 50(a)/6
86	VRI-2	ARSC 51/1
87	Vetori-56	ARSC 51(a)/7
88	39/14	ARSC 52/3
89	Kankadi	ARSC 52(a)/8
90	NDR	ARSC 53/2
91	A-1	ARSC 53(a)/7
92	G-1C	ARSC 54/1
93	H-1B (Hebbari 1) Bold	ARSC 54(a)/7
94	Kottarakara-4B Bold	ARSC 55/1
95	K-7B	ARSC 55(a)/6
96	K-3C (Cluster)	ARSC 56/1
97	K-2B	ARSC 56(a)/7
98	K-6BC	ARSC 57/1
99	Kottarakara-5B	ARSC 57(a)/7
100	CKD-1 (Chikka Dasara Halli)	ARSC 58/14/1
101	CKD-2	ARSC 59/14/1
102	CKD-3	ARSC 60/1
103	CKD-4	ARSC 61/1
104	CKD-5	ARSC 63/14/1

3.2. Morphological evaluation of cashew germplasm source

Phenotypic observations was recorded on 10 quantitative characters in two replications (2008 and 2009), five plants per replication on hundred and four cashew germplasm source of 22 years planted and maintained in five ha at AICRP, ARS, Chintamani. The procedure for recording quantitative traits is described below.

1. Tree height (m)

The height of the tree was recorded from the ground level to the tip of the tree by using a bamboo stick exactly marked in meters.

2. Tree spread (m) {mean of diameter in two directions (EWxNS)}

The spread of the tree was recorded in two directions (EWxNS) by using a bamboo stick exactly marked in meters.

3. Size of cashew apple (cm^3) (mean of 10)

The size of cashew apple was recorded by multiplying length, breadth and width of apple by using a scale in centimeters.

4. Weight of cashew apple (g) (mean of 10)

The weight of cashew apple was recorded by taking total weight of the apples and divided by number of apples in weighing balance and expressed in grams.

5. Nut dimension (cm^3) (LxWxT)

The nut dimension was recorded by multiplying length, width and thickness of the nut by using vernier calipers in centimeters.

6. Nut weight (g) (mean of 100 nuts)

The weight of nut was recorded by taking total weight of the nut and divided by number of nut in weighing balance and expressed in grams.

7. Shelling percentage (mean of 10 nuts)

Nut weight was recorded from plant in grams. Then, the weight of kernels after shelling the nuts of same plant was recorded in grams. The shelling percentage was calculated as

$$\text{Shelling Percentage} = \frac{\text{Weight of nut (g)}}{\text{Weight of kernel (g)}} \times 100$$

8. Shell (pericarp) thickness (mm)

The shell thickness was recorded by using a scale in millimeters.

9. Kernel weight (g) (mean of 10 kernels after shelling dry nuts)

The weight of the total kernels shelled from manual crushing of dry nuts was recorded and expressed in grams.

10. Cumulative yield per plant (Kg) (Avg. yield)

Yield was recorded from all the fallen nuts per plant and expressed in kilograms.

3.3. Statistical analysis for estimation of genetic parameters

Statistical analysis of the data was carried out using statistical program SPSS (correlation), Generes (variability parameters, and diversity analysis) at Department of Genetics and Plant Breeding, University of Agricultural Sciences, GKVK Campus, Bangalore.

3.3.1. Mean

On the basis of individual plant observations, the mean for each character was computed as follows.

$$X = \frac{1}{n} \sum_{i=1}^{n} X_i$$

Where, X = Sample mean

X_i = Individual plant value

n = Number of observations

3.3.2. Range

The minimum and maximum value on the basis of individual plant observations was used to indicate the range for a given character.

3.3.3. Analysis of variance (ANOVA)

The mean replication wise for each character was subjected to analysis of variance (ANOVA) – (Senedecor and Cochran, 1957) to partition tested variabilities for ten quantitative traits attributed to different sources. The structure of ANOVA is as follows.

Source of variation	Df	Mean sum of squares	'F' ratio
Replications	(r-1)	MSSr	MSr/MSe
Genotypes	(t-1)	MSSt	MSt/MSe
Error	(r-1)(t-1)	MSSe	

Where, r = Number of replications

t = Number of genotypes

MSr, MSt, MSe = Mean sum of squares due to replication, treatments and error, respectively.

The significance of differences among all the genotypes was tested by 'F' test. Standard error of mean (SEm) and critical difference (CD) was worked out using appropriate formulae for comparing the means of genotypes.

a) SEm ± was calculated as

$$SEm \pm = \sqrt{\frac{MSe}{r}}$$

Where MSe = mean error variance, r = number of replication

b) Critical difference (CD)

Critical difference was calculated as

CD = √2 X SEm X t

Where, 't' = table value @ error degrees of freedom

3.3.4. A. Phenotypic (PCV) and genotypic (GCV) coefficients of variations

Phenotypic and genotypic coefficients of variations was worked out as suggested by Burton (1953).

$$PCV\ (\%) = \frac{\sqrt{\text{Phenotypic variance}}}{\text{General mean}} \times 100$$

$$GCV\ (\%) = \frac{\sqrt{\text{Genotypic variance}}}{\text{General mean}} \times 100$$

PCV and GCV was classified as per Robinson *et al.* (1949).

0 – 10 %	- Low
10.1 – 20 %	- Moderate
> 20 %	- High

3.3.4. B. Heritability

Broad sense heritability (h_{bs}^2) was estimated for all the characters as the ratio of genotypic variance to the total variance as suggested by Hanson *et al.* (1956) as indicated below.

$h_{bs}^2 = (\sigma_g^2/\sigma_p^2)$ X 100

Where, σ_g^2 is the genotypic variance and σ_p^2 is the phenotypic variance,

Heritability percentage was categorized as per Robinson (1966).

0 – 30 %	- Low
31 – 60 %	- Moderate
> 61 %	- High

3.3.4. C. Genetic advance as per cent mean (GAM)

It was computed using the formula

$$GAM\ (\%) = \frac{\text{Genetic advance}}{\text{General mean of the character}} \times 100$$

Where Genetic Advance = h^2 X σ_p^2 X K ; h^2 = Broad sense heritability, σ_p = Phenotypic standard deviation of the trait, K = Standard selection differential which is 2.06 at 5% selection intensity.

The genetic advance as per cent mean was categorized as suggested by Johnson *et al.* (1955).

0 – 10 %	- Low
11 – 20 %	- Moderate
> 20 %	- High

3.4. Correlation analysis

The correlation coefficients among all possible character combinations at phenotypic (r_p) and genotypic (r_g) level was estimated employing formula of Al-Jibouri *et al.* (1958).

$$\text{Genotypic correlation} = r_{xy}(g) = \frac{cov_{xy}(g)}{\sigma_{(x)}g \times \sigma_{(y)}g}$$

$$\text{Phenotypic correlation} = r_{xy}(p) = \frac{cov_{xy}(p)}{\sigma_{(x)}p \times \sigma_{(y)}p}$$

Where, cov_{xy} (p) and cov_{xy} (g) are phenotypic and genotypic covariances between x and y characters, while σ_x (p) and σ_x (g) represent deviations of characters x and σ_y (p) σ_y (g) denotes variances of character y at phenotypic and genotypic level, respectively.

Significance of association between characters was tested using 't' test.

3.5. Path coefficient analysis

Path coefficient analysis suggested by Wright (1921) and as illustrated by Dewey and Lu (1959) was carried out to know the direct and indirect effect of the morphological traits on yield. The following set of simultaneous equations was formed and solved for estimating various direct and indirect effects.

r_{1y} = a + r_{12}b + r_{13}c + ... + r_{1i}

r_{2y} = r_{21}a + b + r_{23}c + ... + r_{2i}

r_{3y} = r_{31}a + r_{32}b + c + ... + r_{3i}

r_{1y} = r_{11}a + r_{12}b + r_{13}c + ... + iI

Where,

r_{1y} to r_{iy} = Coefficient of correlation between factors 1 to i which dependent characters y

r_{12} to r1i = Coefficient of correlation among causal factors

a,b,c.....i = Direct effects of characters 'a' to 'i' on the dependant character 'y'

Residual effect (R) was computed as follows.

Residual effect (R) = $1- \sqrt{a^2 + b^2 + c^2 + ... i^2 + 2ab_{12}r + 2ac_{13}r + ...}$

3.6. Coefficients of skewness and kurtosis

Skewness, the third degree statistics and kurtosis, the fourth degree statistics was estimated as per Snedecor and Cochran, (1994), to understand the nature of distribution of quantitative traits in germplasm source. Genetic expectations of skewness (-3/4 d^2 h) reveal the nature of genetic control of the traits (Fisher *et al.*, 1932). The parameter 'd' represents additive gene effects and 'h' represents dominance gene effects. Kurtosis indicates the relative number of genes controlling the traits (Robson, 1956). The adjusted mean values of each germplasm source of quantitative traits was used to estimate coefficient of skewness and kurtosis using 'STATISTICA' software program.

3.7. Genetic diversity - Mahalanobis D^2 analysis

Mahalanobis (1936) D^2 –statistic analysis was used for assessing the genetic divergence among the test entries.

The formula for estimation of distance D^2 for the samples.

$$D^2_p = d^1 S^{-1} d$$

Where,

D^2_p = Square of distance considering 'p' variables

d = Vector of observed differences of the mean values of all the characters ($X_{i1} - X_{i2}$)

d^1 = Transpose of vector of observed differences of the mean values of all the characters ($X_{i1} - X_{i2}$)

X_{i1} = Vector of the mean values of all characters

S^{-1} = Inverse of variance and covariance matrix

Since investigating the inverse matrix is complicated, the original correlated variable (x_i) was transformed to non-correlated variables (y_i). The computation of D^2 values reduce to simple summation of the squares of the difference between the values of transformed variables of the two populations.

This transformation was done by Pivotal condensation method. These newly transformed uncorrelated variables was used to calculate the square of distance using the formula.

$$D^2 = (Y_{i1} - Y_{i2})^2$$

Where,

Y = Transformed mean values of 'p' traits

The square root of these D^2 values gives the general distance between the two genotypes. The D^2 values was arranged in a matrix form.

The significance of D^2 values between any two populations is test using the formula.

$$T^2 = \frac{N_1 + N_2}{N_1 \times N_2} \times D^2$$

T^2 is Hotelling T^2 statistic

Using T^2, the F values was calculated

$$F = \frac{N_1 + N_2 - P - 1}{(N_1 \times N_2 - 2)\, P} \times D^2$$

This computed 'F' value was compared with the table 'F' value at five per cent and one per cent level of probability at P and $(N_1 + N_2 - P - 1)$ degrees of freedom.

(i) Clustering of D^2 values

All then n (n-1)/2 D^2 values was clustered using Tocher's method as described by Rao (1952).

(ii) Intra cluster distance

The intra cluster distances was calculated by the formula given by Singh and Chaudhary (1977).

$$\text{Square of intra cluster distance} = \frac{\sum D_i^2}{N}$$

$\sum D_i^2$ = Sum of distance between all possible combinations

N = Number of all possible combinations

(iii) Inter cluster distance

The inter cluster distance was calculated by the formulae described by Singh and Chaudhary (1977).

$$\text{Square of intra cluster distance} = \frac{\sum D_i^2}{n_i n_j}$$

Where,

$\sum D_i^2$ is the sum of distances between all possible combinations ($n_i n_j$) of the entries included in the cluster study.

n_i = Number of entries in cluster i

n_j = Number of entries in cluster j

(iv) Contribution of individual characters towards genetic divergence

The character contribution towards diversity was calculated by the method of Singh and Chaudhary (1977). In all combinations, each character is ranked on the basis of $d_i = y_{1j} - y_{ik}$ values.

Where,

d_i = Mean deviation

y_{1j} = Mean value of the j^{th} genotypes for the i^{th} character

y_{ik} = Mean value of the k^{th} genotypes for the i^{th} character

Rank 'I' is given to the highest mean difference and Rank p is given to the lowest mean difference

Where, P is the total number of characters

3.8. Tagging genes controlling nut traits using markers

3.8.1. Isolation of genomic DNA

Isolation of good quality genomic DNA is one of the important prerequisites for RAPD, ISSR and SSRs. The procedure reported by Mneney *et al.* (1997) for the isolation of genomic DNA from cashew leaves was followed with slight modifications. Young and healthy leaves (one gm) collected from healthy plant, stored at -20^0 C was used for genomic DNA isolation.

Reagents

1) Extraction buffer
   a) 1.4M NaCl - 16.32g/ 200ml
   b) 3% CTAB - 6g/200ml
   c) 20mM EDTA - 1.488g/ 200ml
   d) 100mM Tris HCl, pH: 8 - 2.42g/200ml
      0.2% ß Mercaptoethanol (added fresh)

   2.42g Tris was dissolved in 50ml of water and the pH was adjusted to 8 with concentrated HCl then 20mM EDTA, 1.5M NaCl and CTAB was added. Sequentially adjusted the pH to 8, the volume was made up to 200ml, autoclaved and stored in amber colored bottle.

   (Composition of reagents is provided in Appendix II).

2) 2% PVP (w/v) (Polyvinyl poly pyrrolidone) - 4g/200ml.
3) Chloroform: isoamyl alcohol (24:1) v/v.
4) Absolute alcohol and 76% alcohol, stored at -20^0 C
5) TE (Tris EDTA) buffer:
   a) 10mM Tris-HCl - 0.0605g/50ml.
   b) 1mM EDTA - 0.0372g/100ml.

   Adjusted the pH to 8 and autoclaved.

6) RNase (10mg/ml) powder was dissolved in 10mM Tris –HCl and 15mM NaCl. Adjust the pH 7.5, boil for 5 minutes and cool to room temperature.

7) 7.5 M Ammonium acetate - 57.81g/100ml. Adjusted the pH 7.7 and autoclave.

8) TAE buffer (Stock - 1000ml - 50X) for electrophoresis :

Tris base - 242g

Glacial acetic acid - 57.1ml

0.5M EDTA – 37.2g in 200ml of water

(From this stock 0.5X to be taken for gel running)

9) Bromophenol Blue dye stock

10) Ethidium Bromide solution - 10mg/ml.

3.8.2. Procedure for DNA isolation and purification from cashew leaves

1) 1 g young leaf was weighed and washed with 70 % ethanol and then washed with sterile distilled water.
2) After washing allowed the leaf to dry and ground well using liquid nitrogen in a previously cooled mortar and pestle.
3) About a pinch of PVP was added and ground well.
4) Ground leaf was transfed from the mortar to preheated centrifuge tubes containing 10 ml of extraction buffer and then 20 µl of β-Mercaptoethanol was added.
5) These centrifuge tubes inverted several times and kept in water bath which is maintained at 65^0 C for one hour with intermittent shaking for every 10 minutes.
6) The tubes was cooled to room temperature and to this, mixture of 10 ml chloroform: isoamylalcohol (24:1) was added and mixed gently by inverting the tubes 25-30 minutes to form an emulsion.
7) These tubes was then centrifuged at 5000 rpm for 15 min.

8) After centrifugation the aqueous phase was transferred to fresh centrifuge tubes using cut tips.
9) In this aqueous phase about 10 ml of cold ethanol was added and mixed gently, and then tubes was refrigerated overnight.
10) After refrigeration the centrifuge tubes was spinned at 5000 rpm for 6 min.
11) From these centrifuge tubes the supernatant was discarded and the pellet was washed with 3 ml of 76 % ethanol and again centrifuged at 5000 rpm for 5 min. The washing was repeated twice or more.
12) After ethanol washing and centrifugation the supernatant was discarded and the ethanol was completely removed by blotting on filter paper, dried tubes kept in the desiccator at room temperature with caps opened state for overnight.
13) The desiccated pellet was resuspended with 1 ml of TE buffer and pooled using cut tips. To this RNase at a concentration of 10 µg/ ml was added and incubated at 37^0 C for 30 min.
14) After the incubation with RNase the contents was diluted with 2 ml of TE buffer. Addition of 300 ul 3M Sodium Acetate was followed by 10 ml cold ethanol addition gentle mixing was done to precipitate DNA. The sample was kept for 30 min at -20^0 C in deep freezer.
15) Samples was centrifuged at 5000 rpm for 20 min. at 4^0 C. The supernatant was decanted and the pellet was subjected to air drying in a desiccator and suspended in 1 ml of TE buffer.

3.8.3. Assessing the quality of DNA by agarose gel electrophoresis

The quality of isolated DNA was evaluated through gel electrophoresis (Sambrook *et al.*, 1989).

Reagents

1. Agarose - 0.8 per cent (for genomic DNA samples)
   - 1.5 per cent (for RAPD & ISSR samples)
   - 3.0 per cent (for SSR samples)
2. 50X TAE buffer (pH 8.0)
3. Tracking dye (6X)
4. Ethidium bromide (stock 10 mg/ml; working concentration; 0.5 µg/ml)
5. Electrophoresis unit, power pack, gel casting tray, comb
6. Gel documentation and analysis system

   (Composition of reagents is provided in Appendix III).

The procedure followed for agarose gel electrophoresis is as follows :

1X TAE buffer was prepared from the 50X TAE stock solution. Agarose (0.8 %) was weighed and dissolved in TAE buffer by boiling. While cooling ethidium bromide was added at a concentration of 0.5 µg/ml and mixed well. The open end of gel casting tray was sealed with a cellotape and kept on a horizontal surface. The comb was placed desirably and the dissolved agarose was poured on to the tray. The gel was allowed to set for 30 minutes after which the comb was removed carefully. The tray was kept in the electrophoresis unit well side directed towards the cathode. 1X TAE buffer was added to the tank. Then DNA sample (10 ul) along with the tracking dye (4 ul) was loaded into the wells using a micropipette carefully. λ DNA was used as a molecular marker.

After closing the tank, the anode and cathode ends was connected to the power pack and the gel was run at a constant voltage (100V) and current (50 A). The power was turned off when the tracking dye reach 2/3rd length of the gel.

Then the gel was taken from the electrophoresis unit and viewed under UV light in a transilluminator. The DNA fluoresces under UV light due to ethidium bromide dye. The quality of DNA was judged by clarity of DNA band. The image was documented and saved in gel documentation system.

3.8.4. Assessing the quantity of DNA using flurometer

10X TNE Buffer :

- 1.211g of Tris buffer
- 0.372g of EDTA
- 11.689g of Nacl, make up the volume to100ml.

Hoechst dye stock solution (10 ml, 1mg/ml Hoechst H 33258)

Add 10ml of distilled water to 10 mg H 33258. Do not filter and store at 4^{o} C for up to 6 months in an ambered colored bottle.

Preparation of the assay and DNA standard solutions

Take 10ml of 10 X TNE buffer and dilute with 90 ml of distilled water, add 100 µl of Hoechst dye stock solution.

DNA standard :

Calf thymus - 10mg/ml

1) Set the instrument zero & calibrate the instrument

Prepared 2ml of assay solution (blank) in a dry cuvette. Inserted the cuvette into the well. Closed the lid and pressed <zero>. After zero was displayed, remove the cuvette. 2ml of assay solution was placed in the cuvette and 2 µl of appropriate DNA standard solution (calf thymus DNA) delivered into it, mixed well. Then the cuvette placed in the well. The lid was closed and pressed <Cali B>. (Enter 100 ng/ml for low range assay, 1000 ng/ml for high range assay). 1000 ng/ml for high range assay was entered by pressing <Enter>. After the entered value was displayed, remove the cuvette.

2) Set the instrument zero

Empty and rinse the cuvette by using water and then assay solution. Dry by draining and blotting upside down on a paper towel. 2 ml of same assay solution used in step 2, insert the cuvette and set zero as that of step 2, remove the cuvette.

3) Measure the sample

2 µl of the sample was added and mixed well by using cuvette lid. The cuvette was placed into the well, closed the lid and recorded the measurement that displayed.

4) Measure subsequent samples. Repeated step 4.

3.8.5. Standardization of PCR reaction mixture and amplification condition

The DNA recovery varied widely ranging from 10 – 191 ng/µl/g of leaves in 8/46 Taliparamba, 5/61 Aalangudi and Tree No. 129 respectively (Appendix IV). Average quantity of DNA recovery was 72

ng/µl. DNA was then normalised to 25 ng/µl used as template for RAPD, ISSR and SSR primers.

The PCR amplification conditions was based on basic protocol of Williams *et al.* (1990) and Welsh and Mc Clelland (1990) with slight modifications.

The amplification conditions: Each cycle of PCR consisted of the followed three steps which was repeated for 34 (for RAPD & SSR) and 35 (for ISSR) times.

1. Denaturation at 94^0 C for one minute
2. Annealing at 35^0 C for one minute
3. Extension at 72^0 C for two minute

Different concentrations of template DNA (20ng, 40ng and 50 ng), $MgCl_2$ (1.0mM, 2.0mM and 3.0mM) and dNTPs (100µM, 150µM and 200µM), *Taq* (0.75, 1.5, and 2 U) was tried in PCR (3.4.1). A concentration of 20ng of template DNA, 3.0mM of $MgCl_2$ and 200µM of dNTPs, 1 µM primer, 1.5 U *Taq* was found optimum for obtaining high quality amplification for RAPD, concentration of 20ng of template DNA, 2.0mM of $MgCl_2$ and 200µM of dNTPs, 0.8 µM primer, 0.75 U *Taq* was found optimum for obtaining high quality amplification for ISSR, concentration of 20ng of template DNA, 2.0mM of $MgCl_2$ and 200µM of dNTPs, 0.8 µM primer (F & R), 0.75 U *Taq* was found optimum for obtaining high quality amplification for SSR. However, large changes in concentration of template DNA did affect the amplification, with too little DNA causing either reduced (small bands) or no amplification, and too much DNA producing a smear effect, probably due to nonsequence-specific binding of the primer to the DNA. Therefore, accurate quantification of the DNA was essential for optimal amplification.

3.9. Bulk Segregant Analysis (BSA) for tagging nut traits using DNA markers

The perennial nature of the crop, long phase juvenility, environmental influence and large plant size, straightforward application of principles of plant breeding is seldom possible and identification of DNA markers linked to economic traits is even more difficult due to non availability of right mapping populations for target traits. Due to lack of genomic information, there are no identified markers for any traits in literature BSA was employed in this study for tagging of five polygenic traits in cashew. DNA from the individuals which recorded extreme phenotypic mean expressions during 2008 and 2009 were pooled by normalizing DNA to 25 ng, from each individual 10 µl is dispensed in eppendorf tube (Table 15) for constituting low and high DNA bulk for five important economic characters in cashew. The list of the germplasm source used for constitution of each DNA bulk is presented in Table 15.1 to 15.5.

PCR was carried out as per the procedure followed for screening of primers mentioned in section 3.11.1. The amplified products of five contrasting DNA bulk for all primer was run on 1.5 per cent (for RAPD & ISSRs) and 3.0 per cent (for SSR) agarose gel using 1X TAE buffer along with 100 bp ladder on agarose and 50 bp ladder on 4.5 % denaturing PAGE. The documented profile for 309 RAPD (Table 16 a & b), 15 ISSR (Table c) and 87 SSR primers (Table d) was carefully examined for polymorphism among low and high DNA bulk of five important economic characters of cashew.

The primers which showed polymorphism in low and high bulks and found polymorphic was selected. These short listed polymorphic primers was run on individuals constituting each bulk.

Table 15 : Selection of cashew germplasm source for constitution of DNA bulk contrasting for five important economic traits

Phenotypic character	Range	Criteria f or selection	No. of genotypes selected	Accession nos.
1) Size of Cashew apple (cm^3) (Mean of 10 fruits)	3.85-8.96	Low < 8.96	9	6,22,35,36,53,59,80,81,103
	25.17-80.99	High > 25.17	9	17,30,40,41,45,60,76,82,84
2) Weight of Cashew apple (g) (Mean of 10 fruits)	5-30	Low < 30	9	6,22,30,36,49,53,60,80,103
	84 -110	High > 84	8	16,40,41,76,89,97,101,104
3) Nut weight (g) (Mean of 100 nuts)	3.51- 4.00	Low < 4.00	10	6,12,18,28,48,49,69,83,93,103
	8.88-10.1	High > 8.88	9	10,66,67,70,97,98,99,100,104
4) Shelling percentage (Mean of 10 nuts)	14.81-20.79	Low < 20.79	8	11,13,31,34,65,68,98,99
	33.12-58.3	High > 33.12	10	4,15,19,37,58,59,81,88,90,104
5) Kernel weight (g) (Mean of 10 kernels after shelling dry nuts)	0.68-1.16	Low < 1.16	9	8,11,12,13,18,26,48,68,69
	2.5-9.58	High > 2.5	9	7,38,60,66,67,93,97,100,104

Table 15.1 : List of germplasm source used for constitution of DNA bulk for size of cashew apple (cm^3) (mean of 10 fruits)

Sl. no.	Low bulk (< 8.96 cm^3)		High bulk (> 25.17 cm^3)	
	Accession Nos.	Germplasm source	Accession Nos.	Germplasm source
1	6/1	9/66 Chirala	16(a)/6	3/3 Madhuranthakam
2	19/2	3/67 Chrompet	23/4	H-3-17
3	25(a)/7	BCA-273-1	28/3	M-6/1
4	26/2	T-56 Vittal	28(a)/8	A-18-4
5	34(a)/1	ME-5/3	30(a)/7	2/4 Baruva
6	37(a)/7	Tree No. 129	38/4	2/15
7	48/3	BPP-2	46/3	Veng-3
8	48(a)/7	BPP-3	49/2	BPP-4
9	61/1	CKD-4	50/2	BPP-6

Table 15.2 : List of germplasm source used for constitution of DNA bulk for weight of cashew apple (g) (mean of 10 fruits)

Sl. no.	Low bulk (< 30 g)		High bulk (> 84 g)	
	Accession Nos.	Germplasm source	Accession Nos.	Germplasm source
1	6/1	9/66 Chirala	16/1	5/11 Ullal
2	19/2	3/67 Chrompet	28/3	M-6/1
3	23/4	H-3-17	28(a)/8	A-18-4
4	26/2	T-56 Vittal	46/3	Veng-3
5	32(a)/7	Tree No. 274	52(a)/8	Kankadi
6	34(a)/1	ME-5/3	56(a)/7	K-2B
7	38/4	2/15	59/14/1	CKD-2
8	48/3	BPP-2	63/14/1	CKD-5
9	61/1	CKD-4		

Table 15.3 : List of germplasm source used for constitution of DNA bulk for nut weight (g) (mean of 100 nuts)

Sl. no.	Low bulk (< 4.00 g)		High bulk (> 8.88 g)	
	Accession Nos.	Germplasm source	Accession Nos.	Germplasm source
1	6/1	9/66 Chirala	10/3	6/21 Mudbidri
2	12/3	1/61 Aalangudi	41/3	5/37 Manjery
3	17(a)/6	6/91 Kanhargad	41(a)/6	1/84
4	22/1	Hyb-2/10	43/3	V-3
5	32/3	8/1 Kodur	56(a)/7	K-2B
6	32(a)/7	Tree No. 274	57/1	K-6BC
7	42(a)/6	V-2	57(a)/7	Kottarakara-5B
8	49(a)/7	BPP-5	58/14/1	CKD-1 (Chikka Dasara Halli)
9	54(a)/7	H-1B	63/14/1	CKD-5
10	61/1	CKD-4		

Table 15.4 : List of germplasm source used for constitution of DNA bulk for shelling percentage (mean of 10 nuts)

Sl. no.	Low bulk (< 20.79)		High bulk (> 33.12)	
	Accession Nos.	Germplasm source	Accession Nos.	Germplasm source
1	11/3	4/43 Wyanadu	4/1	1/26 Neeleshwar
2	13/3	4/62 Aalangudi	15/2	1/64 Madhuranthakam
3	23(a)/7	NDR-2-1	17/4	2/61 Aalangudi
4	25/4	K-25-2	26(a)/6	M-44/2
5	40(a)/7	4/48	37/1	Tree No. 40
6	42/2	V-1	37(a)/7	Tree No. 129
7	57/1	K-6BC	48(a)/7	BPP-3
8	57(a)/7	Kottarakara-5B	52/3	39/14
9			53/2	NDR
10			63/14/1	CKD-5

Table 15.5 : List of germplasm source used for constitution of DNA bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

Sl. no.	Low bulk (< 1.16 g)		High bulk (> 2.5 g)	
	Accession Nos.	Germplasm source	Accession Nos.	Germplasm source
1	8/5	2/97 Kottarakara	7/1	2/77 Tuni
2	11/3	4/43 Wyanadu	27/1	Vetori-56
3	12/3	1/61 Aalangudi	38/4	2/15
4	13/3	4/62 Aalangudi	41/3	5/37 Manjery
5	17(a)/6	6/91 Kanhargad	41(a)/6	1/84
6	21/1	Hyb-2/11	54(a)/7	H-1B (Hebbari 1) Bold
7	32/3	8/1 Kodur	56(a)/7	K-2B
8	42/2	V-1	58/14/1	CKD-1 (Chikka Dasara Halli)
9	42(a)/6	V-2	63/14/1	CKD-5

3.10. Primer screening

Good quality genomic DNA isolated from cashew leaf (3.2.2.2) was normalized to 25 ng/ µl and was subjected to RAPD, ISSR and SSR primers. The primers supplied by "Sigma" was used for amplification of DNA.

3.10.1. Screening of RAPD, ISSR and SSR primers

Three hundred and nine RAPD primers in the series from NAPS (Table 16 (a)), C, SB and OPH (Sobir *et al.*, 2007) (Table 16 (b)), fifteen ISSR primers from IS series (Table 16 (c)), eighty seven SSR primers from S [1 to 15 from mango (Schnell *et al.*, 2005), 16 to 50 from almond (Mnejja *et al.*, 2005)], CSSR [51 to 71 from cashew (Croxford *et al.*, 2005)] and LMMA [72 to 87 from mango (Viruel *et al.*, 2005)] series (Table 16 (d)) was screened with DNA of contrasting pools of five important economic characters of cashew (Table 15).

Table 16 (a) : List of NAPS RAPD primer series used for screening on five important contrasting bulks of cashew

Sl. No.	Primer	Nucleotide Sequence (5^1-3^1)
1	NAPS 1	CCTGGGCTTC
2	NAPS 2	CCTGGGCTTG
3	NAPS 3	CCTGGGCTTA
4	NAPS 4	CCTGGGCTGG
5	NAPS 5	CCTGGGTTCC
6	NAPS 6	CCTGGGCCTA
7	NAPS 7	CCTGGGGGTT
8	NAPS 8	CCTGGCGGTA
9	NAPS 9	CCTGCGCTTA
10	NAPS 10	GGGGGGATTA
11	NAPS 11	CCCCCCTTTA
12	NAPS 12	CCTGGGTCCA
13	NAPS 13	CCTGGGTGGA
14	NAPS 14	CCTGGGTTTC
15	NAPS 15	CCTGGGTTTG
16	NAPS 16	GGTGGCGGGA
17	NAPS 17	CCTGGGCCTC
18	NAPS 18	GGGCCGTTTA
19	NAPS 19	GCCCGGTTTA
20	NAPS 20	TCCGGGTTTG
21	NAPS 21	ACCGGGTTTC
22	NAPS 22	CCCTTGGGGG
23	NAPS 23	CCCGCCTTCC
24	NAPS 24	ACAGGGGTGA
25	NAPS 25	ACAGGGCTCA
26	NAPS 26	TTTGGGCCCA

Sl. No.	Primer	Nucleotide Sequence (5^1-3^1)
27	NAPS 27	TTTGGGGGGA
28	NAPS 28	CCGGCCTTAA
29	NAPS 29	CCGGCCTTAC
30	NAPS 30	CCGGCCTTAG
31	NAPS 31	CCGGCCTTCC
32	NAPS 32	GGGGCCTTAA
33	NAPS 33	CCGGCTGGAA
34	NAPS 34	CCGGCCCCAA
35	NAPS 35	CCGGGGTTAA
36	NAPS 36	CCCCCCTTAG
37	NAPS 37	CCGGGGTTTT
38	NAPS 38	CCGGGGAAAA
39	NAPS 39	TTAACCGGGC
40	NAPS 40	TTACCTGGGC
41	NAPS 41	TTAACCGGGG
42	NAPS 42	TTAACCCGGC
43	NAPS 43	AAAACCGGGC
44	NAPS 44	TTACCCCGGC
45	NAPS 45	TTAACCCCGG
46	NAPS 46	TTAAGGGGGC
47	NAPS 47	TTCCCCAAGC
48	NAPS 48	TTAACGGGGA
49	NAPS 49	TTCCCCGAGC
50	NAPS 50	TTCCCCGCGC
51	NAPS 51	CTACCCGTGC
52	NAPS 52	TTCCCGGAGC
53	NAPS 53	CTCCCTGAGC
54	NAPS 54	GTCCCAGAGC

Sl. No.	Primer	Nucleotide Sequence (5^1-3^1)
55	NAPS 55	TCCCTCGTGC
56	NAPS 56	TGCCCCGAGC
57	NAPS 57	TTCCCCGAGG
58	NAPS 58	TTCCCGGAGC
59	NAPS 59	TTCCGGGTGC
60	NAPS 60	TTGGCCGAGC
61	NAPS 61	TTCCCCGACC
62	NAPS 62	TTCCCCGTCG
63	NAPS 63	TTCCCCGCCC
64	NAPS 64	GAGGGCGGGA
65	NAPS 65	AGGGGCGGGA
66	NAPS 66	GAGGGCGTGA
67	NAPS 67	GAGGGCGAGC
68	NAPS 68	GAGCTCGCGA
69	NAPS 69	GAGGGCAAGA
70	NAPS 70	GGGCACGCGA
71	NAPS 71	GAGGGCGAGG
72	NAPS 72	GAGCACGGGA
73	NAPS 73	GGGCACGCGA
74	NAPS 74	GAGCACCTGA
75	NAPS 75	GAGGTCCAGA
76	NAPS 76	GAGCACCAGT
77	NAPS 77	GAGCACCAGG
78	NAPS 78	GAGCACTAGC
79	NAPS 79	GAGCTCGTGT
80	NAPS 80	GTGCTCTAGA
81	NAPS 81	GAGCACGGGG
82	NAPS 82	GGGCCCGAGG

Sl. No.	Primer	Nucleotide Sequence (5^1-3^1)
83	NAPS 83	GGGCTCGTGG
84	NAPS 84	GGGCGCGAGT
85	NAPS 85	GTGCTCGTGC
86	NAPS 86	GGGGGGAAGG
87	NAPS 87	GGGGGGAAGC
88	NAPS 88	CGGGGGATGG
89	NAPS 89	GGGGGCTTGG
90	NAPS 90	GGGGGTTAGG
91	NAPS 91	GGGTGGTTGC
92	NAPS 92	CCTGGGCTTT
93	NAPS 93	GGGGGGAAAG
94	NAPS 94	GGGGGGAACC
95	NAPS 95	GGGGGGTTGG
96	NAPS 96	GGCGGCATGG
97	NAPS 97	ATCTGCGAGC
98	NAPS 98	ATCCTGCCAG
99	NAPS 99	ATCCCCTGGG
100	NAPS 100	ATCGGGTCCG
101	NAPS 101	GCGGCTGGAG
102	NAPS 102	GGTGGGGACT
103	NAPS 103	GTGACGCCGC
104	NAPS 104	GGGCAATGAT
105	NAPS 105	CTCGGGTGGG
106	NAPS 106	CGTCTGCCCG
107	NAPS 107	CTGTCCCTTT
108	NAPS 108	GTATTGCCCT
109	NAPS 109	TGTACGTGAC
110	NAPS 110	TAGCCCGCTT

Sl. No.	Primer	Nucleotide Sequence (5^1-3^1)
111	NAPS 111	AGTAGACGGG
112	NAPS 112	GCTTGTGAAC
113	NAPS 113	ATCCCAAGAG
114	NAPS 114	TGACCGAGAC
115	NAPS 115	TTCCGCGGGC
116	NAPS 116	TACGATGACG
117	NAPS 117	TTAGCGGTCT
118	NAPS 118	CCCGTTTTGT
119	NAPS 119	ATTGGGCGAT
120	NAPS 120	GAATTTCCCC
121	NAPS 121	ATACAGGGAG
121	NAPS 121	GTAGACGAGC
123	NAPS 123	GTCTTTCAGG
124	NAPS 124	ACTCGAAGTC
125	NAPS 125	GCGGTTGAGG
126	NAPS 126	CTTTCGTGCT
127	NAPS 127	ATCTGGCAGC
128	NAPS 128	GCATATTCCG
129	NAPS 129	GCGGTATAGT
130	NAPS 130	GGTTATCCTC
131	NAPS 131	GAAACAGCGT
132	NAPS 132	AGGGATCTCC
133	NAPS 133	GGAAACCTCT
134	NAPS 134	AACACACGAG
135	NAPS 135	AAGCTGCGAG
136	NAPS 136	TACGTCTTGC
137	NAPS 137	GGTCTCTCCC
138	NAPS 138	GCTTCCCCTT

Sl. No.	Primer	Nucleotide Sequence (5^1-3^1)
139	NAPS 139	CCCAATCTTC
140	NAPS 140	GTCGCATTTC
141	NAPS 141	ATCCTGTTCG
142	NAPS 142	ATCTGTTCGG
143	NAPS 143	TCGCAGAACG
144	NAPS 144	AGAGGGTTCT
145	NAPS 145	TGTCGGTTGC
146	NAPS 146	ATGTGTTGCG
147	NAPS 147	GTGCGTCCTC
148	NAPS 148	TGTCCACCAG
149	NAPS 149	AGCAGCGTGG
150	NAPS 150	GAAGGCTCTG
151	NAPS 151	GCTGTAGTGT
152	NAPS 152	CGCACCGCAC
153	NAPS 153	GAGTCACGAG
154	NAPS 154	TCCATGCCGT
155	NAPS 155	CTGGCGGCTG
156	NAPS 156	GCCTGGTTGC
157	NAPS 157	CGTGGGCAGG
158	NAPS 158	TAGCCGTGGC
159	NAPS 159	GAGCCCGTAG
160	NAPS 160	CGATTCAGAG
161	NAPS 161	CGTTATCTCG
162	NAPS 162	AACTTACCGC
163	NAPS 163	CCCCCCAGAT
164	NAPS 164	CCAAGATGCT
165	NAPS 165	GAAGGCACTG
166	NAPS 166	ACTGCTACAG

Sl. No.	Primer	Nucleotide Sequence (5^1-3^1)
167	NAPS 167	CCAATTCACG
168	NAPS 168	CTAGATGTGC
169	NAPS 169	ACGACGTAGG
170	NAPS 170	ATCTCTCCTG
171	NAPS 171	TGACCCCTCC
172	NAPS 172	ACCGTCGTAG
173	NAPS 173	CAGGCGGCGT
174	NAPS 174	AACGGGCAGC
175	NAPS 175	TGGTGCTGAT
176	NAPS 176	CAAGGGAGGT
177	NAPS 177	TCAGGCAGTC
178	NAPS 178	CCGTCATTGG
179	NAPS 179	TCACTGTACG
180	NAPS 180	GGGCCACGCT
181	NAPS 181	ATGACGACGG
182	NAPS 182	GTTCTCGTGT
183	NAPS 183	CGTGATTGCT
184	NAPS 184	CAAACGGCAC
185	NAPS 185	GTGTCTTCAC
186	NAPS 186	GTGCGTCGCT
187	NAPS 187	AACGGGGGAG
188	NAPS 188	GCTGGACATC
189	NAPS 189	TGCTAGCCTC
190	NAPS 190	AGAATCCGCC
191	NAPS 191	CGATGGCTTT
192	NAPS 192	GCAAGTCACT
193	NAPS 193	TGCTGGCTTT
194	NAPS 194	AGGACGTGCC

Sl. No.	Primer	Nucleotide Sequence (5^1-3^1)
195	NAPS 195	GATCTCAGCG
196	NAPS 196	CTCCTCCCCC
197	NAPS 197	TCCCCGTTCC
198	NAPS 198	GCAGGACTGC
199	NAPS 199	GCTCCCCCAC
200	NAPS 200	TCGGGATATG
201	NAPS 201	CTGGGGATTT
202	NAPS 202	GAGCACTTAC
203	NAPS 203	CACGGCGAGT
204	NAPS 204	TTCGGGCCGT
205	NAPS 205	CGGTTTGGAA
206	NAPS 206	GAGGACGTCC
207	NAPS 207	CATATCAGGG
208	NAPS 208	ACGGCCGACC
209	NAPS 209	TGCACTGGAG
210	NAPS 210	GCACCGAGAG
211	NAPS 211	GAAGCGCGAT
212	NAPS 212	GCTGCGTGAC
213	NAPS 213	CAGCGAACTA
214	NAPS 214	CATGTGCTTG
215	NAPS 215	TCACACGTGC
216	NAPS 216	CATAGACTCC
217	NAPS 217	ACAGGTAGAC
218	NAPS 218	CTCAGCCCAG
219	NAPS 219	GTGACCTCAG
220	NAPS 220	GTCGATGTCG
221	NAPS 221	CCCGTCAATA
222	NAPS 222	AAGCCTCCCC

Sl. No.	Primer	Nucleotide Sequence (5^1-3^1)
223	NAPS 223	GATCCATTGC
224	NAPS 224	TCTCCGGTAT
225	NAPS 225	CGACTCACAG
226	NAPS 226	GGGCCTCTAT
227	NAPS 227	CTAGAGGTCC
228	NAPS 228	GCTGGGCCGA
229	NAPS 229	CCACCCAGAG
230	NAPS 230	CGTCGCCCAT
231	NAPS 231	AGGGAGTTCC
232	NAPS 232	CGGTGACATC
233	NAPS 233	CTATGCGCGC
234	NAPS 234	TCCACGGACG
235	NAPS 235	CTGAGGCAAA
236	NAPS 236	ATCGTACGTG
237	NAPS 237	CGACCAGAGC
238	NAPS 238	CTGTCCAGCA
239	NAPS 239	CTGAAGCGGA
240	NAPS 240	ATGTTCCAGG
241	NAPS 241	GCCCGACGCG
242	NAPS 242	CACTCTTTGC
243	NAPS 243	GGGTGAACCG
244	NAPS 244	CAGCCAACCG
245	NAPS 245	CGCGTGCCAG
246	NAPS 246	TATGGTCCGG
247	NAPS 247	TACCGACGGA
248	NAPS 248	GAGTAAGCGG
249	NAPS 249	GCATCTACCG
250	NAPS 250	CGACAGTCCC

Sl. No.	Primer	Nucleotide Sequence (5^1-3^1)
251	NAPS 251	CTTGACGGGG
252	NAPS 252	CTGGTGATGT
253	NAPS 253	CCGTGCAGTA
254	NAPS 254	CGCCCCCATT
255	NAPS 255	TTCCTCCGGA
256	NAPS 256	TGCAGTCGAA
257	NAPS 257	CGTCACCGTT
258	NAPS 258	CAGGATACCA
259	NAPS 259	GGTACGTACT
260	NAPS 260	TCTCAGCTAC
261	NAPS 261	CTGGCGTGAC
262	NAPS 262	CGCCCCCAGT
263	NAPS 263	TTAGAGACGG
264	NAPS 264	TCCACCGAGC
265	NAPS 265	CAGCTGTTCA
266	NAPS 266	CCACTCACCG
267	NAPS 267	CCATCTTGTG
268	NAPS 268	AGGCCGCTTA
269	NAPS 269	CCAGTTCGCC
270	NAPS 270	TGCGCGCGGG
271	NAPS 271	GCCATCAAGA
272	NAPS 272	AGCGGGCCAA
273	NAPS 273	AATGTCGCCA
274	NAPS 274	GTTCCCGAGT
275	NAPS 275	CCGGGCAAGC
276	NAPS 276	AGGATCAAGC
277	NAPS 277	AGGAAGGTGC
278	NAPS 278	GGTTCCAGCT

Sl. No.	Primer	Nucleotide Sequence (5^1-3^1)
279	NAPS 279	AGACATTAGA
280	NAPS 280	CTGGGAGTGG
281	NAPS 281	GAGAGTGGAA
282	NAPS 282	GGGAAAGCAG
283	NAPS 283	CGGCCACCGT
284	NAPS 284	CAGGCGCACA
285	NAPS 285	GGGCGCCTAG
286	NAPS 286	CGGAGCCGGC
287	NAPS 287	CGAACGGCGG
288	NAPS 288	CCTCCTTGAC
289	NAPS 289	ATCAAGCTGC
290	NAPS 290	CCGCGAGCAC
291	NAPS 291	AGCTGAAGAG
292	NAPS 292	AAACAGCCCG
293	NAPS 293	TCGTGTTGCT
294	NAPS 294	TGATTGGCCA
295	NAPS 295	CGCGTTCCTG
296	NAPS 296	CCGCTGGGAG
297	NAPS 297	GCGCATTAGA
298	NAPS 298	CCGTACGGAC
299	NAPS 299	TGTCAGCGGT
300	NAPS 300	GGCTAGGGCG

Table 16 (b) : List of C, SB and OPH RAPD primer series used for screening on five important contrasting bulks of cashew

Sl. No.	Primer	Nucleotide Sequence (5^1-3^1)
1	C 301	ATGTAGCGTGGCGAAACTG
2	C 302	AAGTAACTGACTCCGCTGCGAC
3	C 303	CCCAGCAACTGATCGCACAC
4	C 304	AGGACTCGATAACAGGCTCG
5	SB 13	AGTCAGCCAC
6	SB 19	CAGCACCCAC
7	OPH 12	ACGCGCATGT
8	OPH 13	GACGCCACAC
9	OPH 18	GAATCGGCCA

Table 16 (c) : List of IS ISSR primer series used for screening on five important contrasting bulks of cashew

Sl. No.	Primer number	Repeat motif	Annealing temperature (°C)	Melting temperature (Tm)
1	IS 7	$(AG)_8T$	55	54.78
2	IS 10	$(GA)_8T$	55	54.78
3	IS 42	$(GA)_8YG$	55	58.76
4	IS 12	$(GA)_8A$	55	54.78
5	IS 17	$(CA)_8A$	55	54.78
6	IS 20	$(GT)_8C$	55	57.19
7	IS 25	$(AC)_8T$	55	54.78
8	IS 27	$(AC)_8G$	55	57.19
9	IS 65	$(CCG)_6$	60	78.12
10	IS 34	$(AG)_8YT$	55	56.48
11	IS 66	$(CTC)_6$	60	64.46
12	IS 56	$(AC)_6YA$	55	56.48
13	IS 67	$(GGC)_6$	60	78.12
14	IS 61	$(ACC)_6$	60	64.46
15	IS 55	$(AC)_8YT$	55	56.48

Y = (C+T).

Table 16 (d) : List of S, CSSR and LMMA SSR primer series used for screening on five important contrasting bulks of cashew

Sl. No.	Primer number	Nucleotide Sequence (5^1-3^1)	Annealing temperature (°C)	Repeat motif	Expected size (bp)
1	S 1F	TAACAGCTTTGCTTGCCTCC	50	$(CT/AG)_{14}$	191-207
	S 1R	TCCGCCGATAAACATCAGAC			
2	S 2F	CCACGAATATCAACTGCTGCC	57	$(CT/AG)_{11}$	121-131
	S 2R	TCTGACACTGCTCTTCCACC			
3	S 3F	AAACGAGGAAACAGAGCAC	50	$(AAC/GTT)_8$	90-111
	S 3R	CAAGTACCTGCTGCAACTAG			
4	S 4F	AGGTCTTTTATCTTCGGCCC	65	$(TATG/CATA)_7$	199-203
	S 4R	AAACGAAAAAGCAGCCCA			
5	S 5F	TGTAGTCTCTGTTTGCTTC	57	$(GTT/AAC)_6$	260-275
	S 5R	TTCTGTGTCGTCAAACTC			
6	S 6F	CAACTTGGCAACATAGAC	46	$(TG/CA)_9$	174-182
	S 6R	ATACAGGAATCCAGCTTC			
7	S 7F	AGAATAAAGGGGACACCAGAC	51	$(GTTGTGT/ACACAAC)_3$	222
	S 7R	CCATCATCGCCCACTCAG			
8	S 8F	TTGATGCAACTTTCTGCC	53	$(CA/TG)_9$	200-224
	S 8R	ATGTGATTGTTAGAATGAACTT			
9	S 9F	CGAGGAAGAGGAAGATTATGAC	46	$(CGG/CCT)_7$	236-248
	S 9R	CGAATACCATCCAGCAAAATAC			
10	S 10F	TGTGAAATGGAAGGTTGAG	46	$(GTT)_5GCA(GTT)_5$	228
	S 10R	ACAGCAATCGTTGCATTC			
11	S 11F	GTTTTCATTCTCAAAATGTGTG	50	$(CT/AG)_{15}$	174-190
	S 11R	CTTTCATGTTCATAGATGCAA			
12	S 12F	CTCGCATTTCTCGCAGTC	46	$(AG/CT)_9$	127-132
	S 12R	TCCCTCCATTTAACCCTCC			
13	S 13F	GAACGAGAAATCGGGAAC	53	$(GTT/AAC)_8$	348-369
	S 13R	GCAGCCATTGAATACAGCG			
14	S 14F	AACCCATCTAGCCAACCC	57	$(TC/GA)_{11}(TA)_{10}$ $(CA/TG)_9(TA)_3(CA/TG)_3$	253-260
	S 14R	TTGACAGTTACCAAACCAGAC			
15	S 15F	TTTACCAAGCTAGGGTCA	57	$(GA/TC)_{15}$	201-226
	S 15R	CACTCTTAAACTATTCAACCA			
16	S 16F	CCTGCGTAGAAGAAGGTAGCA	47	$(GA)_{30}$	165
	S 16R	GTATGAAATGCCTGGCCACT			
17	S 17F	CAGCAATGTTTATGCAGGGTAA	47	$(CT)_{27}$	147
	S 17R	TGAATATTTGGATTGCGAAGG			
18	S 18F	GGCGTCGCTGAATGTAGTTT	62	$(GA)_{10}$-$(GA)_{11}$	167
	S 18R	ACTCACTCCGCATTTCATCA			
19	S 19F	TCTCAGGTTCGTATCCCCTCTCT	62	$(CT)_{19}$	151
	S 19R	GCCCATTTTGTGTGTGTCA			
20	S 20F	TTCAAGGAGAAGGCCTGAAA	62	$(CT)_{14}$	114
	S 20R	ATTGTGGGTTCCAACCAATG			
21	S 21F	GTCCTCCTCCCAGCTTCTCT	62	$(CT)_{12}$-$(CT)_5$	190
	S 21R	GGTTTAGCGCAAAAGCTTCA			
22	S 22F	TGCAAGTTGAATGTGGCAAT	62	$(GA)_{19}$	164
	S 22R	CTTTGGGTAGTGCAGGGATG			
23	S 23F	GAAGCAGCGATTCCTAGTGC	62	$(GA)_{18}$	191
	S 23R	TGTTTATGGACCTTAGTAGTCT			
24	S 24F	GCTTGGAAAAGGGTCTCCTA	52	$(GA)_{21}$	183
	S 24R	CCACCTCAGTTTTGACAATGAA			

Sl. No.	Primer number	Nucleotide Sequence (5^1-3^1)	Annealing temperature (°C)	Repeat motif	Expected size (bp)
25	S 25F	AAGACAGAGGGGACAGAGCA	62	$(CT)_5$-$(CT)_9$-$(CT)_5$	189
	S 25R	CGCGCGGAGAGATAATAGAC			
26	S 26F	ATGGTCTAAAAACCGCGAAG	62	$(TC)_6$-$(CT)_8$	176
	S 26R	GGAGATCAAGACCGCCTGT			
27	S 27F	CAGACCGTCGTGTTGAAGTC	62	$(GA)_{12}$	198
	S 27R	GACCCGAATCGGAGTTGTAA			
28	S 28F	GTTTTAGAAACCTCATTCCAACTT	62	$(CT)_{14}$	100
	S 28R	AATTCTAACACTGGGGTATTGT			
29	S 29F	TGCAAAGAAAAACGGAGAGG	62	$(GA)_{25}$	154
	S 29R	GAAACTCAGTGGCACAATCG			
30	S 30F	GAAACTCAGTGGCACAATCG	62	$(CT)_{20}$	158
	S 30R	GCAGGAGTTTCGAAAGGAAG			
31	S 31F	GGAAACCTGATTAGGGCACTT	62	$(GA)_{19}$	196
	S 31R	GGTCTGCTATACTGACCTAGGATT			
32	S 32F	CGTGCCACGAGAATGAGAAT	62	$(GA)_{13}$	179
	S 32R	CCAGGACTTAGGAGGTGTCG			
33	S 33F	AATTTCTTTTGTTAGGATAATACA	47	$(CT)_{15}$	200
	S 33R	TTTGCATATTGAAAATTTGTGG			
34	S 34F	AAAACTCCTCTCCTTTTCCCTTT	62	$(CT)_{24}$	153
	S 34R	TCTTCCTCACCACCTCAAGC			
35	S 35F	TTGAATCGGAGTTGGAAAGAA	62	$(CT)_5$-$(TC)_4$-$(TC)_6$-$(CT)_5$	199
	S 35R	CGGTGCTGGGAGAATCGT			
36	S 36F	GATGGTACCTGAAGCGGAGGA	52	$(GA)_8$-$(GA)_4$	200
	S 36R	TGGTCTAAATACCGCGAAGG			
37	S 37F	TGATCGGCGTCTCCTTTATC	62	$(CT)_{17}$	152
	S 37R	AAAGCAAGCAGGCAAATGAA			
38	S 38F	GTGGCAAATGTTGGCAAAG	62	$(GA)_9$	172
	S 38R	AACACAAAGCAGCACCAAGA			
39	S 39F	TGAAATCTTTAAATCACCCGCAT	62	$(CT)_{19}$-$(CT)_3$	188
	S 39R	CTTGCTTGCTTGCTTCACCT			
40	S 40F	GACCTCATCAGCATCACCAA	62	$(CT)_{10}$	172
	S 40R	TTCCCTAACGTCCCTGACAC			
41	S 41F	AGGCTGAAGCTCCAGCACTC	58	$(GA)_{15}$	132
	S 41R	GAGGTGGAAGCCATGTTTG			
42	S 42F	TGAGGAGAGCACTGGAGGAG	62	$(CT)_{19}$	174
	S 42R	CAACCGATCCCTCTAGACCA			
43	S 43F	TGAACGTTGCACTCCTTCAC	62	$(GA)_{19}$	171
	S 43R	ACCACCACCATAACCACCAT			
44	S 44F	CAATCAAACCACCACAACCA	62	$(GA)_{21}$	193
	S 44R	CGCAACGCTGTTTCTCTTTT			
45	S 45F	CAAGGGGCTCTCAAAAGGAT	58	$(GA)_{18}$	192
	S 45R	TTCTCCTGATTCCCATTCG			
46	S 46F	AATTCATAAATCAACAAATCAACA	62	$(GA)_{22}$	179
	S 46R	GCAGAGCTTTTGGGTCAACT			
47	S 47F	TCAGCTCTCTTTCTCCTCACG	62	$(CT)_5$-$(CT)_6$-$(CT)_6$-$(CT)_6$	185
	S 47R	GGAAATCGGCTAGCCTTGAT			
48	S 48F	CAAAACACAAAAACCCACCA	62	$(CT)_{18}$	132
	S 48R	ATTCGGGGAGTCAATCAGG			
49	S 49F	GAGAACCTTTTGTTTGGCCTTA	62	$(CT)_4$-$(CT)_7$-$(CT)_6$	165
	S 49R	CGTCGTATTTAGTGCCGTTG			
50	S 50F	TCGAAGGAGGATGAAGTTGC	62	$(GA)_{17}$	146
	S 50R	ATATCACGAGGGGCAAAATG			
51	CSSR 1F	GGCCATGGGAAACAACAA	58.2	$(CA)_{10}(TA)_6$	366-375
	CSSR 1R	GGAAGGGCATTATGGGTAAG			

Sl. No.	Primer number	Nucleotide Sequence (5^1-3^1)	Annealing temperature (°C)	Repeat motif	Expected size (bp)
52	CSSR 2F	CAGAACCGTCACTCCACTCC	60.3	$(AC)_{12}(AAAAT)_2$	241-247
	CSSR 2R	ATCCAGACGAAGAAGCGATG			
53	CSSR 3F	CAAAACTAGCCGGAATCTAGC	58.2	$(AT)_5(GT)_{12}$	143-157
	CSSR 3R	CCCCATCAAACCCTTATGAC			
54	CSSR 4F	AACCTTCACTCCTCTGAAGC	58.2	$(AT)_2(GT)_5(AT)(GT)_5$	178-181
	CSSR 4R	GTGAATCCAAAGCGTGTG			
55	CSSR 5F	ATCCAACAGCCACAATCCTC	60.3	$(AT)_3(AC)_{16}$	234-236
	CSSR 5R	CTTACAGCCCCAAACTCTCG			
56	CSSR 6F	TCACCAAGATTGTGCTCCTG	58.2	$(AC)_{12}ATAC(AT)_4$	324-336
	CSSR 6R	AAACTACGTCCGGTCACACA			
57	CSSR 7F	GGAGAAAGCAGTGGAGTTGC	60.3	$(GT)_8(TA)_{17}(GT)_3$	256-268
	CSSR 7R	CAAGTGAGTCCTCTCACTCTCA			
58	CSSR 8F	GCAATGTGCAGACATGGTTC	56.1	$(GA)_{24}$	124-159
	CSSR 8R	GGTTTCGCATGGAAGAAGAG			
59	CSSR 9F	TCCACAAAATCAGCCTCCAC	60.3	$(TA)_5CA(TG)_6$	414-416
	CSSR 9R	GAGCGCTCGTGTCCTGTACT			
60	CSSR 10F	GGAGAAGAAAAGTTAGGTTTGAC	58.2	$(TG)_{10}$	316-320
	CSSR 10R	CGTCTTCTTCCACATGCTTC			
61	CSSR 11F	CATCCTTTTGCCAATTAAAAACA	56.1	$(CT)_{18}(AT)_{19}$	354-356
	CSSR 11R	CACGTGTATTGTGCTCACTCG			
62	CSSR 12F	CTTTCGTTCCAATGCTCCTC	58.2	$(AG)_{14}$	165-169
	CSSR 12R	CATGTGACAGTTCGGCTGTT			
63	CSSR 13F	GCTTAGCCGGCACGATATTA	58.2	$(GGT)_8$	151-161
	CSSR 13R	AGCTCACCTCGTTTCGTTTC			
64	CSSR 14F	ACTGTCACGTCAATGGCATC	60.3	$(CAT)_9TAT(CTT)_7$	197-206
	CSSR 14R	GCGAAGGTCAAAGAGCAGTC			
65	CSSR 15F	CACGTTCGCATCATCCAA	58.2	$GTG(GT)_3GCT(GGT)_4$	256-263
	CSSR 15R	CGTCAGAGATTACGGCATTG			
66	CSSR 16F	CGGCGTCGTTAAAGCAGT	58.2	$(ACC)_7(AC)_3$	217-221
	CSSR 16R	TCCTCCTCCGTCTCACTTTC			
67	CSSR 17F	AAGAGCTGCGACCAATGTTT	58.2	$(TAAA)_2(TA)_7(AAT)_5$	161-173
	CSSR 17R	CTTGAACTTGACACTTCATCCA			
68	CSSR 18F	CAGCGAGTGGCTTACGAAAT	58.2	$(GAA)_6(GA)_3$	172-178
	CSSR 18R	GACCATGGGCTTGATACGTC			
69	CSSR 19F	GCTATGACCCTTGGGAACTC	58.2	$(GT)_{16}(TA)_2$	191-203
	CSSR 19R	GTGACACAACCAAAACCACA			
70	CSSR 20F	TGACTTTCAAATGCCACAAC	58.2	$(AT)_6CT(AC)_5$	104-114
	CSSR 20R	CTCAAGCTTTCATGGGGATT			
71	CSSR 21F	TCCGCCCCTACTCCTATATT	51.8	$(AT)_7(GT)_{14}$	317-327
	CSSR 21R	TGGTGTCGACTGCTTCTTGT			
72	LMMA 1F	ATGGAGACTAGAATGTACAGAG	55	$(GA)_{13}$	202
	LMMA 1R	ATTAAATCTCGTCCACAAGT			
73	LMMA 2F	AAATAAGATGAAGCAACTAAAG	55	$(GA)_{11}$	287
	LMMA 2R	TTAGTGATTTTGTATGTTCTTG			
74	LMMA 3F	AAAAACCTTACATAAGTGAATC	55	$(GA)_{16}$	207
	LMMA 3R	CAGTTAACCTGTTACCTTTTT			
75	LMMA 4F	AGATTTAAAGCTCAAGAAAAA	55	$(GA)_{13}$	241
	LMMA 4R	AAAGACTAATGTGTTTCCTTC			
76	LMMA 5F	AGAATAAGCTGATACTCACAC	55	$(GA)_9$	283
	LMMA 5R	TAACAAATATCTAATTGACAGG			
77	LMMA 6F	ATATCTCAGGCTTCGAATGA	55	$(GA)_{14}$	118
	LMMA 6R	TATTAATTTTCACAGACTATGTTCA			
78	LMMA 7F	ATTTAACTCTTCAACTTTCAAC	55	$(CT)_{15}$	212
	LMMA 7R	AGATTTAGTTTTGATTATGGAG			

Sl. No.	Primer number	Nucleotide Sequence (5^1-3^1)	Annealing temperature (°C)	Repeat motif	Expected size (bp)
79	LMMA 8F	CATGGAGTTGTGATACCTAC	55	$(GA)_{12}$	271
	LMMA 8R	CAGAGTTAGCCATATAGAGTG			
80	LMMA 9F	TTGCAACTGATAACAAATATAG	55	$(GA)_{13}$	185
	LMMA 9R	TTCACATGACAGATATACACTT			
81	LMMA 10F	TTCTTTAGACTAAGAGCACATT	55	$(GA)_{10}$	191
	LMMA 10R	AGTTACAGATCTTCTCCAATT			
82	LMMA 11F	ATTATTTACCCTACAGAGTGC	55	$(GA)_{12}$	244
	LMMA 11R	GTATTATCGGTAATGTCTTCAT			
83	LMMA 12F	AAAGATAGCATTTAATTAAGGA	55	$(GA)_{13}$	206
	LMMA 12R	GTAAGTATCGCTGTTTGTTATT			
84	LMMA 13F	CACAGCTCAATAAACTCTATG	55	$(GA)_{17}$	172
	LMMA 13R	CATTATCCCTAATCTAATCATC			
85	LMMA 14F	ATTATCCCTATAATGCCCTAT	55	$(CT)_{10}$	170
	LMMA 14R	CTCGGTTAACCTTTGACTAC			
86	LMMA 15F	AACTACTGTGGCTGACATAT	55	$(CT)_{11}$	215
	LMMA 15R	CTGATTAACATAATGACCATCT			
87	LMMA 16F	ATAGATTCATATCTTCTTGCAT	55	$(GA)_{17}$	233
	LMMA 16R	TATAAATTATCATCTTCACTGC			

Genomic DNA at the concentration of 25ng/µl was normalized by using millipore water and subjected to amplification using these primers.

The amplification reaction was carried out in a Corbett thermocycler. A master mix without the template DNA and primer was prepared using the reaction mixture for the required number of reactions. From this, master mix, for RAPD 8.2 µl was pipetted into each PCR tube. Template DNA 0.8 µl and primer 1 µl was added. PCR amplification was performed in a 10 µl reaction mixture as constituted below:

Composition of the PCR reaction mixture for RAPD (10.0 µl)

		(1 X) for 10 µl
Sterile water	-	5.0
Taq assay buffer	(1 X)	1.0
$MgCl_2$	(3 mM)	0.6
dNTP mix	(0.2 mM)	1.0
Primer	(1 µM)	1.0
Taq polymerase	(1.5 U)	0.6
Genomic DNA	(25 ng)	0.8

The PCR tubes along with PCR reaction mixture was kept in the thermal cycler and was run in the following programme:

95^0 C for 2 minute - Initial denaturation

94^0 C for 1 minute - Denaturation

35^0 C for 1 minute - Primer annealing

72^0 C for 2 minute - Primer extension

For 34 cycles

72^0 C - 6 minute - Final extension

4^0 C for infinity to hold the sample

A master mix without the template DNA and primer was prepared using the reaction mixture for the required number of reaction. From this, master mix, for ISSR 8.4 µl was pipetted into each PCR tube. Template DNA 0.8 µl and primer 0.8 µl was added. PCR amplification was performed in a 10 µl reaction mixture as constituted below:

Composition of the PCR reaction mixture for ISSR (10.0 µl)

		(1 X) for 10 µl
Sterile water	-	5.95
Taq assay buffer	(1 X)	1.0
$MgCl_2$	(2 mM)	0.2
dNTP mix	(0.2 mM)	1.0
Primer	(0.8 µM)	0.8
Taq polymerase	(0.75 U)	0.25
Genomic DNA	(25 ng)	0.8

The PCR tubes along with PCR reaction mixture was kept in the thermal cycler and was run in the following programme:

95^0 C for 2 minute - Initial denaturation
94^0 C for 1 minute - Denaturation
55^0 C for 1 minute - Primer annealing
72^0 C for 2 minute - Primer extension
For 35 cycles
72^0 C - 6 minute - Final extension
4^0 C for infinity to hold the sample

A master mix without the template DNA and primer was prepared using the reaction mixture for the required number of reaction. From this, master mix, for SSR 7.2 µl was pipetted into each PCR tube. Template DNA 0.8 µl, primer (F) 0.8 µl and (R) 0.8 µl was added. PCR amplification was performed in a 10 µl reaction mixture as constituted below:

Composition of the PCR reaction mixture for SSR (10.0 µl)

		(1 X) for 10 µl
Sterile water	-	4.00
Taq assay buffer	(1 X)	1.0
$MgCl_2$	(2 mM)	0.8
dNTP mix	(0.2 mM)	1.0
Primer (F)	(0.8 µM)	1.0
(R)	(0.8 µM)	1.0
Taq polymerase	(0.75 U)	0.4
Genomic DNA	(25 ng)	0.8

The PCR tubes along with PCR reaction mixture was kept in the thermal cycler and was run in the following programme:

95^0 C for 2 minute - Initial denaturation
94^0 C for 1 minute - Denaturation
58^0 C for 1 minute - Primer annealing
72^0 C for 2 minute - Primer extension
For 34 cycles
72^0 C - 6 minute - Final extension
4^0 C for infinity to hold the sample

The amplified products was run on 1.5 per cent (for RAPD & ISSRs) and 3.0 per cent (for SSRs) agarose gel using 1X TAE with ethidium bromide along with 100 bp ladder. The profile was visualized and documented using gel documentation system Molecular imager ^{R} Gel Doc ^{TM} XR Imaging System from Bio-RAD. The documented profiles was carefully examined for polymorphism.

3.10.2. Solutions prepared for SSRs on PAGE

1. 10x TBE
   - -Tris Base 121g
   - -Boric Acid 51.3g
   - -EDTA 3.7g (80ml 0.25M EDTA pH 8)
   - -Distilled water to 1 litre

   Heat sterilized and stored at room temperature.

2. Acrylamide Mix 4.5%
   - -Urea 126g
   - -10x TBE 30 ml
   - -Acrylamide mix 40% 33.7 ml
   - -Distilled water to 300ml litre

   Stored at 4° C.

3. Acrylamide gel loading dye
   - -98% Formamide
   - -0.025% Bromophenol blue
   - -0.025% Xylene cyanol
   - -10mM EDTA, pH 8

   Stored at room temperature.

3.10.3. Denaturation of SSR product

Three µl of stop loading dye (SLD) was added to five µl of each sample. Denaturation was carried out by heating to 94^0 C for 5 min and then cooling to 10^0 C for 5 min. Finally the product was stored at -20^0 C.

3.10.4. Visualizing SSR products

SSR products was visualized on silver stained polyacrylamide gel.

3.10.5. POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)

i. Plate preparation:

Solutions:

Bind silane stock solution: 100 % ethanol 40 ml + 150µl of Bind silane.
Bind silane working solution: 40 ml of stock Bind solution + 1ml 10 % acetic acid. Store at 4°C.

Repelcote working solution: Repelcote 300 µl + 95 % Aceto ethanol 1ml.

1. Both the plates was cleaned using detergent. The plates was then rinsed with de -ionized water and dried with paper towels.
2. The larger plate surface was treated with repelcote silane by spreading repelcote with paper towel on the surface of the large plate. An ethanol wipe was given over the surface to evenly spread the silane and the final polish was given with second ethanol wipe.
3. The small plate was treated with bind silane by spreading evenly over the whole surface.

4. The spacers was aligned on the edges of large plate. The smaller plate was placed on top and tape was put on both sides to secure both plates together.

ii. Pouring of polyacrylamide gel:

1. Sixty five ml of 4.5% polyacrylamide gel was taken in a pouring bottle and 45µl of chilled TEMED (Tetramethyl ethylenediamine) and 450µl of chilled 10% ammonium persulphate (APS) was added to this.
2. The polyacrylamide was allowed to flow slowly and gently between the plates. The comb was placed in reverse direction between plates.
3. The gel was allowed to polymerize for 1.5 hours.
4. The comb was removed by sliding it out horizontally.
5. The plates was mounted onto the electrophoresis unit, with the longer plate in the outer position. Five hundred ml of 1 x TBE was poured into the top reservoir and also into bottom reservoir.
6. The comb was put into position so that it just touched the gel.
7. Three µl of each denatured DNA sample was loaded.
8. Electrophoresis was carried out at 1600 V for 1 to 2 hours until the dark blue dye ran off.
9. The gel was unmounted and the two plates was separated using plastic wedge. The gel remained in complete contact with the smaller plate.

iii. Silver staining of the gel:

Solutions prepared

a) **Developer** - 3% sodium carbonate was prepared (30g of sodium carbonate in 1 litre of distilled water).

b) Fixer - 10% acetic acid (100 ml acetic acid in 900 ml of distilled water).

c) Silver stain - 1.01 N silver nitrate (60 ml 1.01 N silver nitrate in 1 litre of distilled water). 1.5 ml formaldehyde (40%) was added to this solution and mix.

d) Developing solution - Immediately prior to developing the gel 300 µl of sodium thisulphate (STS) (0.1N) and 3 ml formaldehyde was added to pre-chilled sodium carbonate.

3.10.6. Silver staining procedure

1. The gel was placed in fixer (10% acetic acid) for half an hour, under shaking condition, put fixer back into the pot.
2. The gel was washed 3 times in fresh distilled water and was placed in distilled water for 10 minutes under shaking condition.
3. The gel was placed in silver stain for half an hour, under shaking condition.
4. The gel was placed for 10 seconds in 3 litre of distilled water.
5. It was immediately placed in developing solution and immersed up and down holding the top end of the plate.
6. The reaction was stopped when bands near the bottom of the gel started to show, by adding 1 litre fixer.
7. The gel was then soaked in a tray of distilled water for 10 minutes and left to dry over night, in vertical position.

3.11. Validation of identified markers on germplasm source at Directorate of Cashew Research (DCR), Puttur

Validation of identified markers CSSR 12 for nut weight (g) (mean of 100 nuts) and CSSR 14 for shelling percentage (mean 10 nuts) was done by taking five germplasm source for low DNA bulk and five for high DNA bulk (Table 22) from National Cashew Gene Bank (NCGB), DCR, Puttur.

Table 22 : Cashew germplasm source from DCR, Puttur used for validation of identified markers CSSR 12 for nut weight (g) (mean of 100 nuts) and CSSR 14 for shelling percentage (mean of 10 nuts)

Sl. No.	Character			
	Nut weight (g)		Shelling %	
	(<4.3g) LB	(>12g) HB	(<20) LB	(>35.5) HB
1	NRC 152	NRC 107	NRC 155	NRC 319
2	NRC 153	NRC 278	NRC 156	NRC 081
3	NRC 041	NRC 194	NRC 107	NRC 318
4	NRC 081	NRC 279	NRC 217	NRC 330
5	NRC 168	NRC 112	NRC 335	NRC 324

LB : Low Bulk, **HB :** High Bulk

Experimental Results

4. RESULTS

The results of the present investigation on "Tagging genetic determinants for nut weight and shelling percentage in cashew (*Anacardium occidentale* L.)" are presented under the following heads.

1. Analysis of variance
2. Mean performance of genotypes and descriptive statistics
3. Components of variability and predicted genetic advance
4. Correlation analysis
5. Path coefficient analysis
6. Genetic diversity based on ten quantitative traits
7. Bulk Segregant Analysis (BSA)
8. Validation of identified markers for nut weight and shelling percentage from germplasm source at DCR, Puttur

4.1. Analysis of variance

Mean sum of squares due to genotypes indicated significance differences for seven of the ten quantitative traits (Table 2), indicating wide variation for the character studied among 104 cashew germplasm source.

4.1.1 Pooled analysis of variance for ten quantitative traits among 104 cashew germplasm source

Mean sum of squares due to genotypes indicated significance differences for all the characters (Table 23). Variance due to genotypes, environments & G X E interaction were highly significant for all characters, suggesting mean performance of genotypes is influenced by environment.

Table 2 : Analysis of variance for ten quantitative traits among 104 cashew germplasm source

Mean sum of squares											
Source of variation	**df**	**Tree height (m)**	**Tree spread (m)**	**Size of apple (cm^3)**	**Weight of apple (g)**	**Nut dimension (cm^3)**	**Nut weight (g)**	**Shelling percentage**	**Shell thickness (mm)**	**Kernel weight (g)**	**Cumulative yield (kg)**
Replication	1	3.79	1830.28	1.26	50.63	1.35	0.61	2.49	0.02	0.25	7.46
Genotypes	103	1.17	3022.41**	63.38**	740.57**	8.79**	4.34*	51.48**	0.03	0.41	15.43**
Error	103	0.05	88.95	23.51	52.41	1.57	0.72	10.92	0.02	0.09	0.95

* indicates significant at P = 0.05 and ** indicates significant at P = 0.01

Table 23. Pooled ANOVA for ten quantitative traits among 104 cashew germplasm source

Source	df	Tree height (m)	Tree spread (m)	Size of apple (cm^3)	Weight of apple (g)	Nut dimension (cm^3)	Nut weight (g)	Shelling percentage	Shell thickness (mm)	Kernel weight (g)	Cumulative yield (kg)
Genotypes (G)	103	1.17**	3021.40**	133.28**	739.85**	8.79**	4.34**	51.48**	0.03**	0.40**	15.43**
Environments (E)	1	3.83**	1905.18**	18.33**	50.13**	1.28**	0.56**	3.015**	0.01**	0.28**	7.45**
G X E	103	0.05**	90.18**	7.38**	52.36**	1.57**	0.73**	10.84**	0.02**	0.09**	0.95**
Pooled Error	206	0.01**	1.16**	0.20**	0.34**	0.02**	0.02**	0.47**	0.01**	0.01**	0.02**

* indicates significant at P = 0.05 and ** indicates significant at P = 0.01

Table 17 : Descriptive statistics for selected morphological characters of cashew genetic resource maintained at AICRP, ARS, Chintamani

Sl. No.	Parameters	Tree height (m)	Tree spread (m)	Size of apple (cm^3)	Weight of apple (g)	Nut dimension (cm^3)	Nut weight (g)	Shelling percentage	Shell thickness (mm)	Kernel weight (g)	Cumulative yield (kg)
1	Mean	5.00	105.57	16.14	53.58	2.26	6.32	27.88	0.31	1.85	85.17
2	Standard Error	0.08	3.74	0.97	2.06	0.24	0.17	0.56	0.02	0.09	9.59
3	Range	4.10	181.84	77.14	105.00	14.48	6.59	43.49	2.40	8.90	901.65
4	Minimum	3.30	18.36	3.85	5.00	0.24	3.51	14.81	0.10	0.68	10.35
5	Maximum	7.40	200.20	80.99	110.00	14.72	10.10	58.30	2.50	9.58	912.00
6	Kurtosis	0.62	-0.24	25.90	0.38	14.11	-0.68	6.76	72.89	49.34	49.92
7	Skewness	0.51	0.38	4.50	0.47	3.46	0.34	1.35	7.81	5.98	6.17

4.2. Mean performance of genotypes and descriptive statistics

The mean value of the genotypes evaluated (Appendix V) and descriptive statistics for 10 quantitative traits is presented in (Table 17).

1. Plant height (m)

Lowest plant height of 3.40 m was observed in genotype 3/3 Madhuranthakam and the highest of 7.45 m was observed in genotype ME-4/4. The coefficient of skewness was 0.51 and that of kurtosis was 0.62 (Fig. 1).

2. Tree spread (m) mean of diameter in two directions (EWxNS)

Lowest tree spread of 19.25 m was observed in genotype Vetori-56 and the highest of 208 m was observed in genotype H-2/3. The coefficient of skewness was 0.38 and that of kurtosis was -0.24 (Fig. 2).

3. Size of cashew apple (cm^3) (mean of 10)

Lowest size of cashew apple of 4.51 cm^3 was observed in genotype Tree No. 129 (Plate 2) and the highest of 39.51 cm^3 was observed in genotype BPP-4. The coefficient of skewness was 4.50 and that of kurtosis was 25.9 (Fig. 3).

4. Weight of cashew apple (g) (mean of 10)

Lowest weight of cashew apple of 10 g was observed in genotype VRI-2 and the highest of 100.8 g was observed in genotype A-18-4. The coefficient of skewness was 0.47 and that of kurtosis was 0.38 (Fig. 4).

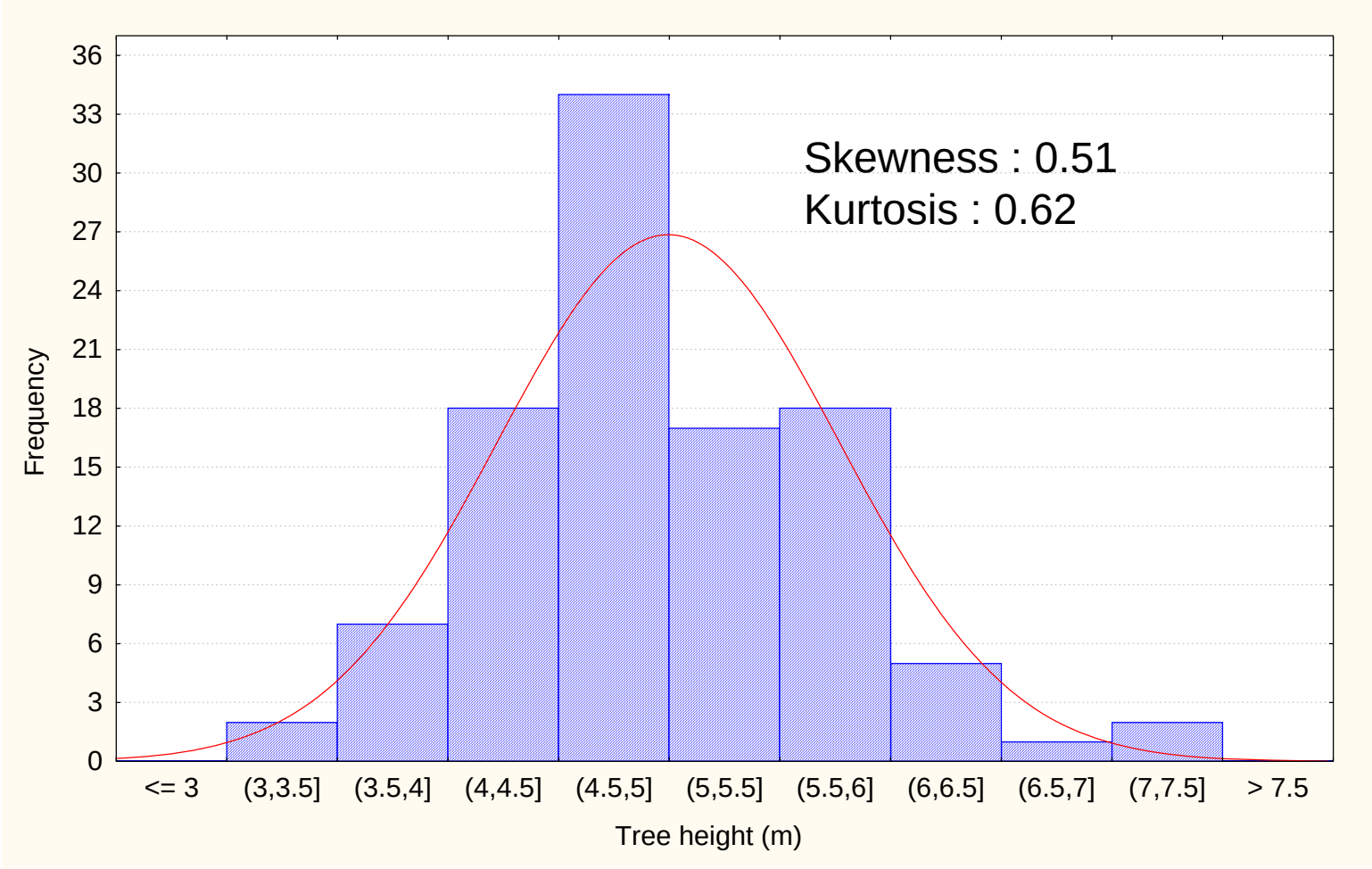


Fig. 1: Leptokurtic and positively skewed distribution of plant height (m) among cashew germplasm source

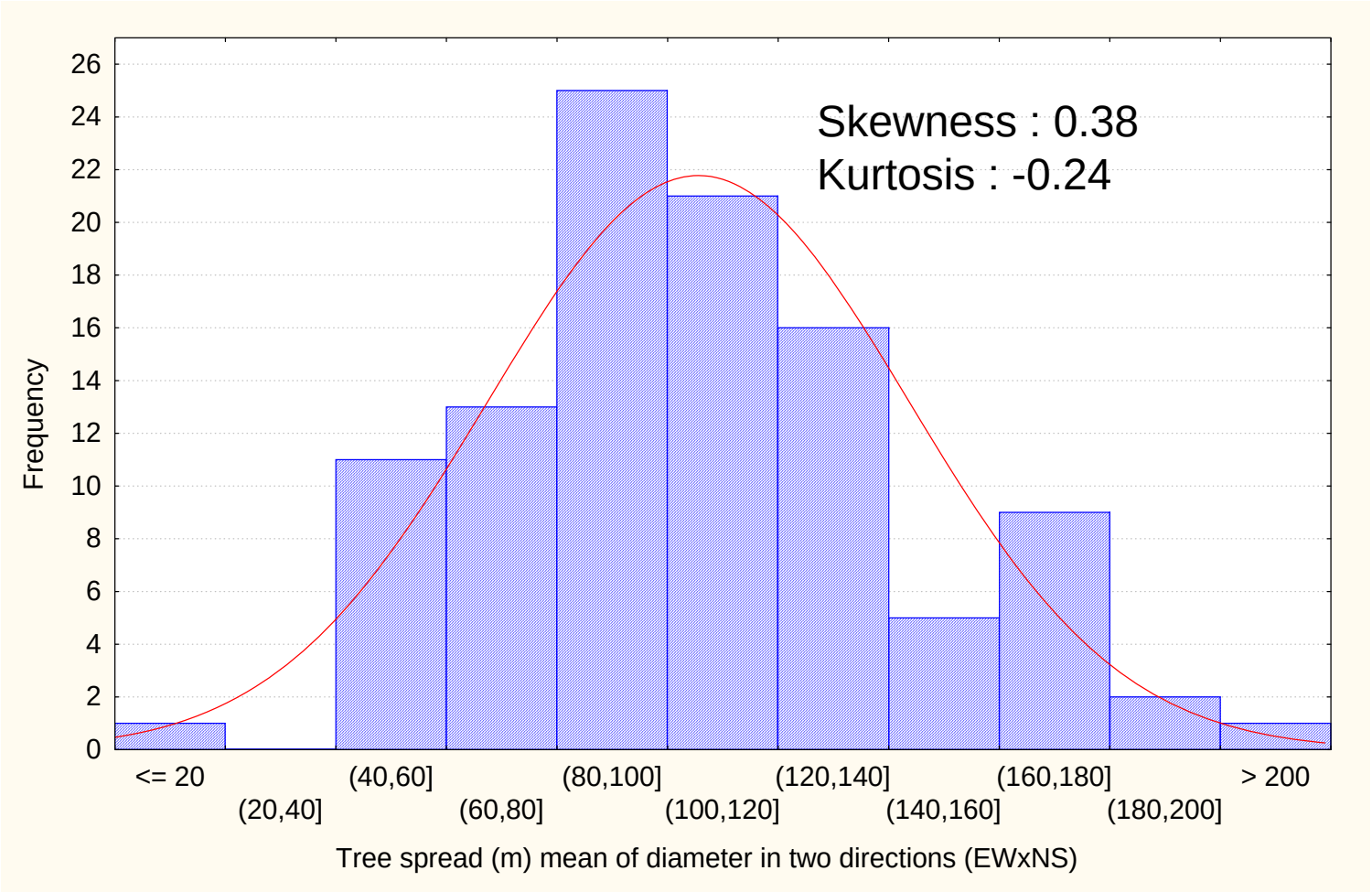
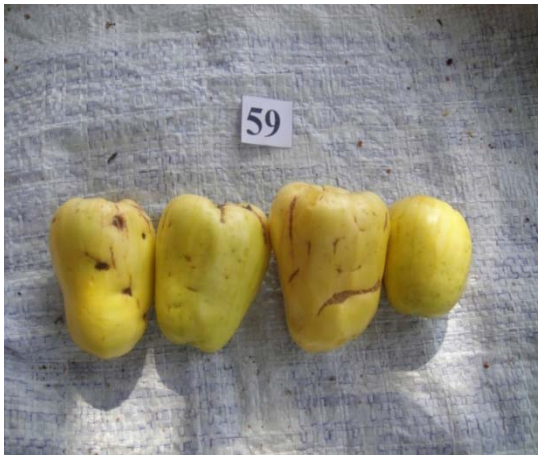


Fig. 2: Platykurtic and positively skewed distribution of tree spread (m) {mean of diameter in two directions (EWxNS)} among cashew germplasm source



Lower fruit size **Higher fruit size**

Plate 2. Variation observed in size of cashew apple (cm^3) (Trait 1)

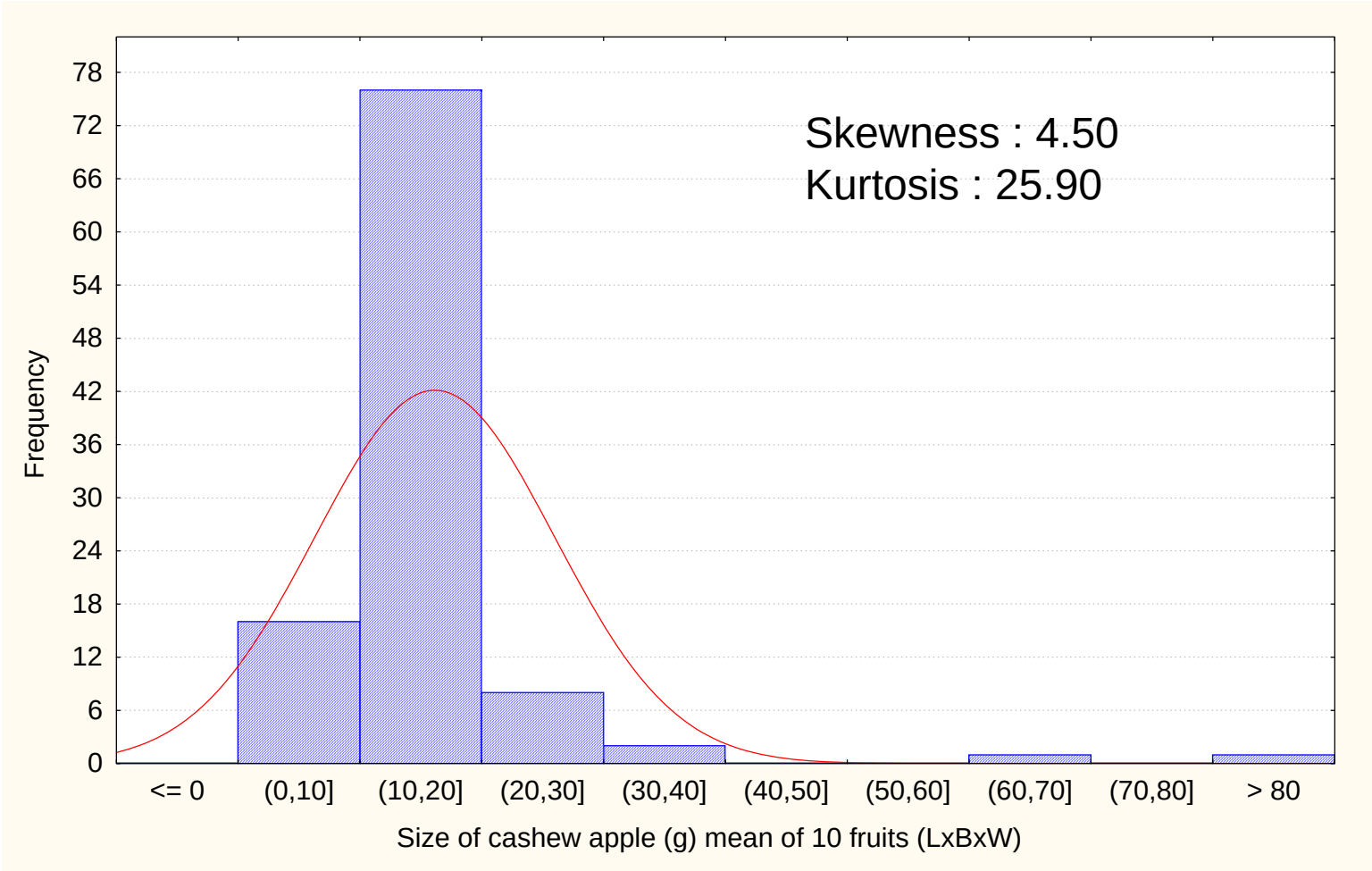


Fig. 3: Leptokurtic and positively skewed distribution of size of cashew apple (cm^3) mean of 10 fruits (LxBxW) among cashew germplasm source

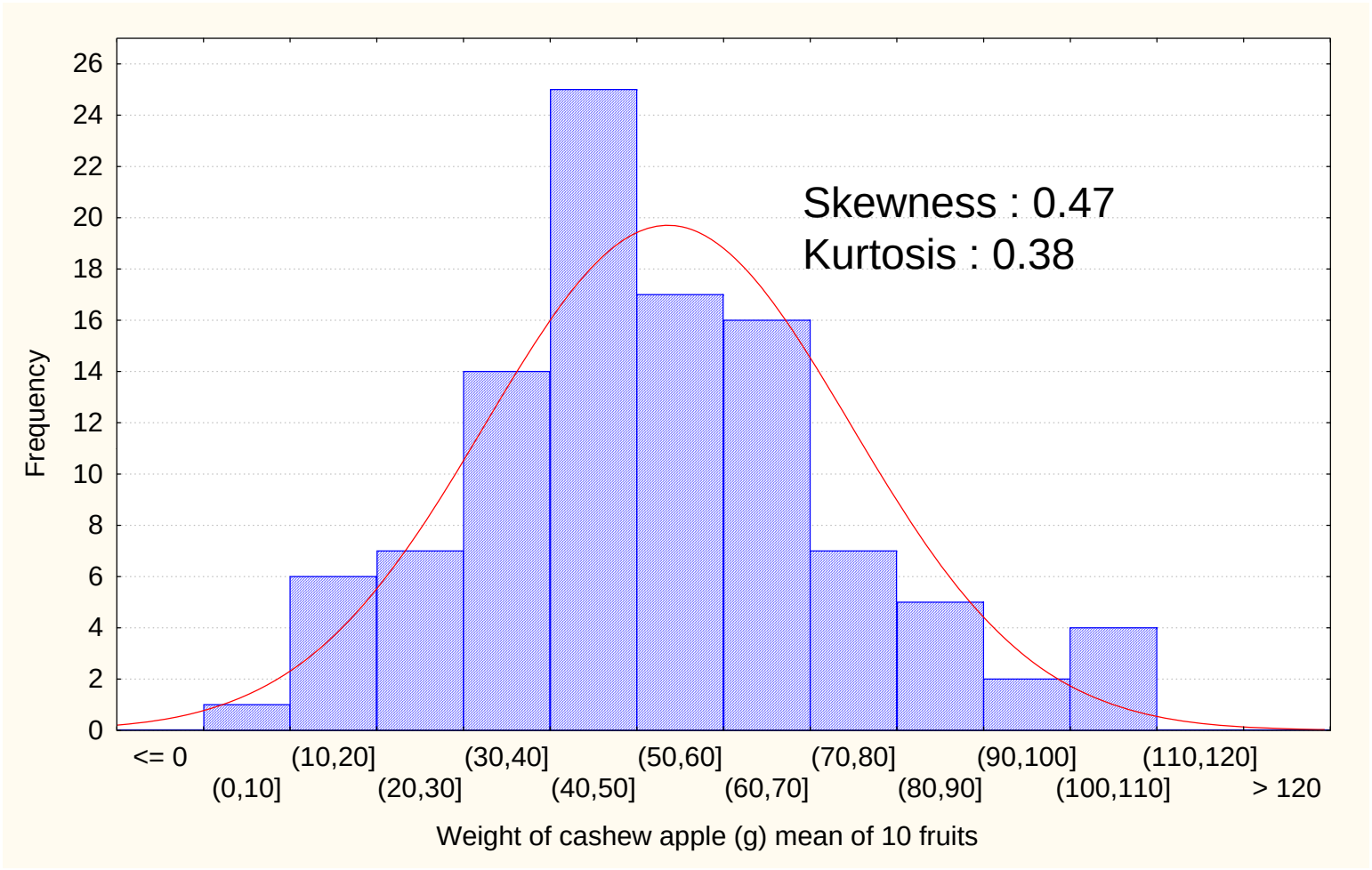


Fig. 4: Leptokurtic and positively skewed distribution of weight of cashew apple (g) mean of 10 fruits among cashew germplasm source

5. Cashew nut dimension (cm^3) (LxWxT)

Lowest cashew nut dimension of 0.25 cm^3 was observed in genotype 6/91 Kanhargad and the highest of 14.27 cm^3 was observed in genotype Kankadi. The coefficient of skewness was 3.46 and that of kurtosis was 14.11 (Fig. 5).

6. Nut weight (g) (mean of 100 nuts)

Lowest nut weight of 3.36 g was observed in genotype 9/66 Chirala (Plate 3) and the highest of 9.85 g was observed in genotype CKD-5. The coefficient of skewness was 0.34 and that of kurtosis was -0.68 (Fig. 6).

7. Shelling percentage (mean of 10 nuts)

Lowest shelling percentage of 13.41 per cent was observed in genotype 4/62 Aalangudi and the highest of 41.15 per cent was observed in genotype 1/26 Neeleshwar. The coefficient of skewness was 1.35 and that of kurtosis was 6.76 (Fig. 7).

8. Shell (pericarp) thickness (mm)

Lowest shell (pericarp) thickness of 0.15 mm was observed in genotype 4/43 Wyanadu and the highest of 1.45 mm was observed in genotype 8/61 Aalangudi. The coefficient of skewness was 7.8 and that of kurtosis was 72.89 (Fig. 8).

9. Kernel weight (g) (mean of 10 kernels after shelling dry nuts)

Lowest kernel weight of 0.54 g was observed in genotype 4/62 Aalangudi and the highest of 3.42 g was observed in genotype CKD-5. The coefficient of skewness was 5.98 and that of kurtosis was 49.34 (Fig. 9).

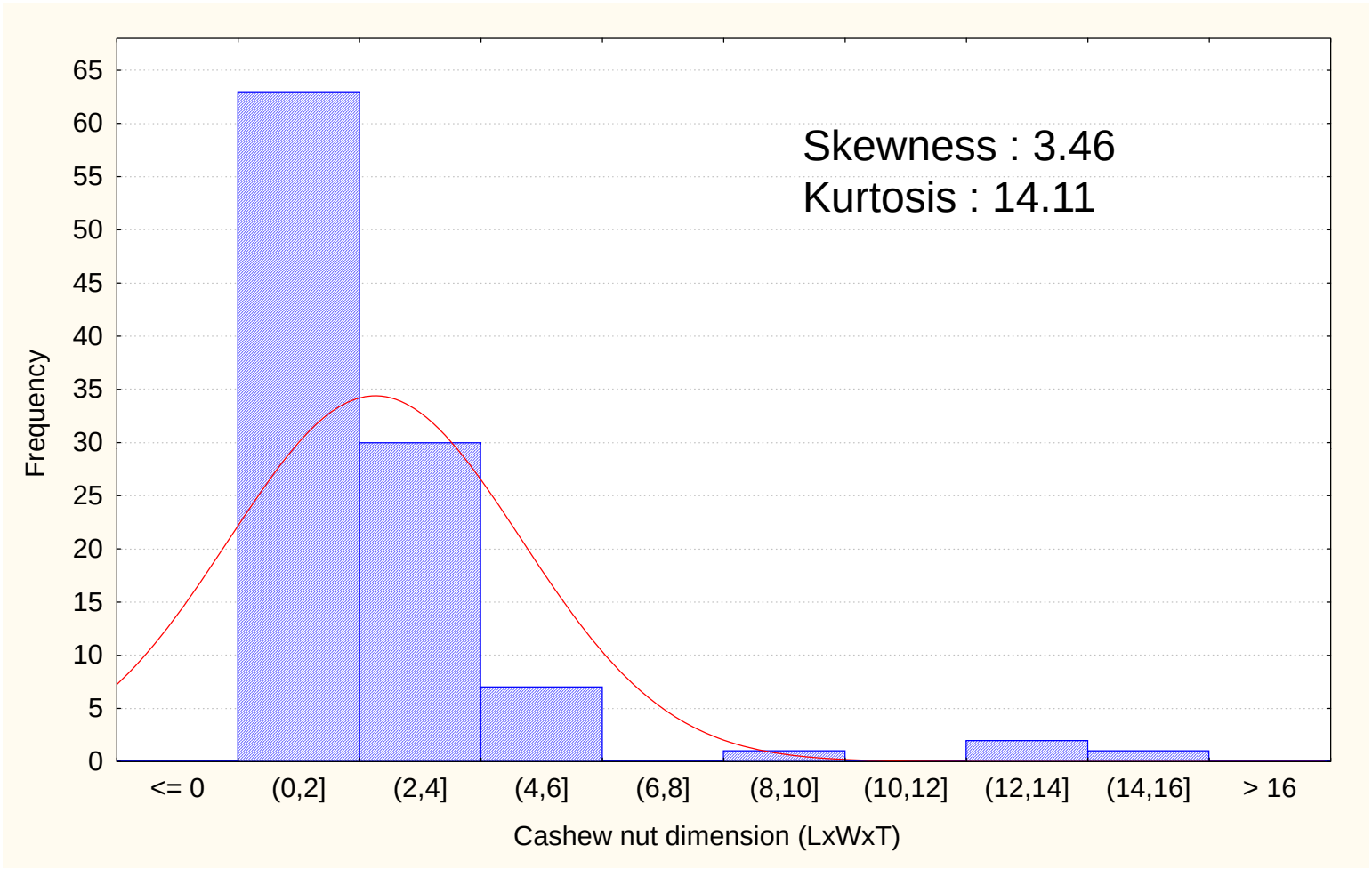
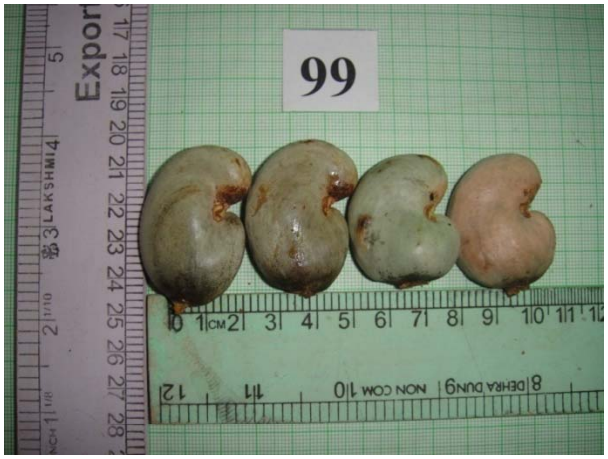


Fig. 5: Leptokurtic and positively skewed distribution of nut dimension (cm^3) (LxWxT) among cashew germplasm source



Small nut **Bold nut**

Plate 3. Variation observed in nut weight (g) (Trait 3)

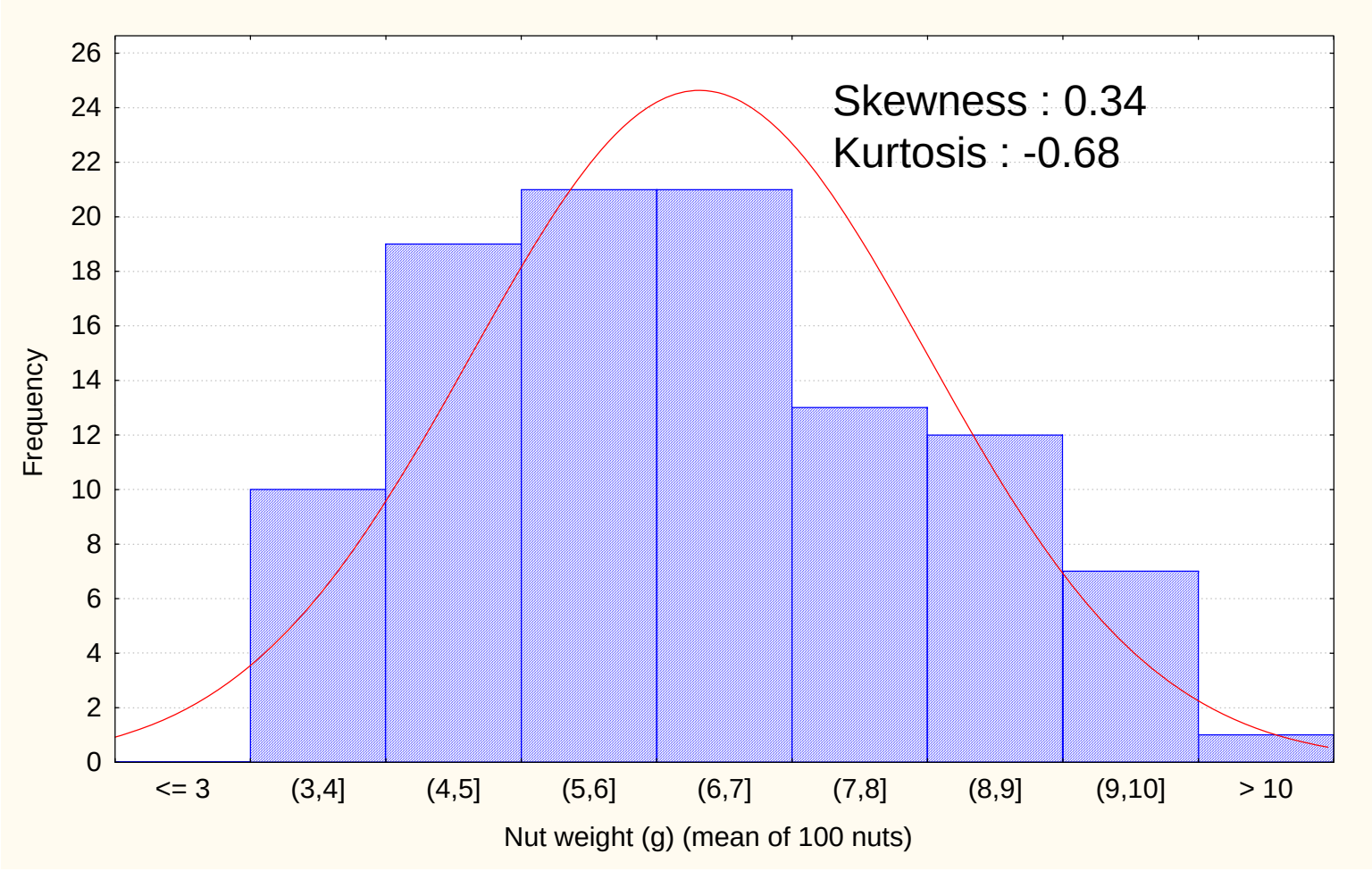


Fig. 6: Platykurtic and positively skewed distribution of nut weight (g) (mean of 100 nuts) among cashew germplasm source

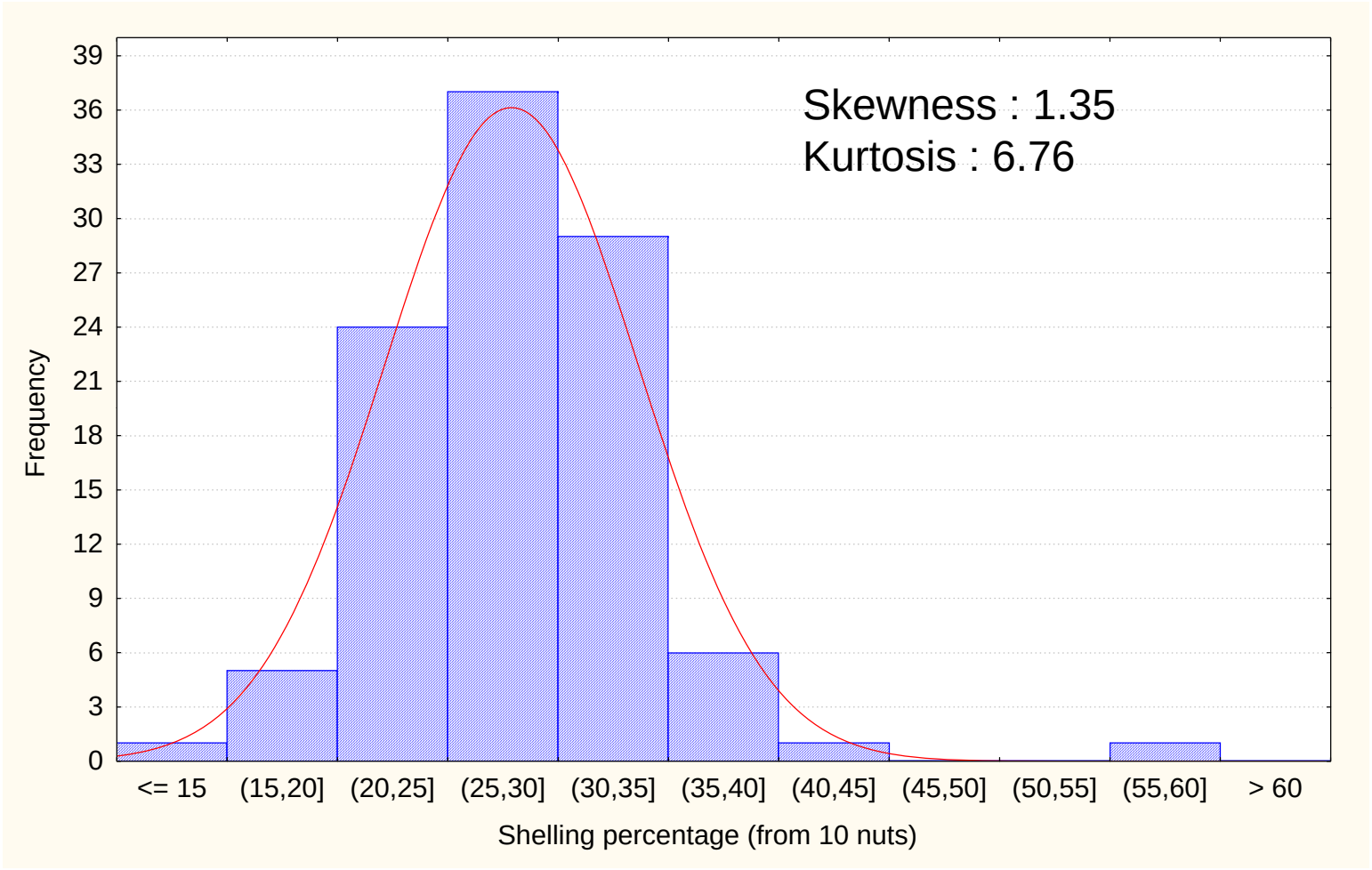


Fig. 7: Leptokurtic and positively skewed distribution of shelling percentage (from 10 nuts) among cashew germplasm source

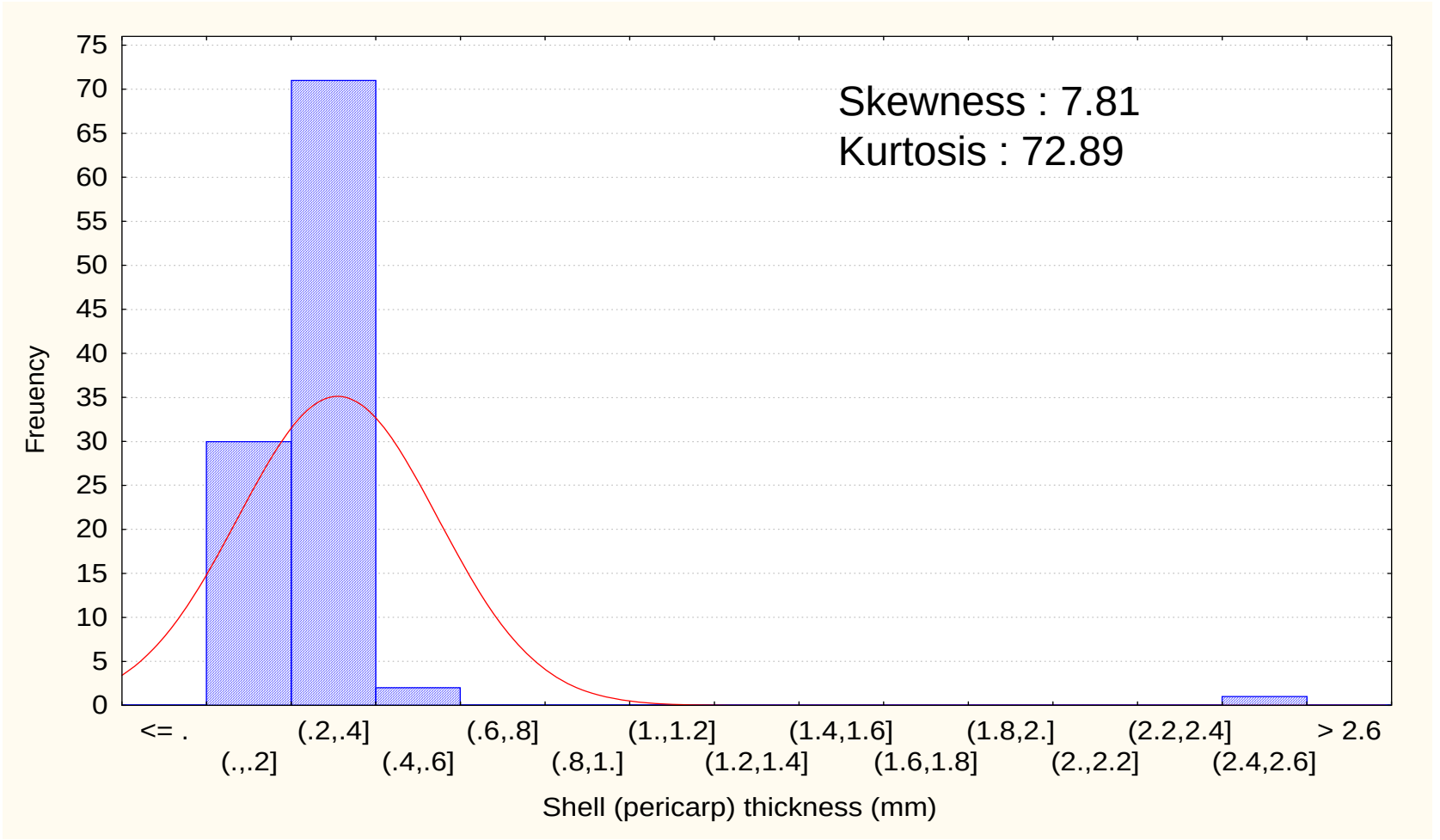


Fig. 8: Leptokurtic and positively skewed distribution of shell (pericarp) thickness (mm) among cashew germplasm source

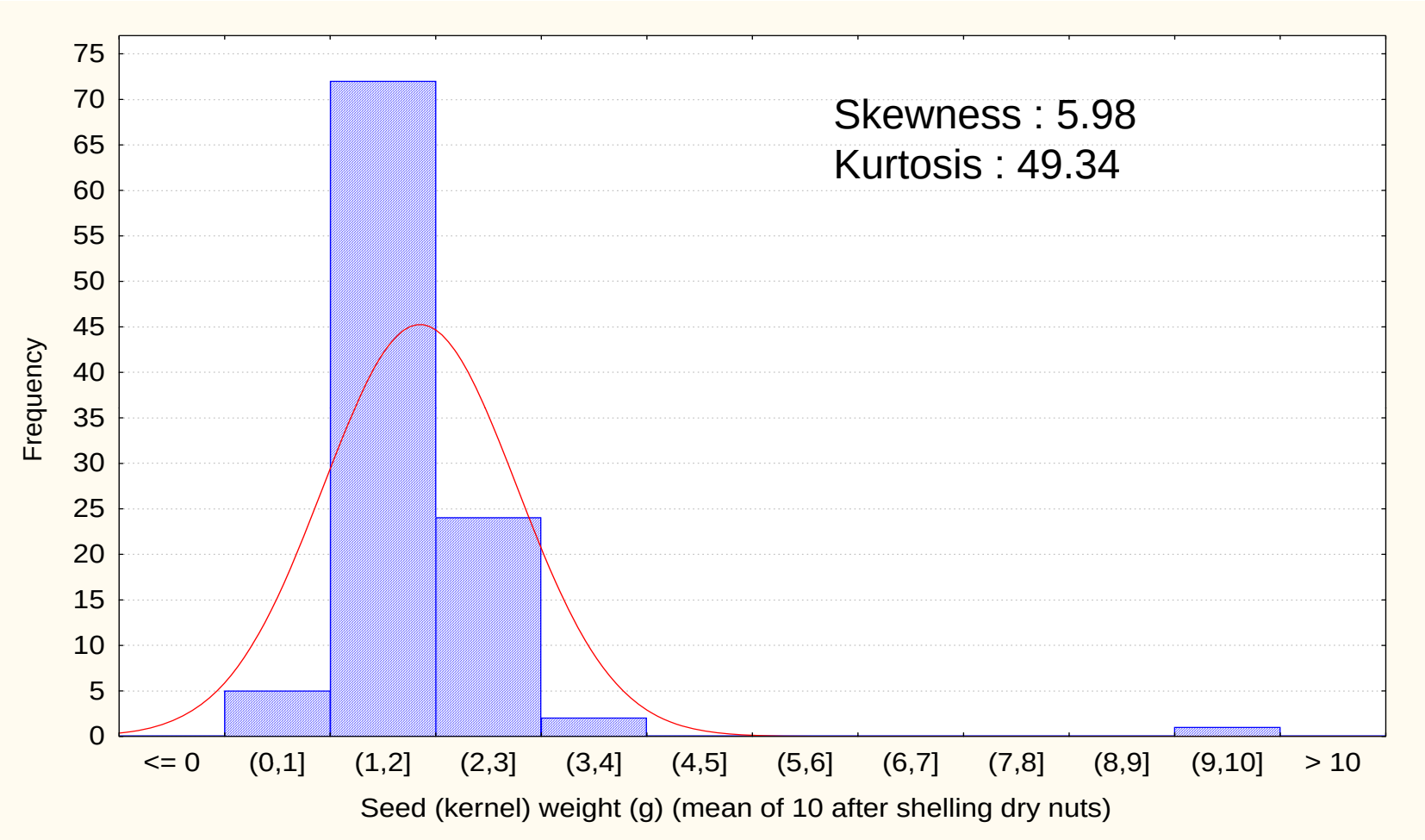


Fig. 9: Leptokurtic and positively skewed distribution of kernel weight (g) (mean of 10 kernels after shelling dry nuts) among cashew germplasm source

10. Cumulative yield (kg)

Lowest cumulative yield of 0.65 kg was observed in genotype 6/91 Kanhargad and the highest of 15.2 kg was observed in genotype V-5 Kottarakara. The coefficient of skewness was 6.17 and that of kurtosis was 49.92 (Fig. 10).

4.3. Components of variability and predicted genetic advance

The results of these components of variability and predicted genetic advance of different characters are presented in Table 3 and briefly described here under.

1. Tree height (m)

The character tree height exhibited moderate PCV and GCV of 15.30 and 14.57 per cent respectively, which had high heritability of 90 per cent along with high genetic advance (28.59 %).

2. Tree spread (m)

Tree spread showed high value of PCV (36.33 %) and high GCV (35.28 %), along with high heritability (94 %) and genetic advance (70.57 %).

3. Size of cashew apple (cm^3)

Size of cashew apple (cm^3) showed high PCV (43.36 %) and high GCV (29.37 %) along with moderate heritability value of 45 per cent whereas, genetic advance was high (40.98 %).

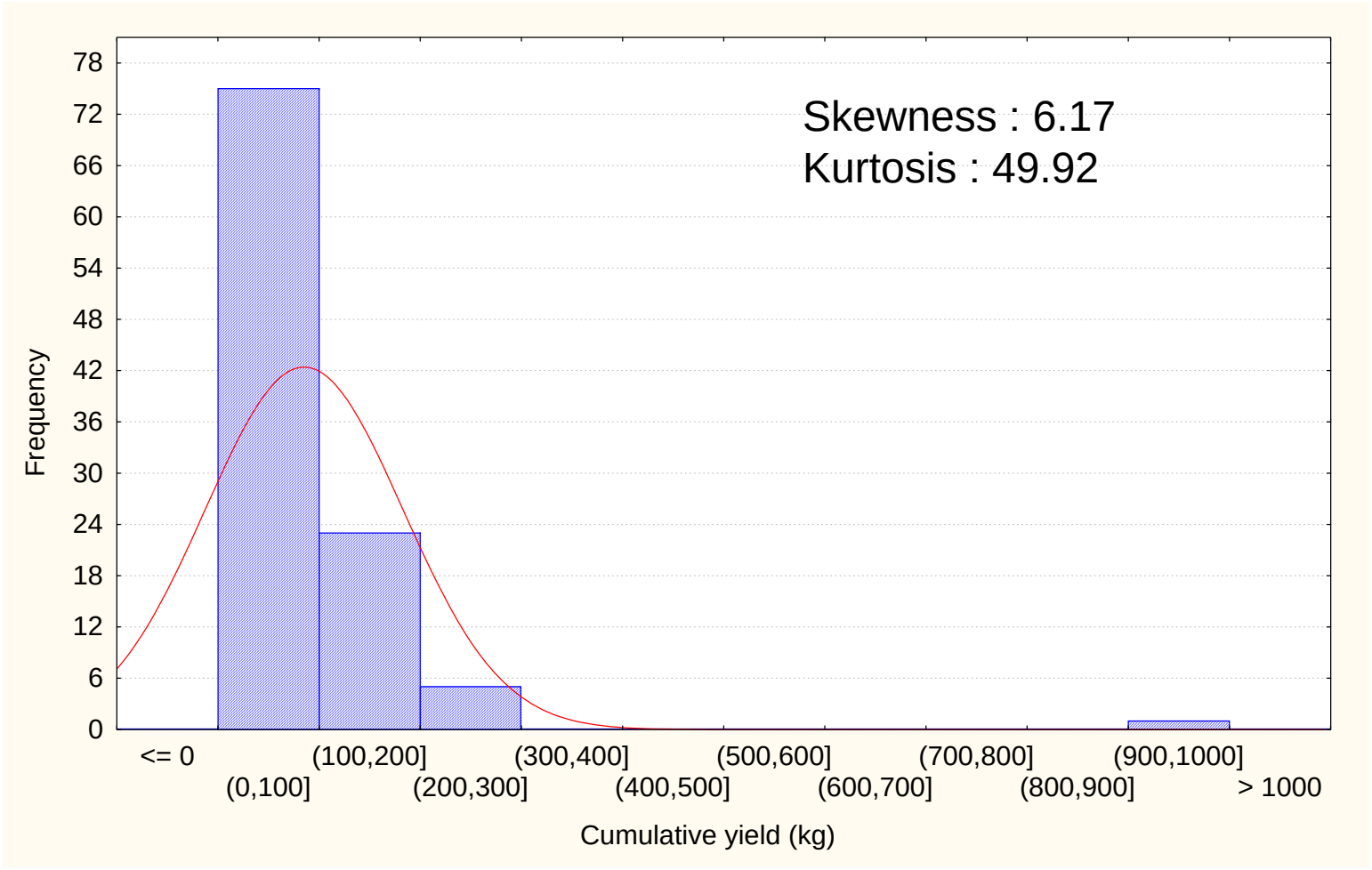


Fig. 10: Leptokurtic and positively skewed distribution of cumulative yield (kg) among cashew germplasm source

Table 3 : Estimation of *per se* performance and genetic parameters in respect of 10 quantitative traits in 104 cashew germplasm source

Sl. No.	Character	Mean ± SEm	Range	Phenotypic Coefficient of Variation (%)	Genotypic Coefficient of Variation (%)	Broad sense Heritability (%)	Genetic advance on per cent mean
1	Tree height (m)	5.13± 0.15	3.40- 7.45	15.30	14.57	90	28.59
2	Tree spread (m)	108.60± 6.66	19.25- 208	36.33	35.28	94	70.57
3	Size of cashew apple (cm^3)	15.33± 3.42	4.51- 39.51	43.36	29.37	45	40.98
4	Weight of cashew apple (g)	54.05± 5.11	10- 100.8	36.80	34.33	86	65.89
5	Nut dimension (cm^3)	2.25± 0.87	0.25-14.27	105.51	88.08	69	151.46
6	Nut weight (g)	6.24± 0.60	3.36-9.85	25.51	21.53	71	37.43
7	Shelling percentage	27.92± 2.33	13.41- 41.15	19.99	16.12	64	26.77
8	Shell (pericarp) thickness (mm)	0.30± 0.10	0.15- 1.45	58.55	22.57	14	17.92
9	Kernel weight (g)	1.81± 0.21	0.54- 3.42	27.70	22.07	63	36.22
10	Cumulative yield (Kg)	5.03± 0.68	0.65- 15.2	57.45	53.99	88	104.51

4. **Weight of cashew apple (g)**

Weight of cashew apple (g) showed high PCV and GCV values of 36.8 and 34.33 per cent respectively along with high heritability value of 86 per cent with high genetic advance (65.89 %).

5. **Nut dimension (cm^3)**

Nut dimension (cm^3) exhibited high PCV (105.51 %) and GCV (88.08 %) along with the high heritability value of 69 per cent and high genetic advance of 151.46 per cent.

6. **Nut weight (g)**

Nut weight (g) exhibited high PCV (25.51 %) and GCV (21.53 %) along with the high heritability value of 71 per cent and high genetic advance of 37.43 per cent.

7. **Shelling percentage**

Shelling percentage exhibited moderate PCV (19.99 %) and GCV (16.12 %) along with the high heritability value of 64 per cent and high genetic advance of 26.77 per cent.

8. **Shell (pericarp) thickness (mm)**

Shell (pericarp) thickness (mm) exhibited high PCV (58.55 %) and GCV (22.57 %) with the low heritability value of 14 per cent and moderate genetic advance of 17.92 per cent.

9. Kernel weight (g)

Kernel weight (g) exhibited high PCV (27.70 %) and GCV (22.07 %) along with the high heritability value of 63 per cent and high genetic advance of 36.22 per cent.

10. Cumulative yield (Kg)

Cumulative yield (Kg) exhibited high PCV (57.45 %) and GCV (53.99 %) along with the high heritability value of 88 per cent and high genetic advance of 104.51 per cent.

4.4. Correlation analysis

To aid the selection process, it is always essential to have the information on nature of association of characters with yield. Hence, the correlation co-efficient between the characters was computed both at the genotypic and phenotypic levels. The nature of genotypic and phenotypic association of 10 quantitative traits is presented in Table 4 and 5 respectively and briefly described below.

4.4.1. Genotypic correlation coefficient of 10 quantitative traits in 104 cashew germplasm source

Among ten quantitative parameters studied, tree spread had positive significant association with tree height (0.63) and cumulative yield had a positive significance relationship with tree spread (0.18).

Table 4 : Estimation of genotypic correlation coefficient matrix of 10 quantitative traits in 104 cashew germplasm source

Character	Tree height (m)	Tree spread (m)	Size of apple (cm^3)	Weight of apple (g)	Nut dimension (cm^3)	Nut weight (g)	Shelling percentage	Shell thickness (mm)	Kernel weight (g)	Cumulative yield (kg)
Tree height (m)	1	0.63**	-0.09	0.08	-0.04	0.01	0.06	-0.04	0.01	0.05
Tree spread (m)		1	-0.10	0.10	0.08	0.15	-0.12	0.13	0.13	0.18**
Size of apple (cm^3)			1	0.61**	0.52**	0.38**	-0.12	0.03	0.27**	-0.01
Weight of apple (g)				1	0.32**	0.38**	-0.11	-0.08	0.32**	0.04
Nut dimension (cm^3)					1	0.41**	-0.08	0.01	0.31**	0.02
Nut weight (g)						1	-0.11	0.20**	0.64**	0.07
Shelling percentage							1	-0.19	0.48**	0.05
Shell thickness (mm)								1	0.59**	0.01
Kernel weight (g)									1	0.21**
Cumulative yield (kg)										1

*Significance at P = 0.05 level, **Significance at P = 0.01 level

Table 5 : Estimation of phenotypic correlation coefficient matrix of 10 quantitative traits in 104 cashew germplasm source

Character	Tree height (m)	Tree spread (m)	Size of apple (cm^3)	Weight of apple (g)	Nut dimension (cm^3)	Nut weight (g)	Shelling percentage	Shell thickness (mm)	Kernel weight (g)	Cumulative yield (kg)
Tree height (m)	1	0.61**	-0.06	0.07	-0.04	0.01	0.01	-0.02	0.01	0.05
Tree spread (m)		1	-0.08	0.08	0.05	0.05	-0.12	0.02	0.08	0.17**
Size of apple (cm^3)			1	0.42**	0.24**	0.28**	-0.07	0.04	0.16	-0.01
Weight of apple (g)				1	0.28**	0.39**	-0.05	0.01	0.31**	0.05
Nut dimension (cm^3)					1	0.37**	-0.01	0.01	0.27**	0.01
Nut weight (g)						1	0.01	0.12	0.58**	0.06
Shelling percentage							1	-0.05	0.41	0.02
Shell thickness (mm)								1	0.08	0.01
Kernel weight (g)									1	0.19**
Cumulative yield (kg)										1

*Significance at P = 0.05 level, **Significance at P = 0.01 level

The association between weight of cashew apple, nut dimension, nut weight and kernel weight had positive significant relationship with size of cashew apple with values of 0.61, 0.52, 0.38 and 0.27 respectively.

The association between nut dimension, nut weight and kernel weight had positive significant relationship with weight of cashew apple with values of 0.32, 0.38 and 0.32 respectively.

The association between nut weight and kernel weight had positive significant relationship with nut dimension with values of 0.41 and 0.31 respectively.

The association between shell (pericarp) thickness and kernel weight had positive significant relationship with nut weight with values of 0.20 and 0.64 respectively.

The association between kernel weight and shelling percentage was positive significant with value of 0.48.

The association between kernel weight and shell (pericarp) thickness was positive significant with value of 0.59.

The association between cumulative yield and kernel weight was positive significant with value of 0.21.

4.4.2. Phenotypic correlation coefficient of 10 quantitative traits in 104 cashew germplasm source

Among ten quantitative parameters studied, tree spread had positive significant association with tree height (0.61).

The association between cumulative yield and tree spread was positive significance with value of (0.17).

Weight of cashew apple, nut dimension and nut weight had positive significant relationship with size of cashew apple with values of 0.42, 0.24 and 0.28 respectively.

Nut dimension, nut weight and Kernel weight had positive significant relationship with weight of cashew apple with values of 0.28, 0.39 and 0.31 respectively.

Nut weight and kernel weight had positive significant relationship with nut dimension with values of 0.37 and 0.27 respectively.

Kernel weight had positive significant relationship with nut weight with value of 0.58.

Cumulative yield had positive significant relationship with kernel weight with value of 0.19.

Genotypic and phenotypic correlation of economic traits with other quantitative traits in 104 cashew germplasm source is presented in Table 6.

4.5. Path coefficient analysis

Path coefficient analysis carried out to know the direct and indirect effect of the morphological traits on yield (Table 7). Shelling percentage had highest positive direct effect of 0.61 on yield, whereas nut dimension had lowest positive direct effect of 0.05 on yield, while kernel weight had highest negative direct effect of -0.71 on yield, whereas size of cashew apple had lowest negative direct effect of -0.04 on yield.

Kernel weight had highest positive indirect effect of 0.3 on yield, whereas shell (pericarp) thickness had lowest positive indirect effect of 0.001 on yield, while nut weight had highest negative indirect effect of -0.46 on yield, whereas kernel weight had lowest negative indirect effect of -0.001 on yield.

Table 6 : Estimation of phenotypic and genotypic correlation coefficients of economic traits with other quantitative traits in 104 cashew germplasm source

Character	Size of apple (cm^3)		Weight of apple (g)		Nut weight (g)		Shelling percentage		Kernel weight (g)	
	Phenotypic	Genotypic	Phenotypic	Genotypic	Phenotypic	Genotypic	Phenotypic	Genotypic	Phenotypic	Genotypic
Tree height (m)	-0.06	-0.09	0.07	0.08	0.01	0.01	0.01	0.06	0.01	0.01
Tree spread (m)	-0.08	-0.10	0.08	0.10	0.05	0.15	-0.12	-0.12	0.08	0.13
Nut dimension (cm^3)	0.24**	0.52**	0.28**	0.32**	0.37**	0.41**	-0.01	-0.08	0.27**	0.31**
Shell thickness (mm)	0.04	0.03	0.01	-0.08	0.12	0.20**	-0.05	-0.19	0.08	0.59**
Cumulative yield (kg)	-0.01	-0.01	0.05	0.04	0.06	0.07	0.02	0.05	0.19**	0.21**

Table 7 : Path coefficient analysis (effects table) of quantitative traits on yield in 104 cashew germplasm source

Character	Tree height (m)	Tree spread (m)	Size of apple (cm^3)	Weight of apple (g)	Nut dimension (cm^3)	Nut weight (g)	Shelling percentage	Shell thickness (mm)	Kernel weight (g)
1	**-0.194**	0.211	0.004	0.018	-0.002	0.004	0.036	-0.020	-0.003
2	-0.123	**0.334**	0.004	0.024	0.005	0.056	-0.076	0.063	-0.099
3	0.018	-0.033	**-0.046**	0.143	0.031	0.139	-0.077	0.015	-0.200
4	-0.015	0.035	-0.028	**0.234**	0.019	0.140	-0.068	-0.038	-0.234
5	0.009	0.028	-0.024	0.075	**0.059**	0.152	-0.051	0.005	-0.226
6	-0.002	0.051	-0.017	0.090	0.024	**0.365**	-0.069	0.092	-0.460
7	-0.011	-0.041	0.005	-0.026	-0.004	-0.041	**0.616**	-0.090	-0.349
8	0.008	0.046	-0.001	-0.020	0.001	0.074	-0.122	**0.452**	-0.424
9	-0.001	0.046	-0.013	0.076	0.018	0.234	0.300	0.268	**-0.717**

Residual effect = 1.01

4.6. Genetic diversity based on ten quantitative traits

4.6.1. Mahalanobis generalized distance (D^2)

The genetic diversity among 104 genotypes was estimated by employing D^2 statistics. Of 10 characters studied, cumulative yield (45.42 %) contributed maximum towards the total diversity, followed by tree spread (11.23 %), nut weight (10.54 %), tree height (9.61 %), shelling percentage (8.51 %), kernel weight (5.97 %), weight of cashew apple (4.53 %), shell (pericarp) thickness (1.71 %), nut dimension (1.69 %), size of cashew apple (0.72 %) was low (Table 9).

Table 9 : Per cent contribution of 10 quantitative characters towards total diversity in 104 cashew germplasm source

Character	Per cent contribution towards diversity
Tree height (m)	9.61
Tree spread (m)	11.23
Size of cashew apple (cm^3)	0.72
Weight of cashew apple (g)	4.53
Nut dimension (cm^3)	1.69
Nut weight (g)	10.54
Shelling percentage	8.51
Shell (pericarp) thickness (mm)	1.71
Kernel weight (g)	5.97
Cumulative yield (Kg)	45.42

Based on inter- genotypes D^2 values the genotypes was grouped into thirteen clusters using Tocher's methods described by Rao (1952). All 104 genotypes was grouped in to 13 clusters based on D^2 values. Of the 13 clusters, cluster I was the largest comprising of 50 genotypes followed by cluster III & IV with 13 genotypes each, cluster II & XII with 5 genotypes each and X 4 genotypes, where as cluster V, VI, VII, VIII, IX, XII and XIII 2 genotypes each (Table 8).

4.6.2. Inter cluster distance

The genotypes included are found to be very diverse in nature as they contributed maximum inter cluster distance (D^2) of 337.45 between the clusters VIII and X, while the minimum D^2 value was found between the clusters VI and XII (38.48) (Table 10).

Cluster I showed maximum inter cluster distance with the cluster XIII (209.62) followed by cluster X (196.81), IV (143.95), while it had minimum distance with the cluster VII (70.69). Cluster II had maximum Inter cluster distance with the cluster VIII (213.60) followed by cluster XI (187.38) and is closer to the cluster V (86.30).

Cluster III is more distanced from the cluster VIII (189.66) followed by cluster XI (188.86) and is closer to the cluster II (112.82). Cluster IV has more D^2 distance with the cluster XI (201.25) followed by cluster VI (200.79) and is closer to the cluster V (100.64). Cluster V has more D^2 distance with the cluster X (168.05) followed by cluster XIII (149.03) and less D^2 distance with the cluster VII (77.65). Cluster VI is genetically more distanced with the cluster X (324.62) followed by cluster XIII (244.08), while it is closer to the cluster XII (38.48).

Table 8 : Grouping of 104 cashew germplasm source based on D^2 analysis of ten quantitative characters

Cluster	No. of genotypes	Germplasm source*
I	50	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50
II	5	51, 52, 53, 98, 99
III	13	54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 78
IV	13	66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 79
V	2	94, 95
VI	2	83, 88
VII	2	85, 92
VIII	2	96, 103
IX	2	91, 102
X	4	80, 81, 84, 100
XI	5	82, 86, 87, 97, 104
XII	2	90, 93
XIII	2	89, 101

* Refer Table 1 for respective names of germplasm source

Table 10 : The nearest and farthest clusters from each cluster based on D^2 values in 104 cashew germplasm source

Cluster No.	Nearest cluster with D^2 value	Farthest cluster with D^2 value
I	VII (70.69)	XIII (209.62)
II	V (86.30)	VIII (213.60)
III	II (112.82)	VIII (189.66)
IV	V (100.64)	XI (201.25)
V	VII (77.65)	X (168.05)
VI	XII (38.48)	X (324.62)
VII	VIII (60.66)	XIII (215.29)
VIII	VI (44.25)	X (337.45)
IX	VIII (65.50)	X (250.97)
X	III (129.13)	VIII (337.45)
XI	VI (98.76)	X (300.87)
XII	VI (38.48)	X (321.93)
XIII	V (149.03)	VIII (266.21)

Cluster VII is more distanced from the cluster XIII (215.29) followed by cluster X (195.77) and is closer to the cluster VIII (60.66). Cluster VIII has more D^2 distance with the cluster X (337.45) followed by cluster XIII (266.21) and is closer to the cluster VI (44.25). Cluster IX has more D^2 distance with the cluster X (250.97) followed by cluster XIII (189.19) and less D^2 distance with the cluster VIII (65.50). Cluster X is genetically more distanced with the cluster VIII (337.45) followed by cluster XII (321.93), while it is closer to the cluster III (129.13).

Cluster XI is more distanced from the cluster X (300.87) followed by cluster XIII (213.29) and is closer to the cluster VI (98.76). Cluster XII has more D^2 distance with the cluster X (321.93) followed by cluster XIII (236.19) and is closer to the cluster VI (38.48). Cluster XIII has more D^2 distance with the cluster VIII (266.21) followed by cluster VI (244.08) and less D^2 distance with the cluster V (149.03).

4.6.3. Intra cluster distance

All the clusters exhibited more intra cluster distances and comprised more than one genotype. Intra cluster distance was highest in the cluster XI (153.81), followed by the cluster II (129.78), cluster IV (115.94), cluster X (113.13), cluster III (94.20), cluster I (93.28), cluster XIII (77.19), cluster XII (70.66), cluster IX (34.27), cluster VIII (29.70), cluster VII (26.51), cluster VI (18.70) and cluster V (14.57). Intra cluster D^2 and D values are presented in the Table 11 and 12.

4.6.4. Cluster mean analysis

The mean values for all 10 characters over thirteen clusters was calculated and presented in Table 13. The tree height was more in genotypes included in the cluster III (5.76 m) while cluster VIII (4.22 m)

Table 11 : Average intra and inter cluster D^2 values of 104 cashew germplasm source

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
I	**93.28**	121.19	122.93	143.95	80.36	95.56	70.69	124.36	115.02	196.81	134.68	113.90	209.62
II		**129.78**	112.82	131.22	86.30	174.78	126.05	213.60	145.12	150.84	187.38	180.55	184.42
III			**94.20**	113.41	113.91	182.28	115.56	189.66	117.37	129.13	188.86	174.05	183.50
IV				**115.94**	100.64	200.79	127.47	195.20	134.92	143.03	201.25	199.26	169.93
V					**14.57**	85.10	77.65	128.92	103.39	168.05	125.77	110.20	149.03
VI						**18.70**	66.24	44.25	86.23	324.62	98.76	38.48	244.08
VII							**26.51**	60.66	90.74	195.77	101.80	92.40	215.29
VIII								**29.70**	65.50	337.45	111.83	57.06	266.21
IX									**34.27**	250.97	130.63	73.11	189.19
X										**113.13**	300.87	321.93	232.55
XI											**153.81**	102.53	213.29
XII												**70.66**	236.19
XIII													**77.19**

Note : Diagonal values are intra cluster D^2 values

Table 12 : Average intra and inter cluster D values of 104 cashew germplasm source

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
I	**9.65**	11.00	11.08	11.99	8.96	9.77	8.40	11.15	10.72	14.02	11.60	10.67	14.47
II		**11.39**	10.62	11.45	9.29	13.22	11.22	14.61	12.04	12.28	13.68	13.43	13.58
III			**9.70**	10.65	10.67	13.50	10.75	13.77	10.83	11.36	13.74	13.19	13.54
IV				**10.76**	10.03	14.17	11.29	13.97	11.61	11.96	14.18	14.11	13.03
V					**3.81**	9.22	8.81	11.35	10.16	12.96	11.21	10.49	12.20
VI						**4.32**	8.13	6.65	9.28	18.01	9.93	6.20	15.62
VII							**5.14**	7.78	9.52	13.99	10.09	9.61	14.67
VIII								**5.45**	8.09	18.37	10.57	7.55	16.31
IX									**5.85**	15.84	11.43	8.55	13.75
X										**10.63**	17.34	17.94	15.25
XI											**12.40**	10.12	14.60
XII												**8.40**	15.36
XIII													**8.78**

Note : Diagonal values are intra cluster D values

Table 13 : The mean values of different genotypes clusters for 10 quantitative traits in 104 cashew germplasm source

Clusters	Tree height (m)	Tree spread (m)	Size of apple (cm^3)	Weight of apple (g)	Nut dimension (cm^3)	Nut weight (g)	Shelling percentage	Shell thickness (mm)	Kernel weight (g)	Tree height (m)
I	5.00	98.67	14.38	49.13	1.71	5.83	27.71	0.31	1.68	3.77
II	5.70	126.78	15.92	63.09	2.35	6.87	24.81	0.32	1.85	3.45
III	5.76	136.04	15.05	50.37	1.84	6.10	29.63	0.28	1.99	6.16
IV	5.22	136.43	16.92	61.64	2.04	6.88	25.35	0.28	1.81	7.33
V	4.75	106.45	20.56	77.90	1.30	7.12	24.29	0.30	1.41	3.51
VI	4.37	60.72	14.43	57.54	1.29	4.87	32.40	0.20	1.44	4.45
VII	4.50	82.32	9.79	31.75	1.79	6.06	25.11	0.25	1.63	5.24
VIII	4.22	53.32	10.76	50.00	1.06	6.01	31.28	0.30	1.72	9.09
IX	5.67	91.80	14.82	65.57	1.06	6.39	28.51	0.27	1.75	10.01
X	5.55	164.95	9.91	49.84	2.42	7.50	31.66	0.31	2.27	4.17
XI	4.58	67.13	22.01	61.87	4.83	7.26	29.23	0.36	2.30	5.65
XII	4.77	64.14	19.32	68.75	1.65	5.87	34.45	0.25	2.33	6.71
XIII	5.25	132.48	18.64	89.00	13.03	7.60	27.77	0.22	2.02	6.84

comprised of genotypes with less tree height. The tree spread was more in genotypes included in the cluster IV (136.43 m) while cluster VIII (53.32 m) comprised of genotypes with less tree spread. The size of cashew apple was more in genotypes included in the cluster XI (22.01 cm^3) while cluster VII (9.79 cm^3) comprised of genotypes with less apple size. The weight of cashew apple was more in genotypes included in the cluster XIII (89.00 g) while cluster VII (31.75 g) comprised of genotypes with less apple weight. The nut dimension was more in genotypes included in the cluster XIII (13.03 cm^3) while cluster VIII and IX (1.06 cm^3) comprised of genotypes with less nut dimension.

With respect to the nut weight was more in genotypes included in the cluster XIII (7.60 g) while cluster VI (4.87 g) comprised of genotypes with less nut weight. The shelling percentage was more in genotypes included in the cluster XII (34.45 %) while cluster V (24.29 %) comprised of genotypes with less shelling percentage. The shell (pericarp) thickness was more in genotypes included in the cluster XI (0.36 mm) while cluster VI (0.20 mm) comprised of genotypes with less shell (pericarp) thickness. The kernel weight was more in genotypes included in the cluster XII (2.33 g) while cluster V (1.41 g) comprised of genotypes with less kernel weight. The cumulative yield was more in genotypes included in the cluster IX (10.01 kg) while cluster II (3.45 kg) comprised of genotypes with less cumulative yield.

4.7. Bulk Segregant Analysis (BSA)

After isolation of good quality genomic DNA and constitution of bulks, the cashew genotypes was subjected to RAPD, ISSR and SSR primer screening. The different experiments carried out under this include screening of random primers, and, ISSR and SSR primers on low

and high DNA bulk constituted for five important economic characters in cashew (Table 15).

4.8.1. Screening of RAPD primers on low and high DNA bulk constituted for five important characters in cashew

The three hundred and nine RAPD primers belongs to NAPS, C, SB and OPH series used to screen on high and low bulk of germplasm source to determine the polymorphism existing among the germplasm source for five important characters is presented in (Table 16a & 16b). Among three hundred and nine RAPD primers twenty four polymorphic primers showed polymorphism for five important selected characters (Table 18). The polymorphic random primers was NAPS 3, NAPS 11, NAPS 27, NAPS 32, NAPS 16, NAPS 22, NAPS 40, NAPS 56, NAPS 31, NAPS 34, NAPS 39, NAPS 68, NAPS 75, NAPS 257, NAPS 24, NAPS 63, NAPS 65, NAPS 252, NAPS 13, NAPS 29, NAPS 66, NAPS 288, C 301 and C 302. These 24 polymorphic RAPD primers was validated on individuals constituting each bulk of the concern trait.

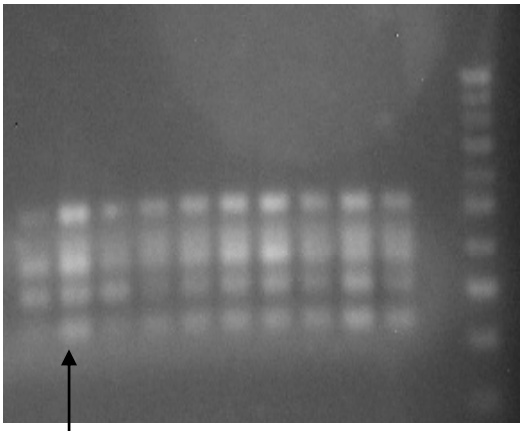
1) Screening with NAPS 3 primer (CCTGGGCTTA)

PCR amplification with RAPD primer NAPS 3 produced five loci in DNA bulk constituted for size of cashew apple on 1.5 per cent agarose gel (Plate 4 a) in high bulk and four loci in low bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 150 bp. However, the results was inconsistent in individual constituents of bulk.

Table 18 : Polymorphic RAPD primers showing polymorphism between HB and LB for each of five traits

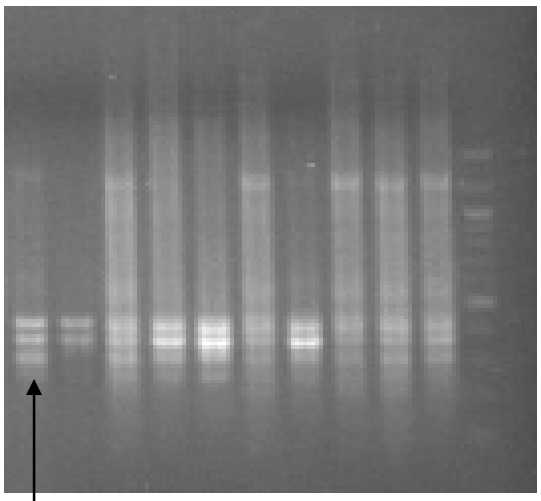
Sl. No.	Primer	Trait	No. of bands (High Bulk)	No. of bands (Low Bulk)	Polymorphic loci present in	Polymorphic loci size (bp)
1	NAPS 3	1st	5	4	High Bulk	150
2	NAPS 11	1st	2	3	Low Bulk	300
3	NAPS 27	1st	6	4	High Bulk	700 & 1000
4	NAPS 32	1st	3	2	High Bulk	700
5	NAPS 16	2nd	3	2	High Bulk	300
6	NAPS 22	2nd	6	5	Low Bulk	250
7	NAPS 40	2nd	3	4	Low Bulk	700
8	NAPS 56	2nd	3	4	Low Bulk	600
9	NAPS 31	3rd	5	6	Low Bulk	1500
10	NAPS 34	3rd	3	2	High Bulk	300
11	NAPS 39	3rd	8	9	Low Bulk	1000
12	NAPS 68	3rd	8	7	High Bulk	700
13	NAPS 75	3rd	5	3	High Bulk	1000 & 1400
14	NAPS 257	3rd	7	6	High Bulk	800
15	NAPS 24	4th	2	1	Low Bulk	300
16	NAPS 63	4th	5	2	Low Bulk	600, 700 & 1400
17	NAPS 65	4th	7	6	High Bulk	700
18	NAPS 252	4th	4	3	High Bulk	1000
19	NAPS 13	5th	3	2	High Bulk	900
20	NAPS 29	5th	3	2	High Bulk	100
21	NAPS 66	5th	4	3	High Bulk	1000
22	NAPS 288	5th	7	6	High Bulk	1200
23	C 301	5th	1	2	Low Bulk	-
24	C 302	5th	2	4	Low Bulk	-

1L 1H 2L 2H 3L 3H 4L 4H 5L 5H M



150 bp **a) Gel profile with primer NAPS 3 (CCTGGGCTTA)**

1L 1H 2L 2H 3L 3H 4L 4H 5L 5H M



300 bp **b) Gel profile with primer NAPS 11 (CCCCCCTTTA)**

1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

LB: Low Bulk, HB: High Bulk
M =100 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 4. BSA with NAPS 3 (Plate 4a) and NAPS11 (Plate 4b) RAPD primers on LB and HB for size of cashew apple (cm^3) (mean of 10 fruits) (Trait 1)

2) Screening with NAPS 11 primer (CCCCCCTTTA)

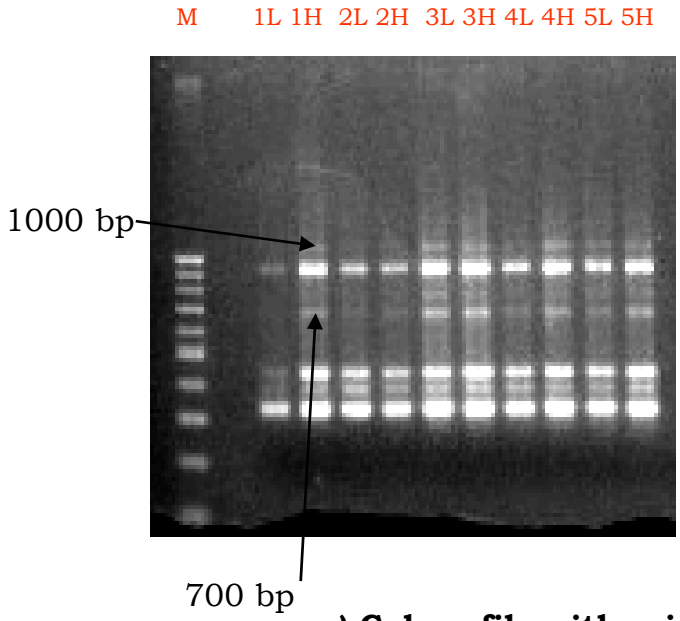
PCR amplification with RAPD primer NAPS 11 produced three loci in DNA bulk constituted for size of cashew apple on 1.5 per cent agarose gel (Plate 4 b) in low bulk and two loci in high bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 300 bp. However, the results was inconsistent in individual constituents of bulk.

3) Screening with NAPS 27 primer (TTTGGGGGGA)

PCR amplification with RAPD primer NAPS 27 produced six loci in DNA bulk constituted for size of cashew apple on 1.5 per cent agarose gel (Plate 5 a) in high bulk and four loci in low bulk. Two loci was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 700 and 1000 bp. However, the results was inconsistent in individual constituents of bulk.

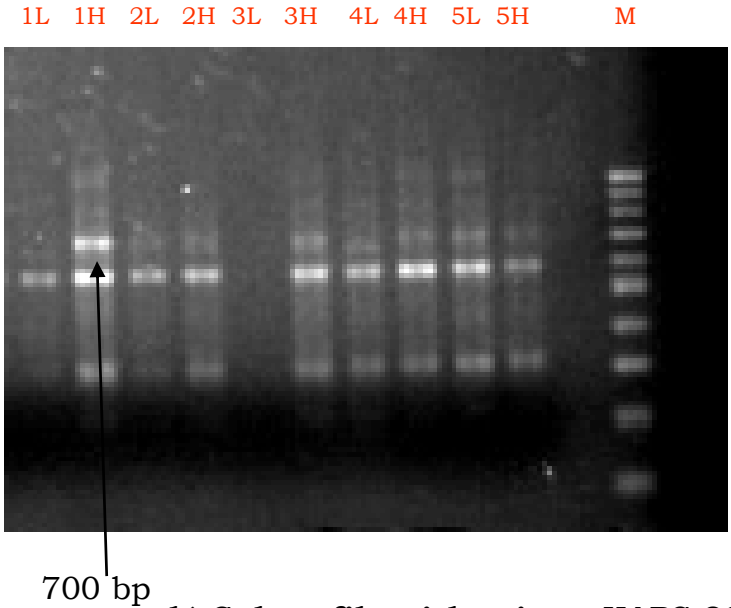
4) Screening with NAPS 32 primer (GGGGCCTTAA)

PCR amplification with RAPD primer NAPS 32 produced three loci in DNA bulk constituted for size of cashew apple on 1.5 per cent agarose gel (Plate 5 b) in high bulk and two loci in low bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 700 bp. However, the results was inconsistent in individual constituents of bulk.



1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

a) Gel profile with primer NAPS 27 (TTTGGGGGGA)



b) Gel profile with primer NAPS 32 (GGGGCCTTAA)

LB: Low Bulk, HB: High Bulk
M =100 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 5. BSA with NAPS 27 (Plate 5a) and NAPS 32 (Plate 5b) RAPD primers on LB and HB for size of cashew apple (cm^3) (mean of 10 fruits) (Trait 1)

5) Screening with NAPS 16 primer (GGTGGCGGGA)

PCR amplification with RAPD primer NAPS 16 produced three loci in DNA bulk constituted for weight of cashew apple on 1.5 per cent agarose gel (Plate 6 a) in high bulk and two loci in low bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 300 bp. However, the results was inconsistent in individual constituents of bulk.

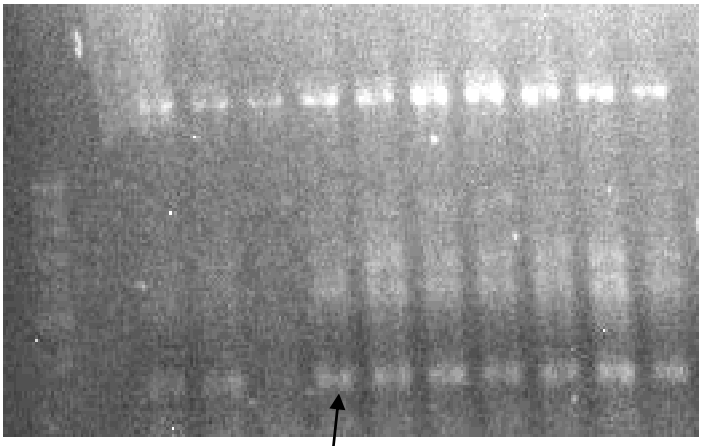
6) Screening with NAPS 22 primer (CCCTTGGGGG)

PCR amplification with RAPD primer NAPS 22 produced six loci in DNA bulk constituted for weight of cashew apple on 1.5 per cent agarose gel (Plate 6 b) in high bulk and five loci in low bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 250 bp. However, the results was inconsistent in individual constituents of bulk.

7) Screening with NAPS 40 primer (TTACCTGGGC)

PCR amplification with RAPD primer NAPS 40 produced three loci in DNA bulk constituted for weight of cashew apple on 1.5 per cent agarose gel (Plate 7 a) in high bulk and four loci in low bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 700 bp. However, the results was inconsistent in individual constituents of bulk.

M 1L 1H 2L 2H 3L 3H 4L 4H 5L 5H

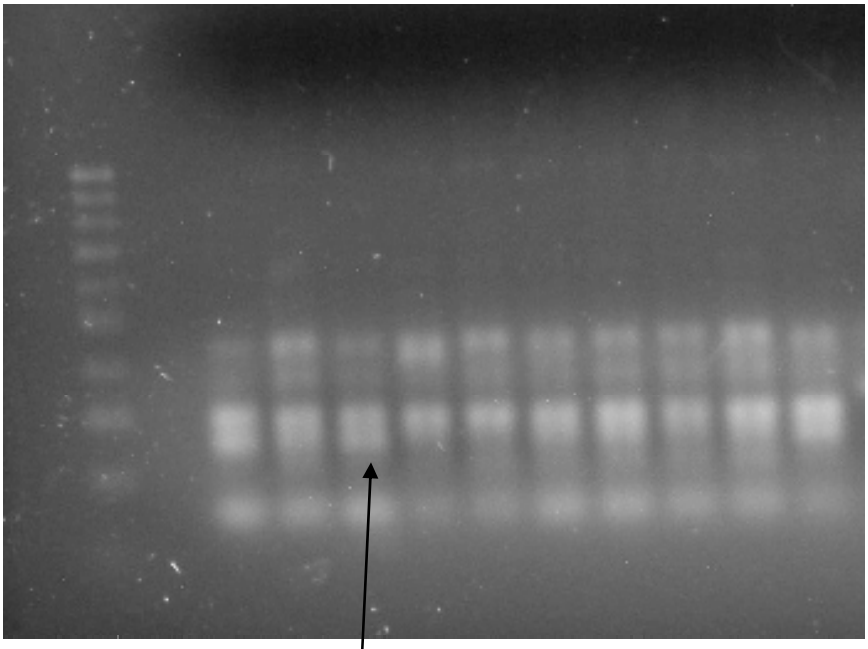


300 bp

1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

a) Gel profile with primer NAPS 16 (GGTGGCGGGA)

M 1L 1H 2L 2H 3L 3H 4L 4H 5L 5H



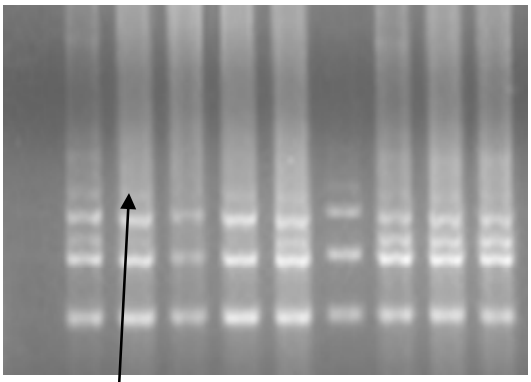
250 bp

b) Gel profile with primer NAPS 22 (CCCTTGGGGG)

LB: Low Bulk, HB: High Bulk
M =100 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 6. BSA with NAPS 16 (Plate 6a) and NAPS 22 (Plate 6b) RAPD primers on LB and HB for weight of cashew apple (g) (mean of 10 fruits) (Trait 2)

1L 1H 2L 2H 3L 3H 4L 4H 5L 5H

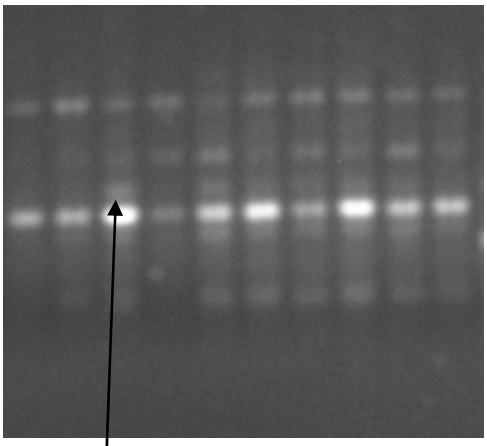


1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

700 bp

a) Gel profile with primer NAPS 40 (TTACCTGGGC)

1L 1H 2L 2H 3L 3H 4L 4H 5L 5H



600 bp

b) Gel profile with primer NAPS 56 (TGCCCCGAGC)

LB: Low Bulk, HB: High Bulk
M =100 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 7. BSA with NAPS 40 (Plate 7a) and NAPS 56 (Plate 7b) RAPD primers on LB and HB for weight of cashew apple (g) (mean of 10 fruits) (Trait 2)

8) Screening with NAPS 56 primer (TGCCCCGAGC)

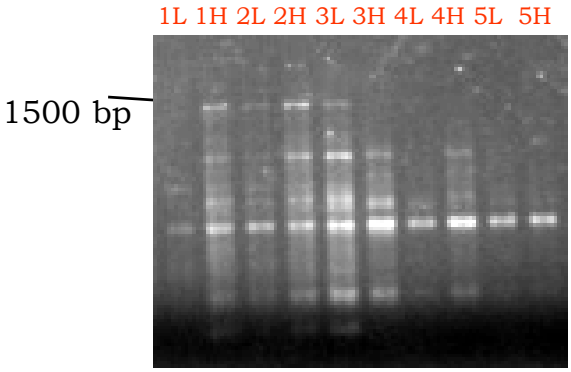
PCR amplification with RAPD primer NAPS 56 produced four loci in DNA bulk constituted for weight of cashew apple on 1.5 per cent agarose gel (Plate 7 b) in low bulk and three loci in high bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 600 bp. However, the results was inconsistent in individual constituents of bulk.

9) Screening with NAPS 31 primer (CCGGCCTTCC)

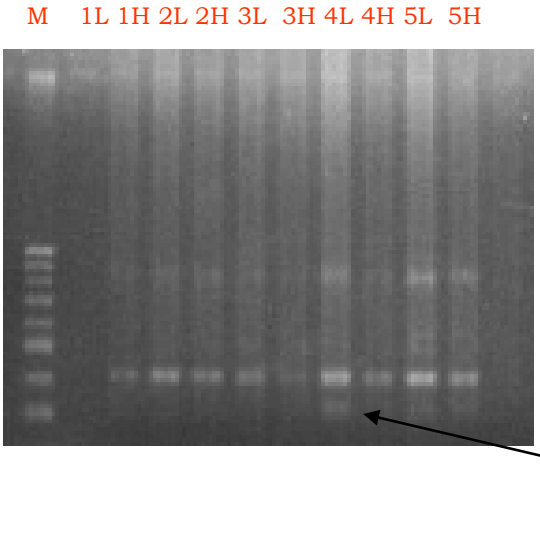
PCR amplification with RAPD primer NAPS 31 produced six loci in DNA bulk constituted for nut weight on 1.5 per cent agarose gel (Plate 8 a) in low bulk and five loci in high bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 1500 bp. However, the results was inconsistent in individual constituents of bulk.

10) Screening with NAPS 34 primer (CCGGCCCCAA)

PCR amplification with RAPD primer NAPS 34 produced three loci in DNA bulk constituted for nut weight on 1.5 per cent agarose gel (Plate 8 b) in high bulk and two loci in low bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 300 bp. However, the results was inconsistent in individual constituents of bulk.



a) Gel profile with primer NAPS 31 (CCGGCCTTCC)



1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

b) Gel profile with primer NAPS 34 (CCGGCCCCAA)

LB: Low Bulk, HB: High Bulk
M =100 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 8. BSA with NAPS 31 (Plate 8a) and NAPS 34 (Plate 8b) RAPD primers on LB and HB for nut weight (g) (mean of 100 nuts) (Trait 3)

11) Screening with NAPS 39 primer (TTAACCGGGC)

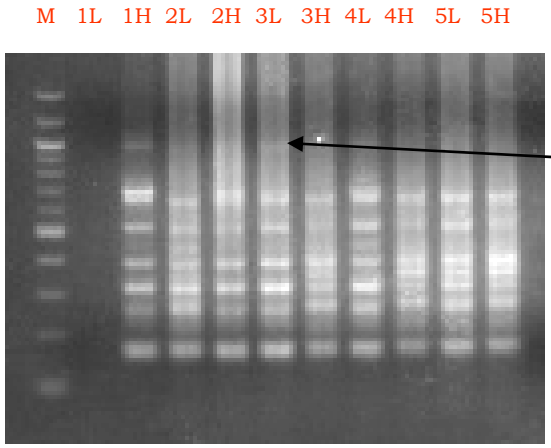
PCR amplification with RAPD primer NAPS 39 produced nine loci in DNA bulk constituted for nut weight on 1.5 per cent agarose gel (Plate 9 a) in low bulk and eight loci in high bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 1000 bp. However, the results was inconsistent in individual constituents of bulk.

12) Screening with NAPS 68 primer (GAGCTCGCGA)

PCR amplification with RAPD primer NAPS 68 produced eight loci in DNA bulk constituted for nut weight on 1.5 per cent agarose gel (Plate 9 b) in high bulk and seven loci in low bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 700 bp. However, the results was inconsistent in individual constituents of bulk.

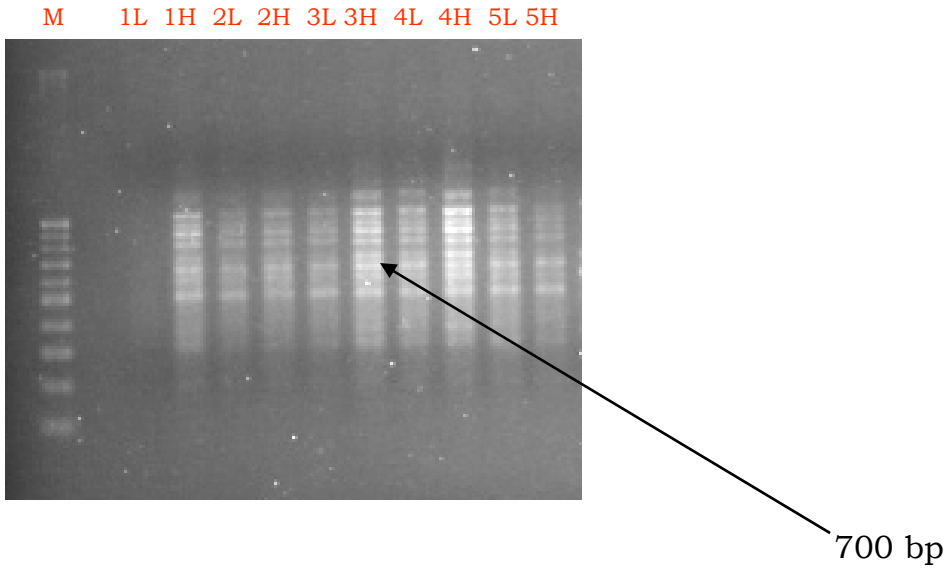
13) Screening with NAPS 75 primer (GAGGTCCAGA)

PCR amplification with RAPD primer NAPS 75 produced five loci in DNA bulk constituted for nut weight on 1.5 per cent agarose gel (Plate 10 a) in high bulk and three loci in low bulk. Two loci was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 1000 and 1400 bp. However, the results was inconsistent in individual constituents of bulk.



1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

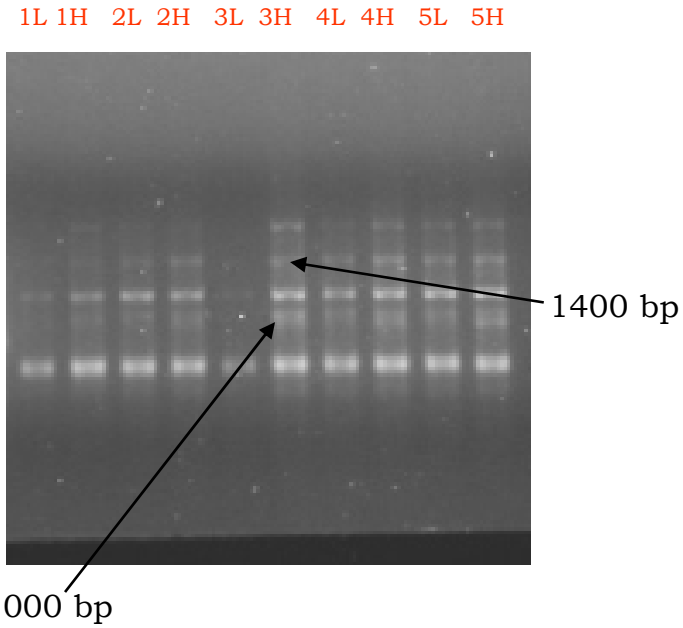
a) Gel profile with primer NAPS 39 (TTAACCGGGC)



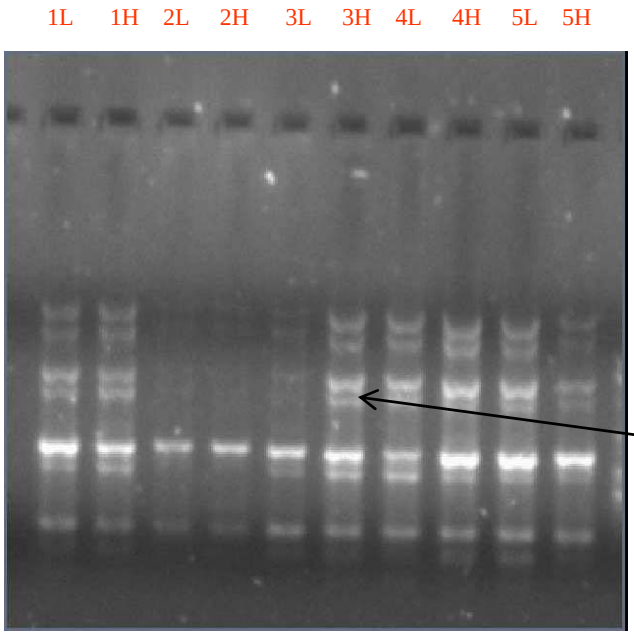
b) Gel profile with primer NAPS 68 (GAGCTCGCGA)

LB: Low Bulk, HB: High Bulk
M =100 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 9. BSA with NAPS 39 (Plate 9a) and NAPS 68 (Plate 9b) RAPD primers on LB and HB for nut weight (g) (mean of 100 nuts) (Trait 3)



a) Gel profile with primer NAPS 75 (GAGGTCCAGA)



1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

b) Gel profile with primer NAPS 257 (CGTCACCGTT)

LB: Low Bulk, HB: High Bulk
M =100 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 10. BSA with NAPS 75 (Plate 10 a) and NAPS 257 (Plate 10 b) RAPD primers on LB and HB for nut weight (g) (mean of 100 nuts) (Trait 3)

14) Screening with NAPS 257 primer (CGTCACCGTT)

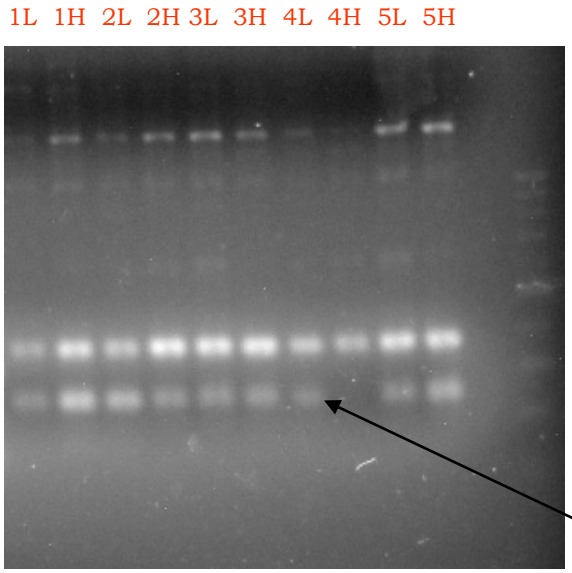
PCR amplification with RAPD primer NAPS 257 produced seven loci in DNA bulk constituted for nut weight on 1.5 per cent agarose gel (Plate 10 b) in high bulk and six loci in low bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 800 bp. However, the results was inconsistent in individual constituents of bulk.

15) Screening with NAPS 24 primer (ACAGGGGTGA)

PCR amplification with RAPD primer NAPS 24 produced two loci in DNA bulk constituted for shelling percentage on 1.5 per cent agarose gel (Plate 11 a) in high bulk and one locus in low bulk. Only one locus was polymorphic between the contrasting bulks. Polymorphism was observed at 300 bp. However, the results was inconsistent in individual constituents of bulk.

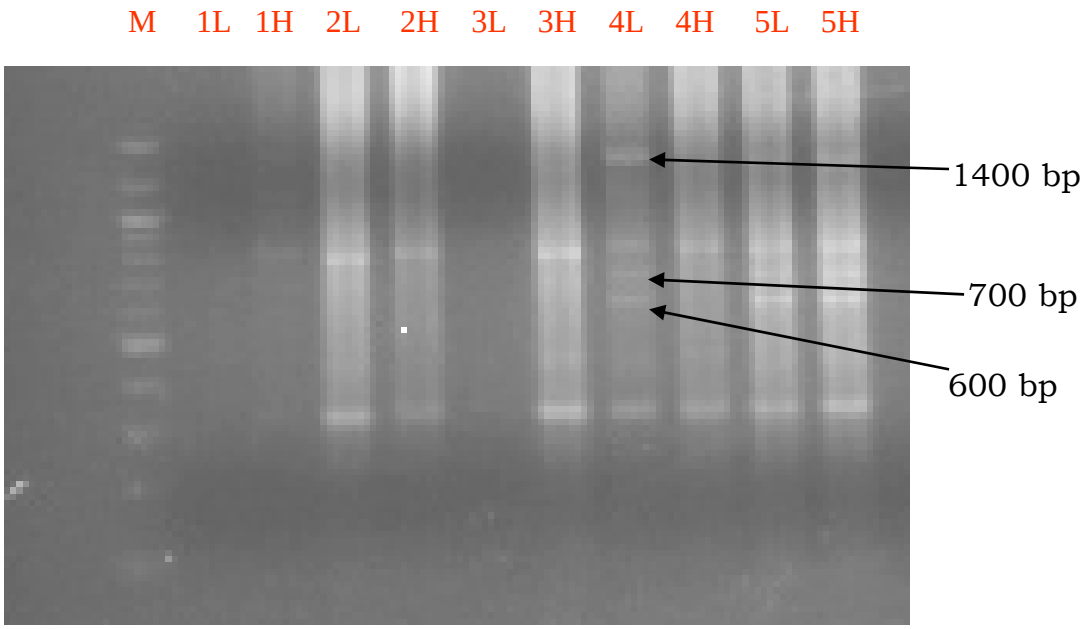
16) Screening with NAPS 63 primer (TTCCCCGCCC)

PCR amplification with RAPD primer NAPS 63 produced five loci in DNA bulk constituted for shelling percentage on 1.5 per cent agarose gel (Plate 11 b) in high bulk and two loci in low bulk. Three loci was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 600, 700 and 800 bp. However, the results was inconsistent in individual constituents of bulk.



1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

a) Gel profile with primer NAPS 24 (ACAGGGGTGA)



b) Gel profile with primer NAPS 63 (TTCCCCGCCC)

LB: Low Bulk, HB: High Bulk
M =100 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 11. BSA with NAPS 24 (Plate 11a) and NAPS 63 (Plate 11b) RAPD primers on LB and HB for shelling percentage (mean of 10 nuts) (Trait 4)

17) Screening with NAPS 65 primer (AGGGGCGGGA)

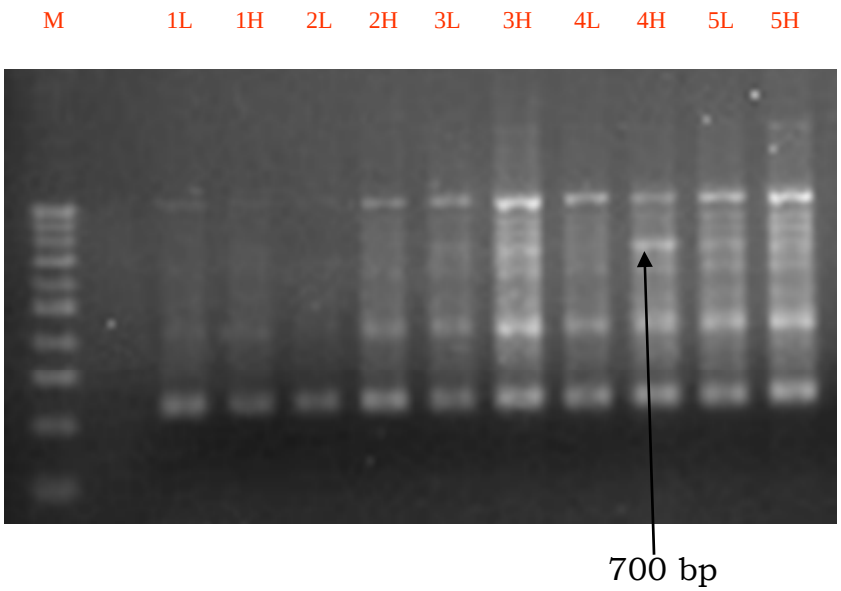
PCR amplification with RAPD primer NAPS 65 produced seven loci in DNA bulk constituted for shelling percentage on 1.5 per cent agarose gel (Plate 12 a) in high bulk and six loci in low bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 700 bp. However, the results was inconsistent in individual constituents of bulk.

18) Screening with NAPS 252 primer (CTGGTGATGT)

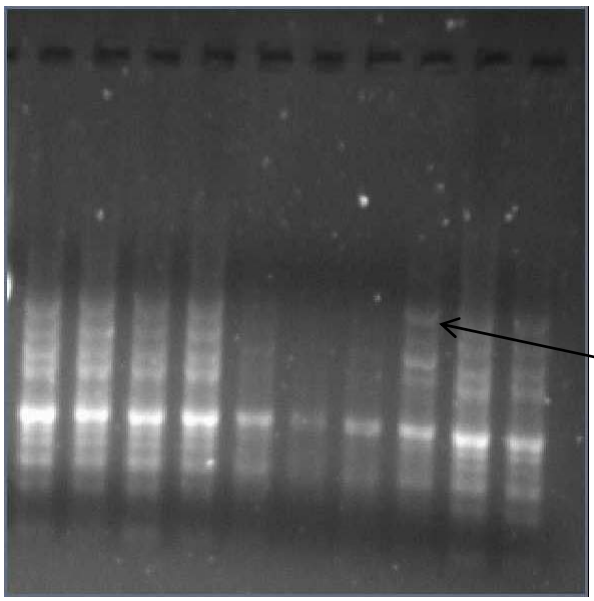
PCR amplification with RAPD primer NAPS 252 produced four loci in DNA bulk constituted for shelling percentage on 1.5 per cent agarose gel (Plate 12 b) in high bulk and three loci in low bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 1000 bp. However, the results was inconsistent in individual constituents of bulk.

19) Screening with NAPS 13 primer (CCTGGGTGGA)

PCR amplification with RAPD primer NAPS 13 produced three loci in DNA bulk constituted for kernel weight on 1.5 per cent agarose gel (Plate 13 a) in high bulk and two loci in low bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 900 bp. However, the results was inconsistent in individual constituents of bulk.



a) Gel profile with primer NAPS 65 (AGGGGCGGGA)

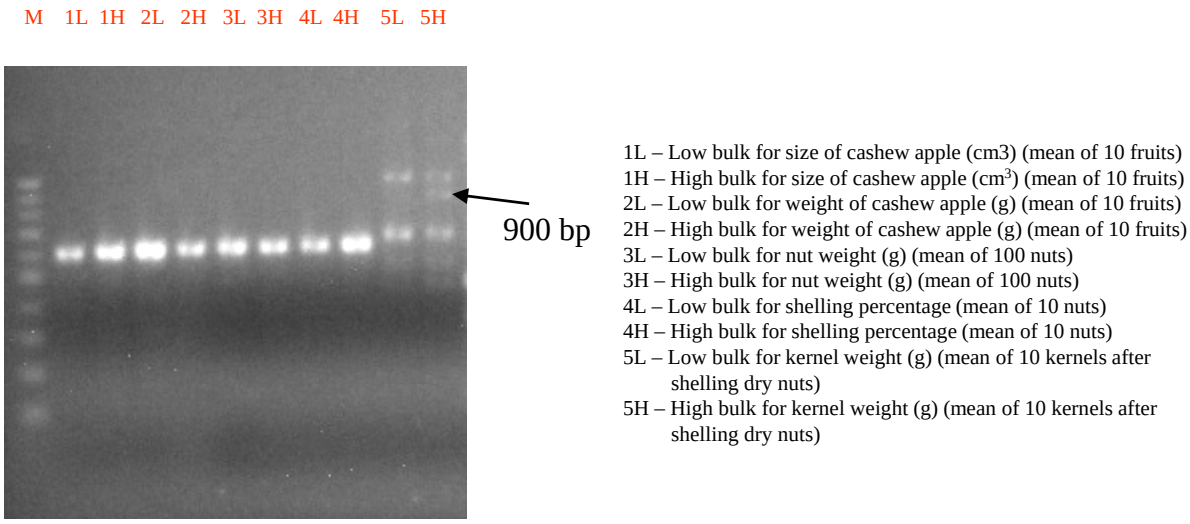


1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

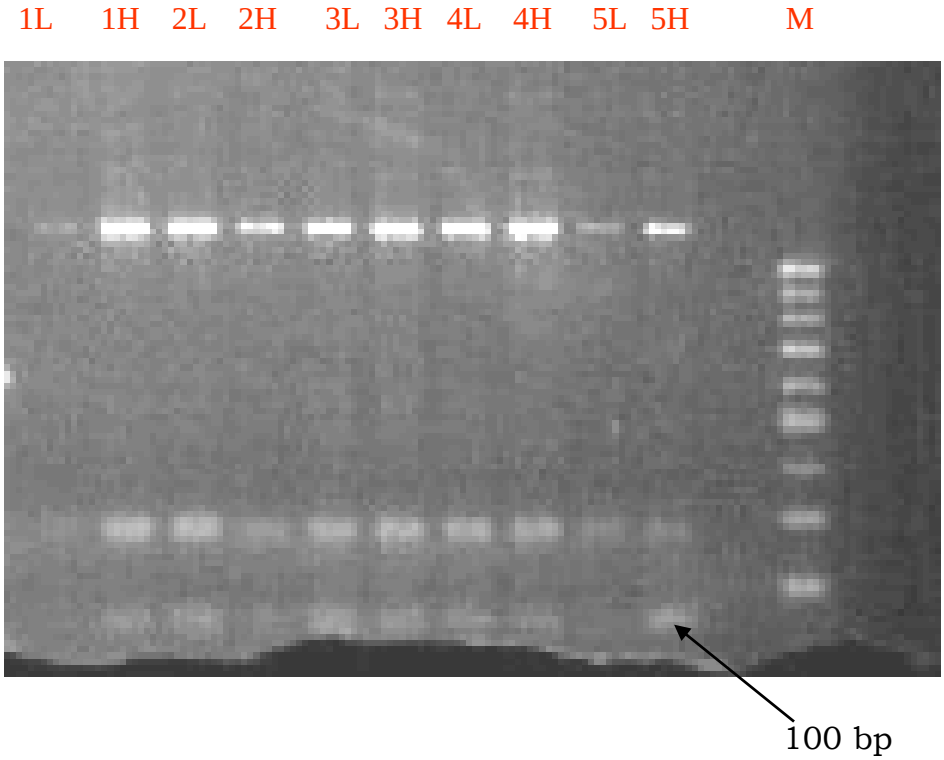
b) Gel profile with primer NAPS 252 (CTGGTGATGT)

LB: Low Bulk, HB: High Bulk
M =100 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 12. BSA with NAPS 65 (Plate 12a) and NAPS 252 (Plate 12b) RAPD primers on LB and HB for shelling percentage (mean of 10 nuts) (Trait 4)



a) Gel profile with primer NAPS 13 (CCTGGGTGGA)



b) Gel profile with primer NAPS 29 (CCGGCCTTAC)

LB: Low Bulk, HB: High Bulk
M =100 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 13. BSA with NAPS 13 (Plate 13a) and NAPS 29 (Plate 13b) RAPD primers on LB and HB for kernel weight (g) (mean of 10 kernels after shelling dry nuts) (Trait 5)

20) Screening with NAPS 29 primer (CCGGCCTTAC)

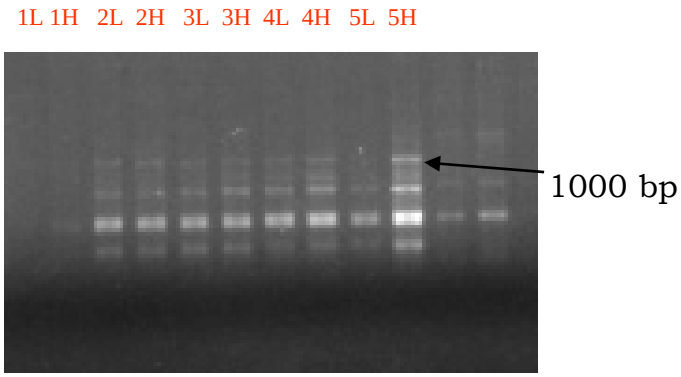
PCR amplification with RAPD primer NAPS 29 produced three loci in DNA bulk constituted for kernel weight on 1.5 per cent agarose gel (Plate 13 b) in high bulk and two loci in low bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 100 bp. However, the results was inconsistent in individual constituents of bulk.

21) Screening with NAPS 66 primer (GAGGGCGTGA)

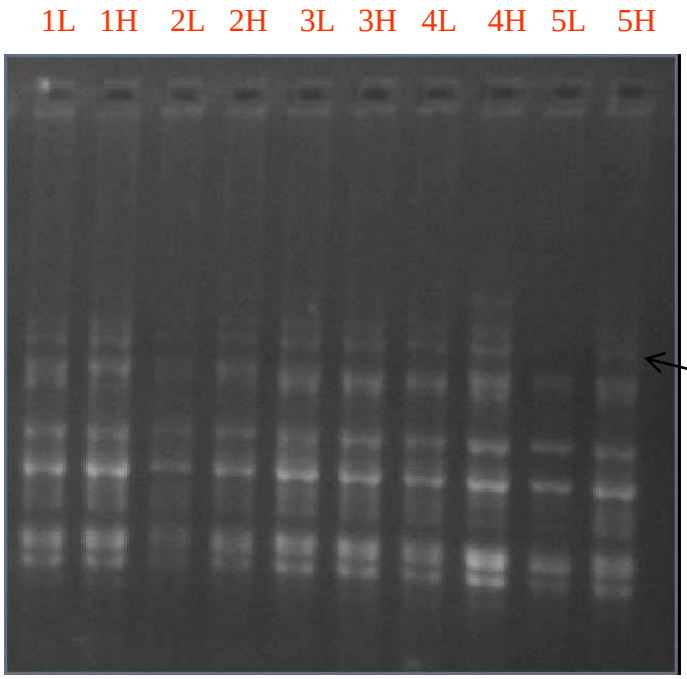
PCR amplification with RAPD primer NAPS 66 produced four loci in DNA bulk constituted for kernel weight on 1.5 per cent agarose gel (Plate 14 a) in high bulk and three loci in low bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 1000 bp. However, the results was inconsistent in individual constituents of bulk.

22) Screening with NAPS 288 primer (CCTCCTTGAC)

PCR amplification with RAPD primer NAPS 288 produced seven loci in DNA bulk constituted for kernel weight on 1.5 per cent agarose gel (Plate 14 b) in high bulk and six loci in low bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 1200 bp. However, the results was inconsistent in individual constituents of bulk.



a) Gel profile with primer NAPS 66 (GAGGGCGTGA)



1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

b) Gel profile with primer NAPS 288 (CCTCCTTGAC)

LB: Low Bulk, HB: High Bulk
M =100 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 14. BSA with NAPS 66 (Plate 14a) and NAPS 288 (Plate 14b) RAPD primers on LB and HB for kernel weight (g) (mean of 10 kernels after shelling dry nuts) (Trait 5)

23) Screening with C 301 (ATGTAGCGTGGCGAAACTG)

PCR amplification with RAPD primer C 301 produced two loci in DNA bulk constituted for kernel weight on 1.5 per cent agarose gel (Plate 15 a) in low bulk and one locus in high bulk. Only one locus was polymorphic between the contrasting bulks. However, the results was inconsistent in individual constituents of bulk.

24) Screening with C 302 (AAGTAACTGACTCCGCTGCGAC)

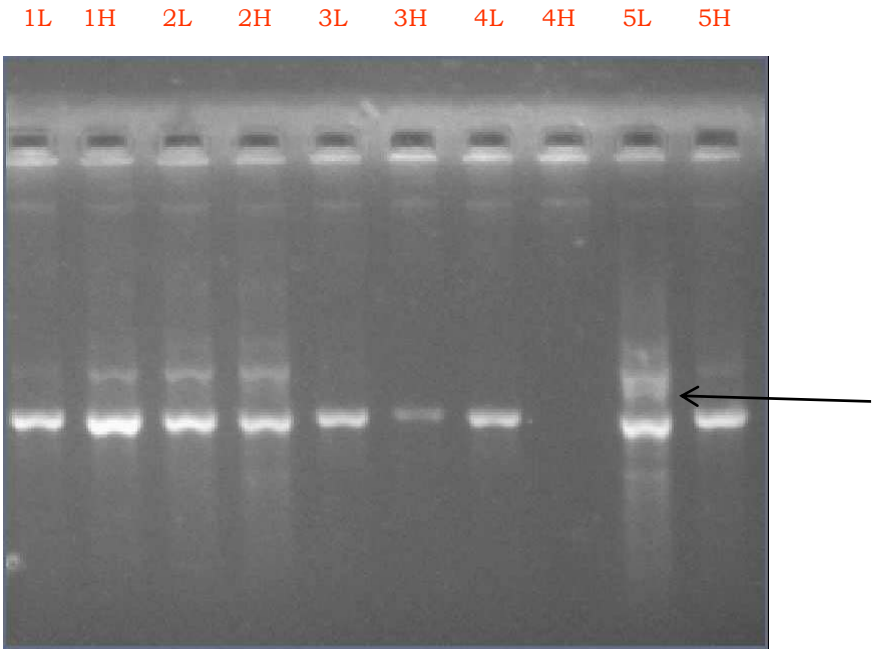
PCR amplification with RAPD primer C 302 produced four loci in DNA bulk constituted for kernel weight on 1.5 per cent agarose gel (Plate 15 b) in low bulk and two loci in high bulk. Two loci was polymorphic and the rest was monomorphic between the contrasting bulks. However, results was inconsistent in individual constituents of bulk.

4.8.2. Screening of ISSR primers on low and high DNA bulk constituted for five important characters in cashew

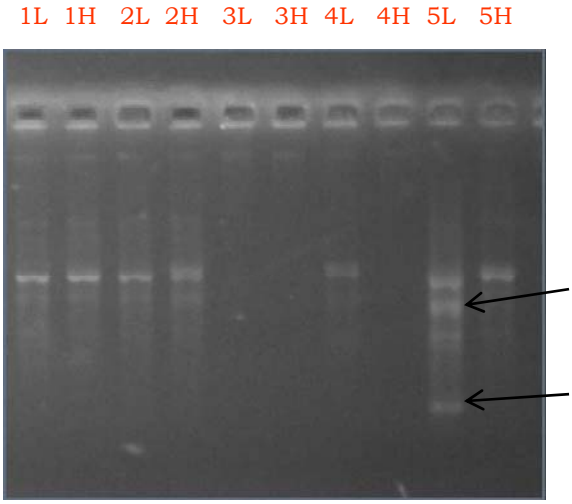
The fifteen ISSR primers belongs to IS series used to screen on high and low bulk of germplasm source to determine the polymorphism existing among the germplasm source for five important characters is presented in (Table 16c). Among fifteen ISSR primers one primer showed polymorphism (Table 19). The selected polymorphic primer was IS 7. This one polymorphic ISSR primer was validated on individuals constituting bulk of the concern trait.

1) Screening with IS 7 primer ((AG)8T)

PCR amplification with ISSR primer IS 7 produced three loci in DNA bulk constituted for size of cashew apple on 1.5 per cent agarose gel



a) Gel profile with primer C 301 (19 mer RAPD) (ATGTAGCGTGGCGAAACTG)



1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

b) Gel profile with primer C 302 (22 mer RAPD) (AAGTAACTGACTCCGCTGCGAC)

LB: Low Bulk, HB: High Bulk
M =100 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 15. BSA with of C 301 (Plate 15a) and C 302 (Plate 15b) RAPD primers on LB and HB for kernel weight (g) (mean of 10 kernels after shelling dry nuts) (Trait 5)

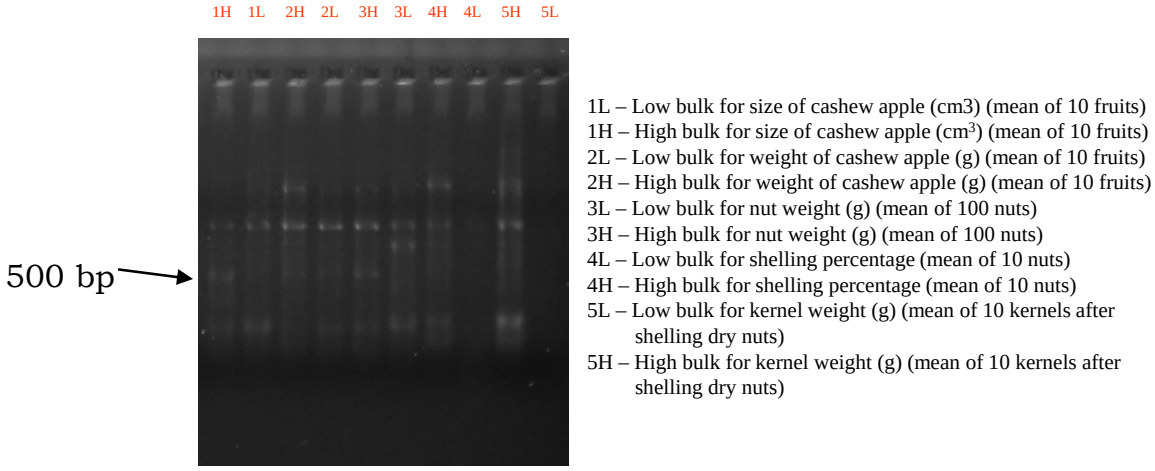
(Plate 16) in high bulk and two loci in low bulk. Only one locus was polymorphic and the rest was monomorphic between the contrasting bulks. Polymorphism was observed at 500 bp. However, the results was inconsistent in individual constituents of bulk.

Table 19 : Polymorphic ISSR primer showing polymorphism between HB and LB for each of five traits

Sl. No.	Primer	Trait	No. of bands (High Bulk)	No. of bands (Low Bulk)	Polymorphism present in	Base pair
1	IS 7	1st	3	2	High Bulk	500

4.8.3. Screening of SSR primers on low and high DNA bulk constituted for five important characters in cashew

The eighty seven SSR primers belongs to S, CSSR and LMMA series used to screen on high and low bulk of germplasm source for five important characters is presented in (Table 16d). All the primers showed monomorphic loci on 3 per cent agarose gel, therefore to resolve the alleles they was run on 4.5 per cent denaturing PAGE where they showed polymorphism between low and high DNA bulks and those found polymorphic is presented in (Table 20). The selected polymorphic SSR primers was CSSR 1, CSSR 12, CSSR 13, CSSR 14, and CSSR 10. These polymorphic SSR primers was validated on individuals constituting each bulk of the concern trait.



Gel profile with primer IS 7 ((AG)8T)

LB: Low Bulk, HB: High Bulk
M =100 bp ladder
(Arrow showing polymorphism at indicated locus)

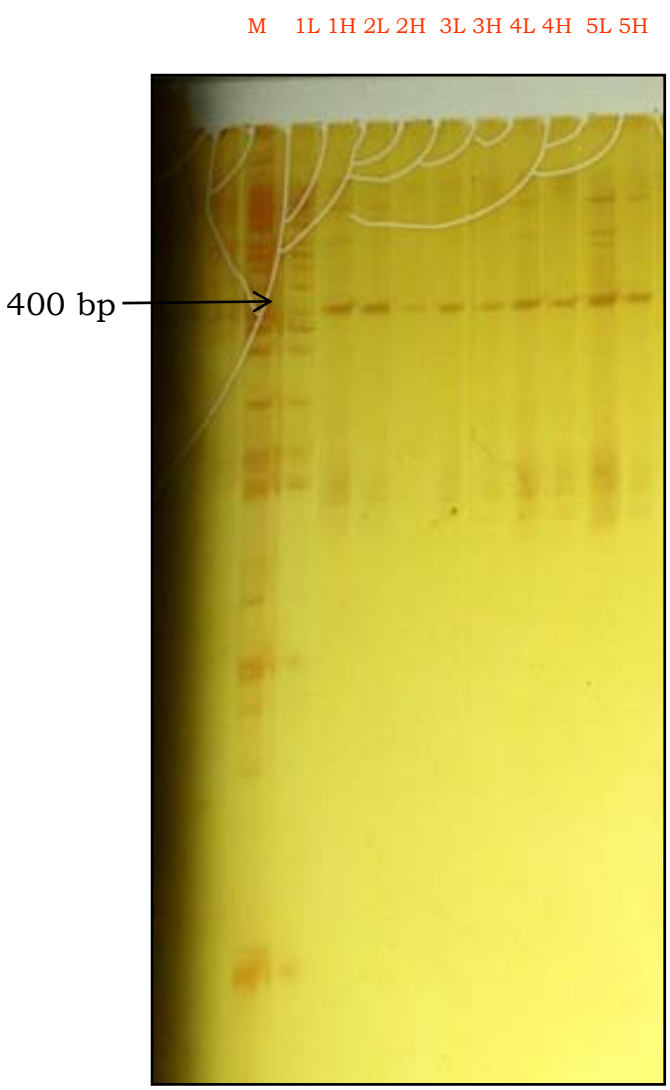
Plate 16. BSA with IS 7 ISSR primer on LB and HB for size of cashew apple (cm^3) (mean of 10) (Trait 1)

Table 20 : Polymorphic SSR primers showing polymorphism between HB and LB for each of five traits

Sl. No.	Primer	Trait	Polymorphism present at (bp)
1	CSSR 1	1st	400
2	CSSR 12	3rd	310
3	CSSR 13	3rd	175
4	CSSR 14	3rd	150
5	CSSR 15	3rd	150
6	CSSR 1	4th	400
7	CSSR 14	4th	280
8	CSSR 16	4th	250
9	CSSR 1	5th	450
10	CSSR 10	5th	500

1) Screening with CSSR 1 primer
(F: GGCCATGGGAAACAACAA
R: GGAAGGGCATTATGGGTAAG)

PCR amplification with SSR primer CSSR 1 (Plate 17) was run on 4.5 per cent denaturing PAGE on DNA bulk constituted for size of cashew apple polymorphism between the loci observed between LB and HB at 400 bp. However, the results was inconsistent in individual constituents of bulk.



1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

Gel profile with primer CSSR 1
(F: GGCCATGGGAAACAACAA
R: GGAAGGGCATTATGGGTAAG)

LB: Low Bulk, HB: High Bulk
M =50 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 17. BSA on 4.5 % PAGE with CSSR 1 SSR primer on LB and HB for size of cashew apple (cm^3) (mean of 10 fruits) (Trait 1)

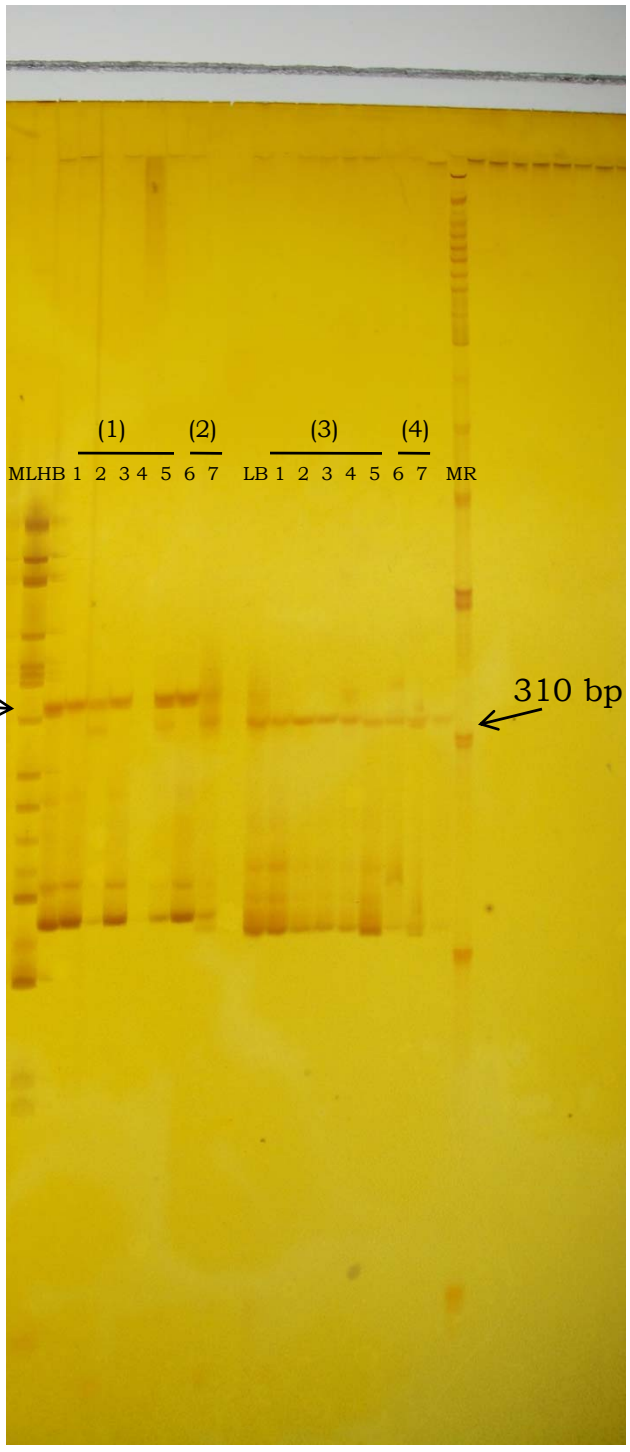
2) Screening with CSSR 12 primer
(F: CTTTCGTTCCAATGCTCCTC
R: CATGTGACAGTTCGGCTGTT)

PCR amplification with SSR primer CSSR 12 was run on 4.5 per cent denaturing PAGE on DNA bulk constituted for nut weight distinct polymorphism between the loci observed between LB and HB at 310 and 320 bp respectively. When CSSR 12 primer was used on the individual constituting the bulk the polymorphism between the loci was found to be consistent.

BSA was carried out with individuals of low bulk and high bulk for nut weight with selected primer CSSR 12. The result is presented in (Plate 25). One locus of size 310 and 320 bp was polymorphic in 5 individuals of low and high bulk (Table 21).

Table 21 : Markers identified for economic traits in cashew

Sl. No.	Trait	Primer number and sequence	Base pair
1	Nut weight (g) (mean of 100 nuts)	CSSR 12 (F: CTTTCGTTCCAATGCTCCTC R: CATGTGACAGTTCGGCTGTT)	310 for LB 320 for HB
2	Shelling percentage (mean of 10 nuts)	CSSR 14 (F: ACTGTCACGTCAATGGCATC R: GCGAAGGTCAAAGAGCAGTC)	280 for LB 310 for HB



(1) Individuals of HB (Chintamani)

1. V-3	9.08
2. CKD-1	9.13
3. K-6BC	9.3
4. 5/37 Manjery	9.62
5. 1/84	10

(2) Individuals from DCR, Puttur (> 12g)

6. NRC 278
7. NRC 194

(3) Individuals of LB (Chintamani)

1. 6/91 Kanhargad	3.51
2. 9/66 Chirala	3.53
3. Hyb-2/10	3.57
4. 8/1 Kodur	3.72
5. Tree No. 274	3.72

(4) Individuals from DCR, Puttur (< 4.3g)

6. NRC 152
7. NRC 168

Gel profile with primer CSSR 12
(F: CTTTCGTTCCAATGCTCCTC
R: CATGTGACAGTTCGGCTGTT)

LB: Low Bulk, HB: High Bulk
ML=50 bp ladder, MR=100 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 25. BSA for nut weight (g) (mean of 100 nuts) (Trait 3) with CSSR 12 SSR primer

3) Screening with CSSR 13 primer
(F: GCTTAGCCGGCACGATATTA
R: AGCTCACCTCGTTTCGTTTC)

PCR amplification with SSR primer CSSR 13 (Plate 18) was run on 4.5 per cent denaturing PAGE on DNA bulk constituted for nut weight polymorphism between the loci observed between LB and HB at 175 bp. However, the results was inconsistent in individual constituents of bulk.

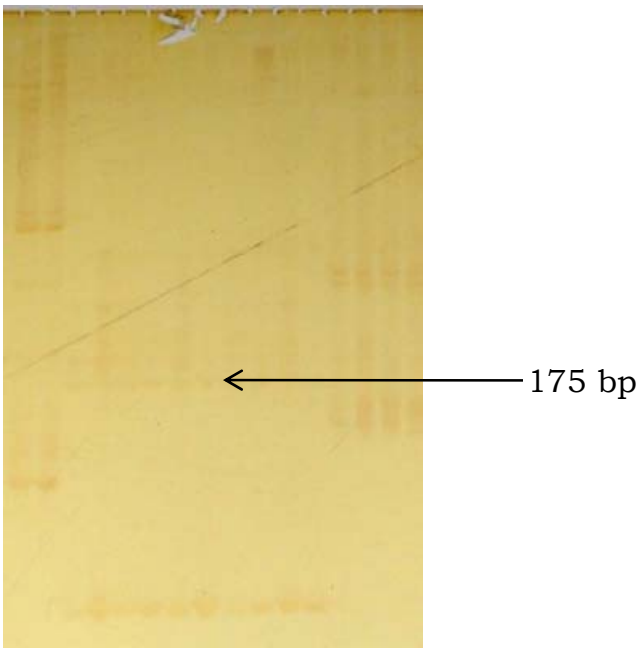
4) Screening with CSSR 14 primer
(F: ACTGTCACGTCAATGGCATC
R: GCGAAGGTCAAAGAGCAGTC)

PCR amplification with SSR primer CSSR 14 (Plate 19) was run on 4.5 per cent denaturing PAGE on DNA bulk constituted for nut weight polymorphism between the loci observed between LB and HB at 150 bp. However, the results was inconsistent in individual constituents of bulk.

5) Screening with CSSR 15 primer
(F: ACTGTCACGTCAATGGCATC
R: GCGAAGGTCAAAGAGCAGTC)

PCR amplification with SSR primer CSSR 15 (Plate 20) was run on 4.5 per cent denaturing PAGE on DNA bulk constituted for nut weight polymorphism between the loci observed between LB and HB at 150 bp. However, the results was inconsistent in individual constituents of bulk.

1L 1H 2L 2H 3L 3H 4L 4H 5L 5H

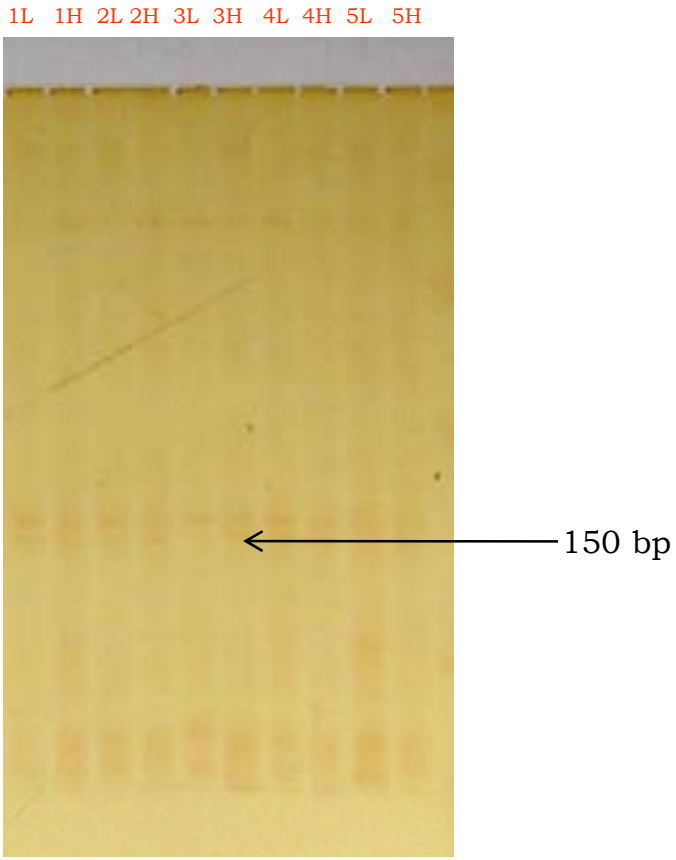


1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

Gel profile with primer CSSR 13
(F: GCTTAGCCGGCACGATATTA
R: AGCTCACCTCGTTTCGTTTC)

LB: Low Bulk, HB: High Bulk
M =50 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 18. BSA on 4.5 % PAGE with CSSR 13 SSR primer on LB and HB for nut weight (g) (mean of 100 nuts) (Trait 3)

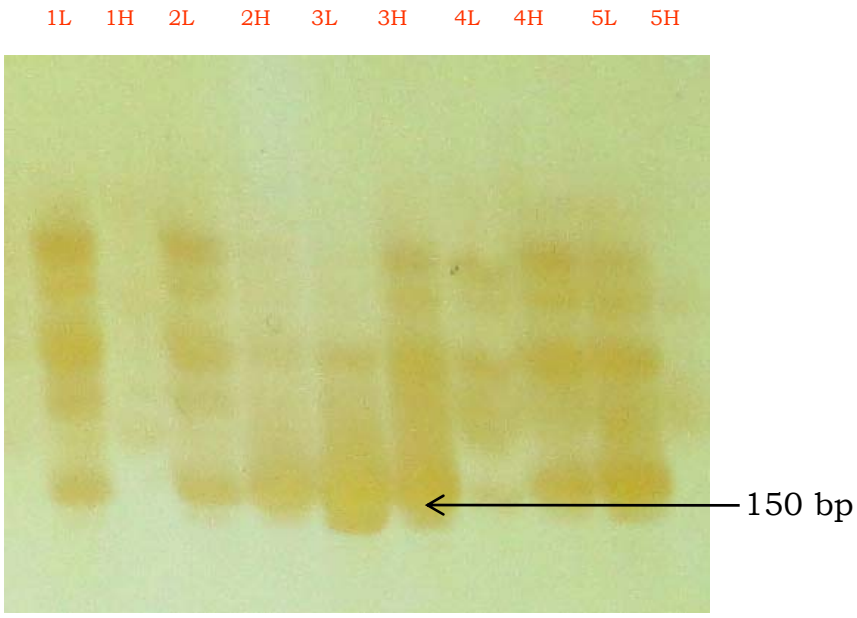


1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

Gel profile with primer CSSR 14
(F: ACTGTCACGTCAATGGCATC
R: GCGAAGGTCAAAGAGCAGTC)

LB: Low Bulk, HB: High Bulk
M =50 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 19. BSA on 4.5 % PAGE with CSSR 14 SSR primer on LB and HB for nut weight (g) (mean of 100 nuts) (Trait 3)



1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

Gel profile with primer CSSR 15
(F: ACTGTCACGTCAATGGCATC
R: GCGAAGGTCAAAGAGCAGTC)

LB: Low Bulk, HB: High Bulk
M =50 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 20. BSA on 4.5 % PAGE with CSSR 15 SSR primer on LB and HB for nut weight (g) (mean of 100 nuts) (Trait 3)

6) Screening with CSSR 1 primer
(F: GGCCATGGGAAACAACAA
R: GGAAGGGCATTATGGGTAAG)

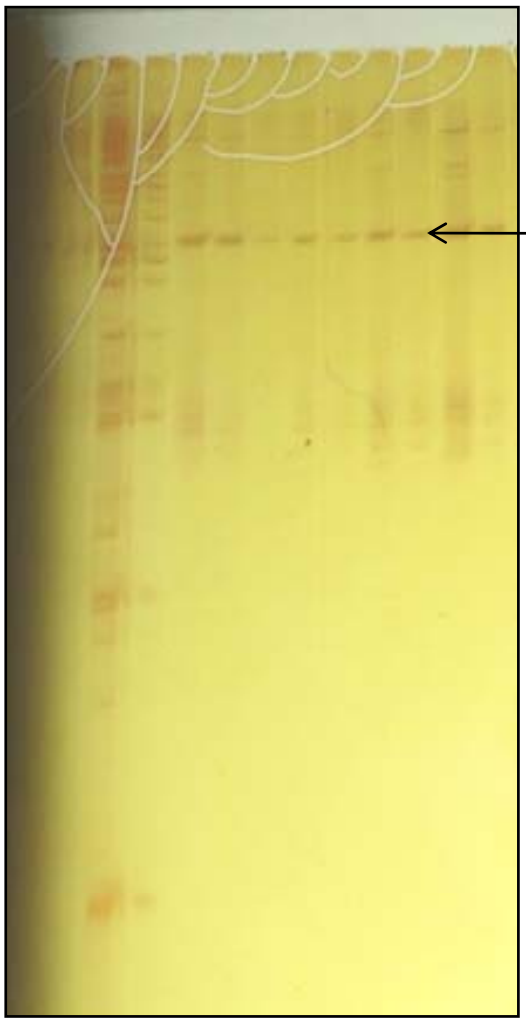
PCR amplification with SSR primer CSSR 1 (Plate 21) was run on 4.5 per cent denaturing PAGE on DNA bulk constituted for shelling percentage polymorphism between the loci observed between LB and HB at 400 bp. However, the results was inconsistent in individual constituents of bulk.

7) Screening with CSSR 14 primer
(F: ACTGTCACGTCAATGGCATC
R: GCGAAGGTCAAAGAGCAGTC)

PCR amplification with SSR primer CSSR 14 was run on 4.5 per cent denaturing PAGE on DNA bulk constituted for shelling percentage distinct polymorphism between the loci observed between LB and HB 280 and 310 bp respectively. When CSSR 14 primer was used on the individual constituting the bulk the polymorphism between the loci was found to be consistent.

BSA was carried out with individuals of low bulk and high bulk for shelling percentage with selected primer CSSR 14. The result is presented in (Plate 26). One locus of size 280 and 310 bp was polymorphic in 5 individuals of low and high bulk (Table 21).

M 1L 1H 2L 2H 3L 3H 4L 4H 5L 5H

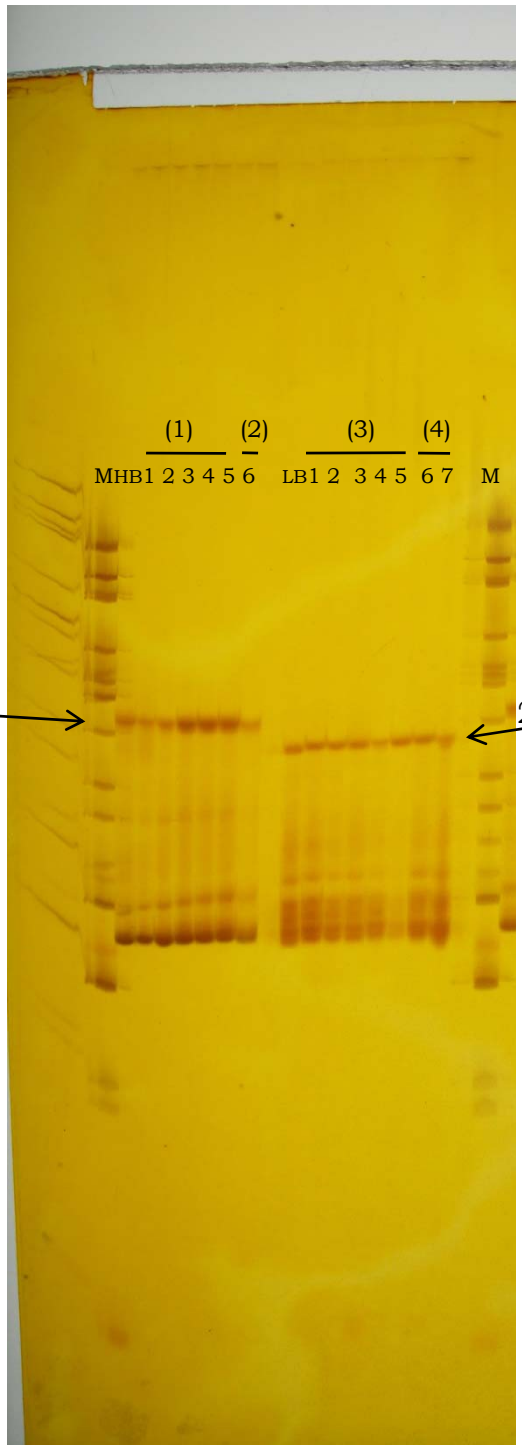


1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

Gel profile with primer CSSR 1
(F: GGCCATGGGAAACAACAA
R: GGAAGGGCATTATGGGTAAG)

LB: Low Bulk, HB: High Bulk
M =50 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 21. BSA on 4.5 % PAGE with CSSR 1 SSR primer on LB and HB for shelling percentage (mean of 10 nuts) (Trait 4)



(1) Individuals of HB (Chintamani)

1. CKD-5	35.71
2. Tree No. 40	36.51
3. 1/64 Madhuranthakam	38.51
4. BPP-3	40.4
5. 1/26 Neeleshwar	58.3

(2) Individuals from DCR, Puttur (> 35.5%)

6. NRC 318

(3) Individuals of LB (Chintamani)

1. 4/62 Aalangudi	14.81
2. 4/48	15.91
3. Kottarakara-5B	17.7
4. V-1	18.44
5. 4/43 Wyanadu	19.62

(4) Individuals from DCR, Puttur (< 20 %)

6. NRC 156
7. NRC 107

Gel profile with primer CSSR 14 (F: ACTGTCACGTCAATGGCATC R: GCGAAGGTCAAAGAGCAGTC)

LB: Low Bulk, HB: High Bulk
M =50 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 26. BSA for shelling percentage (mean of 10 nuts) (Trait 4) with CSSR 14 SSR primer

8) Screening with CSSR 16 primer
(F: GGCCATGGGAAACAACAA
R: GGAAGGGCATTATGGGTAAG)

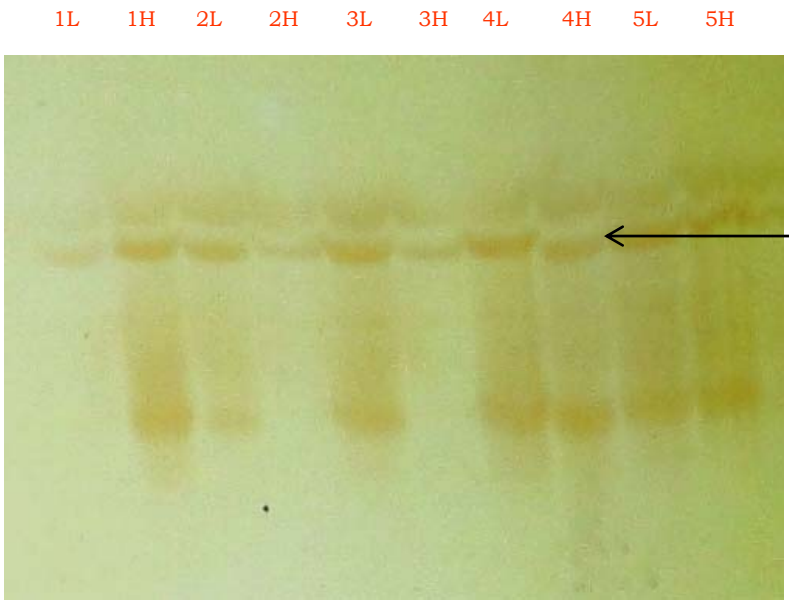
PCR amplification with SSR primer CSSR 16 (Plate 22) was run on 4.5 per cent denaturing PAGE on DNA bulk constituted for shelling percentage polymorphism between the loci observed between LB and HB at 250 bp. However, the results was inconsistent in individual constituents of bulk.

9) Screening with CSSR 1 primer
(F: GGCCATGGGAAACAACAA
R: GGAAGGGCATTATGGGTAAG)

PCR amplification with SSR primer CSSR 1 (Plate 23) was run on 4.5 per cent denaturing PAGE on DNA bulk constituted for kernel weight polymorphism between the loci observed between LB and HB at 450 bp. However, the results was inconsistent in individual constituents of bulk.

10) Screening with CSSR 10 primer
(F: GGAGAAGAAAAGTTAGGTTTGAC
R: CGTCTTCTTCCACATGCTTC)

PCR amplification with SSR primer CSSR 10 (Plate 24) was run on 4.5 per cent denaturing PAGE on DNA bulk constituted for kernel weight polymorphism between the loci observed between LB and HB at 500 bp. However, the results was inconsistent in individual constituents of bulk.

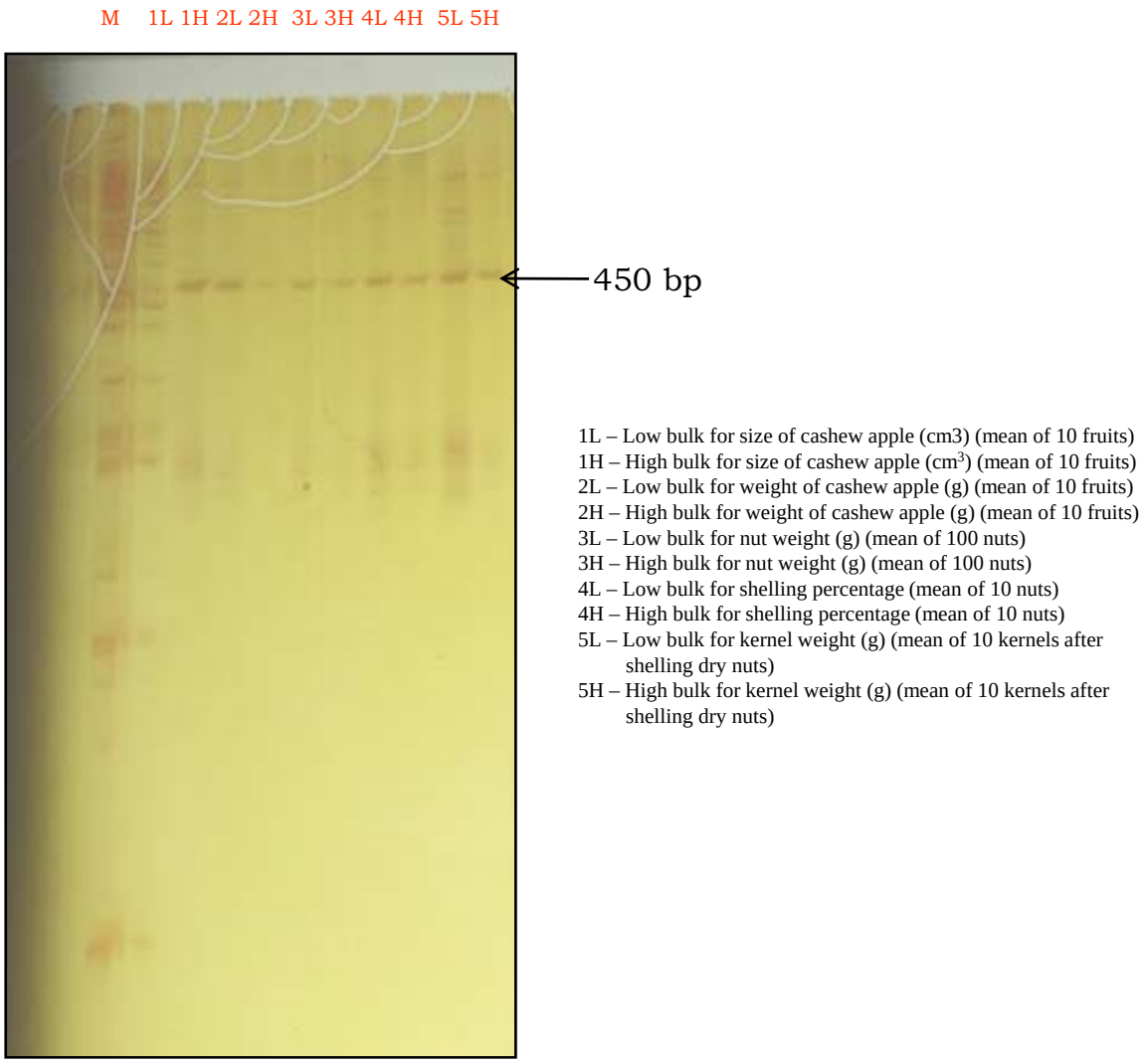


1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

Gel profile with primer CSSR 16
(F: GGCCATGGGAAACAACAA
R: GGAAGGGCATTATGGGTAAG)

LB: Low Bulk, HB: High Bulk
M =50 bp ladder
(Arrow showing polymorphism at indicated locus)

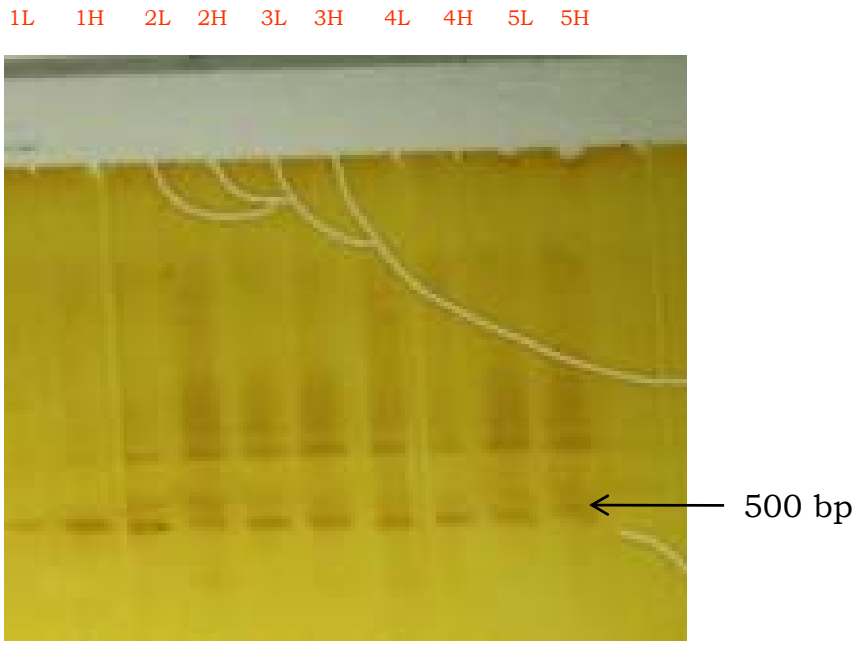
Plate 22. BSA on 4.5 % PAGE with CSSR 16 SSR primer on LB and HB for shelling percentage (mean of 10 nuts) (Trait 4)



Gel profile with primer CSSR 1
(F: GGCCATGGGAAACAACAA
R: GGAAGGGCATTATGGGTAAG)

LB: Low Bulk, HB: High Bulk
M =50 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 23. BSA on 4.5 % PAGE with CSSR 1 SSR primer on LB and HB for kernel weight (g) (mean of 10 kernels after shelling dry nuts) (Trait 5)



1L – Low bulk for size of cashew apple (cm3) (mean of 10 fruits)
1H – High bulk for size of cashew apple (cm^3) (mean of 10 fruits)
2L – Low bulk for weight of cashew apple (g) (mean of 10 fruits)
2H – High bulk for weight of cashew apple (g) (mean of 10 fruits)
3L – Low bulk for nut weight (g) (mean of 100 nuts)
3H – High bulk for nut weight (g) (mean of 100 nuts)
4L – Low bulk for shelling percentage (mean of 10 nuts)
4H – High bulk for shelling percentage (mean of 10 nuts)
5L – Low bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)
5H – High bulk for kernel weight (g) (mean of 10 kernels after shelling dry nuts)

Gel profile with primer CSSR 10
(F: GGAGAAGAAAAGTTAGGTTTGAC
R: CGTCTTCTTCCACATGCTTC)

LB: Low Bulk, HB: High Bulk
M =50 bp ladder
(Arrow showing polymorphism at indicated locus)

Plate 24. BSA on 4.5 % PAGE with CSSR 10 SSR primer on LB and HB for kernel weight (g) (mean of 10 kernels after shelling dry nuts) (Trait 5)

4.8. Validation of identified markers for nut weight and shelling percentage from germplasm source at DCR, Puttur

Validation of identified marker, CSSR 12 for LB and HB at 310 and 320 bp respectively for nut weight (g) (mean of 100 nuts) was done by taking five germplasm source for low bulk and five for high bulk (Table 22). Ten PCR reactions was setup (five for low bulk and five for high bulk) with CSSR 12 primer and run on 4.5 per cent denaturing PAGE. Out of five, NRC 152 and NRC 168 for low bulk and NRC 278 and NRC 194 for high bulk (Plate 25) was found to be consistent with marker identified using Chintamani germplasm source.

Validation of identified marker, CSSR 14 for LB and HB at 280 and 310 bp respectively for shelling percentage (mean of 10 nuts) was done by taking five germplasm source for low bulk and five for high bulk (Table 22). Ten PCR reactions was setup (five for low bulk and five for high bulk) with CSSR 14 primer and run on 4.5 per cent denaturing PAGE. Out of five, NRC 156 and NRC 107 for low bulk and NRC 318 for high bulk (Plate 26) was found to be consistent with marker identified using Chintamani germplasm source.

Discussion

5. DISCUSSION

India was the first country to exploit international trade of cashew kernels in the early part of the 20th century and also the first to initiate research in the early 1950s (Bhaskara and Swamy, 1994). Cashew research received impetus with the inception of Central Plantation Crops Research Institute (CPCRI) and All India Coordinated Spice and Cashew nut improvement project in 1970. Cashew is currently the highest foreign exchange earner for the country, this emphasizes the need for greater efforts for crop improvement. However, the research results/technologies emanated through different cashew research centres in the country have not given the desired increase in production, because of inherent nature (high heterozygosity and cross pollinated nature) of the crop suitable for afforestation and wasteland development.

During early eighties, importance of cashew as a commercial horticultural crop was realized. Subsequently, research efforts in the country have led to the release of 34 cultivars including ten hybrids.

Molecular breeding holds great potential for crop improvement as they promise to expedite the time taken to produce crop varieties with desirable characters. With the use of molecular techniques it would now be possible to hasten the transfer of desirable genes among varieties and to introgress novel genes from related wild species. Techniques which are particularly promising in assisting selection for desirable characters involve the use of molecular markers such as RAPD, SSR, AFLP, SCAR, RFLP, STS and ISSR using F_2, germplasm, backcross populations, NILs, RILs and doubled haploids.

Progress has been made in tagging many agriculturally important genes with molecular markers, which forms the foundation for marker-

assisted selection. Availability of linked markers for important economic characters will help in identifying plants carrying these genes. No doubt, when reliable markers are identified and genes tagged with them they would prove to be very powerful tools, especially in screening and selection for quantitative and qualitative characters, thus reducing the burden of plant breeders to a great extent.

The results obtained on various aspects are discussed under the following heads.

1. Mean performance of genotypes
2. Components of variability and predicted genetic advance
3. Correlation analysis
4. Path coefficient analysis
5. Descriptive statistics for quantitative traits
6. Genetic diversity - Mahalanobis generalized distance
7. Tagging genetic determinants controlling the inheritance of five important economic traits using RAPD, ISSR and SSR markers
8. Validation of identified markers for nut weight and shelling percentage

5.1. Mean performance of genotypes

Mean performance of 104 cashew genotypes for ten characters are presented in Appendix V. Size of cashew apple, weight of cashew apple, nut weight, shelling percentage and kernel weight are the five important economic traits in cashew and selection of genotypes with higher economic yield has practical relevance.

Size of the cashew apple ranged from 4.51 cm^3 to 39.51 cm^3. The genotype which recorded higher size of cashew apple was BPP-4 followed by 2/4 Baruva, Veng-3, 38/4 and H-3-17.

Weight of cashew apple ranged from 10 g to 100.8 g. The genotype which recorded higher weight of cashew apple was A-18-4 followed by Kankadi, CKD-5, K-2B and Veng-3. Nut weight ranged from 3.36 g to 9.85 g. The genotype which recorded higher nut weight was CKD-5 followed by K-2B, K-6BC, CKD-1 and H-19.

Shelling percentage ranged from 13.41 per cent to 41.15 per cent. The genotype which recorded higher shelling percentage was 1/26 Neeleshwar followed by BPP-3, NDR, 1/64 Madhuranthakam and CKD-5. Kernel weight ranged from 0.54 g to 3.42 g. The genotype which recorded higher kernel weight was CKD-5, followed by K-2B, CKD-1 and Veng-3, H-1B.

In general, no single genotype was found to be better for important economic characters under study. However, the top performing genotype was Veng-3 which exhibited higher mean performance for size of cashew apple, weight of cashew apple and kernel weight whereas the genotype CKD-5 exhibited higher mean performance for weight of cashew apple, nut weight and kernel weight and the genotype K-2B exhibited higher mean performance for nut weight and kernel weight (Table 14).

Table 14 : Top performing genotypes for five important economic characters in 104 cashew germplasm source

Sl. No.	Character	Genotypes
1	Size of cashew apple (cm^3)	BPP-4, 2/4 Baruva, Veng-3, 38/4, H-3-17
2	Weight of cashew apple (g)	A-18-4, Kankadi, CKD-5, K-2B, Veng-3
3	Nut weight (g)	CKD-5, K-2B, K-6BC, CKD-1, H-19
4	Shelling percentage	1/26 Neeleshwar, BPP-3, NDR, 1/64 Madhuranthakam, CKD-5
5	Kernel weight (g)	CKD-5, K-2B, CKD-1, Veng-3, H-1B

5.2. Components of variability and predicted genetic advance

Mean sum of squares for ten quantitative traits (Table 2) studied, revealed highly significant differences among the genotypes. This implies the existence of variability among the genotypes for the characters studied. Mere variability will not give clear picture about the heritable portion of variability governed by genes which is actually of more importance than the total variability. Therefore, it is important to study the variation in terms of Genotypic Co-efficient of Variation (GCV) and Phenotypic Co-efficient of Variation (PCV) for individual characters (Table 3).

Genetic estimates for tree spread and weight of cashew apple expressed high amount of PCV and GCV values coupled with high heritability and high genetic advance. The difference between PCV and GCV was narrow indicating less influence of environment on these characters and thus likely to be governed by additive genes. High heritability coupled with high genetic advance indicated that these characters would respond well to selection. The results obtained for weight of cashew apple in the present study are in agreement with the reports of Sena *et al.* (1994).

Genetic estimates for kernel weight and cumulative yield expressed high amount of PCV and GCV values coupled with high heritability and high genetic advance. The difference between PCV and GCV was narrow which indicated that the genotypes possessed more variability and expected response to selection was high.

Nut dimension and nut weight also manifested high PCV, GCV coupled with high heritability and high genetic advance indicating better expected response for selection.

Tree height and shelling percentage manifested moderate PCV and high GCV coupled with high heritability and high genetic advance.

Heritability values was found high for eight characters. Among the eight characters, tree spread and tree height recorded high heritability value and genetic advance as per cent mean was high for nut dimension. On the other hand, low heritability and low genetic advance was recorded for shell (pericarp) thickness. However similar research was not carried out by earlier workers.

5.3. Correlation analysis

Understanding the nature of association among various characters and influence on these characters is important from breeding point of view. The correlation between different characters could arise because of linkage, pleiotropy or developmentally influenced relationships. Correlation between two characters provides information on the extent of variation that could be expected in one trait by altering the other character. When a complex character is associated with a simple trait, it would facilitate correlated response for the complex trait. It may also bring about a balance between the different traits during simultaneous improvement for those traits. If the difference between the genotypic correlation and phenotypic correlation coefficients is narrow, it indicates that the association between the pair of characters is less influenced by environmental factors.

Genotypic and phenotypic correlation of economic traits with other quantitative traits in 104 cashew germplasm source is presented in Table 6.

Size of cashew apple had positive significant correlation with nut dimension (0.24 and 0.52).

Weight of cashew apple had positive significant correlation with nut dimension (0.28 and 0.32). Similar result was reported by Sena *et al*. (1994).

Nut weight had positive significant correlation with nut dimension (0.37 and 0.41) and nut weight has positive significance relation with shell (pericarp) thickness (0.20) of genotypic correlation coefficient.

Kernel weight had positive significant correlation with nut dimension (0.27 and 0.31) and cumulative yield (0.19 and 0.21) while kernel weight has positive significance relation with shell (pericarp) thickness of (0.21) genotypic correlation coefficient.

Therefore, while breeding for yield in cashew equal priority should be given to these quantitative traits and there should be a balance between these traits so as to harness the commercial potentiality of the genotypes.

5.4. Path coefficient analysis

Shelling percentage (0.61) shown highest positive direct effect on yield, whereas nut dimension (0.05) shown lowest positive direct effect on yield, while kernel weight (-0.71) shown highest negative direct effect on yield, whereas size of cashew apple (-0.04) had lowest negative direct effect on yield.

Kernel weight (0.3) shown highest positive indirect effect on yield, whereas shell (pericarp) thickness (0.001) shown lowest positive indirect

effect on yield, while nut weight (-0.46) shown highest negative indirect effect on yield, whereas kernel weight (-0.001) had lowest negative indirect effect on yield (Table 7).

5.5. Descriptive statistics for quantitative traits

The study of distribution properties such as coefficients of skewness and kurtosis provides insight about the nature of gene action (Fisher *et al.*, 1932) and number of genes controlling the traits (Robson, 1956), respectively. Genetic analysis of quantitative traits is based on first degree (gene effects through generation mean analysis) and second degree (components of genetic variances through diallel, line × tester analysis, etc) statistics. Skewness and kurtosis are more powerful than first and second degree statistics which reveal genetic interaction effects (Choo and Reinbergs, 1982). The skewed distribution of a trait in general suggests that the trait is under the control of non-additive gene action, especially epistasis and is influenced by environmental variables (Pooni *et al.*, 1977). Positive skewness is associated with complementary gene interactions, negative skewness is associated with duplicate (additive × additive) gene interactions. The genes controlling the trait with skewed distribution tend to be predominantly dominant irrespective of whether they have increasing or decreasing effect on the trait. Kurtosis is negative or close to zero in the absence of gene interaction and is positive in the presence of gene interactions (Pooni *et al.*, 1977; Choo and Reinbergs 1982).

Descriptive statistical analysis on the phenotypic characters of hundred and four germplasm source at AICRP, ARS, Chintamani has revealed leptokurtic, platykurtic and positively skewed distribution for various quantitative traits studied.

Leptokurtic and positively skewed distribution suggested the involvement of relatively fewer number of segregating genes with majority of them had decreasing effects with complementary type of interaction in the inheritance of plant height (Fig. 1), size of cashew apple (Fig. 3), weight of cashew apple (Fig. 4), cashew nut dimension (Fig. 5), shelling percentage (Fig. 7), shell (pericarp) thickness (Fig. 8), kernel weight (Fig. 9) and cumulative yield (Fig. 10).

Platykurtic and positively skewed distribution suggested the involvement of very large numbers of genes with majority of them had increasing effects with complementary type of interaction in the inheritance of tree spread (Fig. 2) and nut weight (Fig. 6).

5.6. Genetic diversity - Mahalanobis generalized distance

Whenever germplasm is evaluated for diversity, it will be useful if they are grouped into clusters which would help to choose the genotypes for hybridization. For this purpose, D^2 statistic is an effective tool for estimating genetic divergence among the genotypes. Being a numerical estimate, it has the added advantage over other criteria allowing better comparison among all possible pairs of genotypes selected for the study.

In the present study on 104 cashew genotypes, all characters studied contributed to the total genetic divergence. Highest contribution was made by cumulative yield, followed by tree spread, nut weight, tree height, shelling percentage, kernel weight, weight of cashew apple, shell (pericarp) thickness, nut dimension lowest contribution was by size of cashew apple.

Based on D^2 values, 104 genotypes was grouped into thirteen clusters. Maximum number of genotypes fell in the cluster I (50

genotypes) followed by cluster III & IV (13 genotypes each), cluster II & XII (5 genotypes each) and X (4 genotypes), while cluster V, VI, VII, VIII, IX, XII and XIII had only two genotypes each.

The genotypes falling in a particular cluster will have close genetic background with smaller intracluster distance among themselves within a cluster. The genotypes between the clusters have more D^2 value with more genetic distance. Further, genotypes present in the more distanced clusters will serve as good sources of divergent genes which is very much required for breeding to exploit heterosis as reported by Gill *et al.* (1982) or/and to get good transgressants in the segregating population.

Maximum intercluster distance was observed between the cluster VIII and X indicating that the genotypes included in those clusters are highly divergent compared to genotypes in each clusters separately. The cluster mean values for each character in the clusters VIII and X also indicated large differences between the cluster means for many characters. It is also evident from the cluster mean differences between clusters VIII and X for other characters.

Minimum intercluster distance was observed between the cluster VI and XII. Cluster X showing high D^2 distance with other clusters indicating that genotypes in the cluster X are more divergent from genotypes of other clusters. Intracluster D^2 values was small in the cluster V with only two genotypes whereas cluster XI recorded maximum intracluster D^2 value indicating that five genotypes in the cluster XI was not closely related compared to the genotypes in the cluster V followed by the cluster VI. When the genotypes select for hybridization, it is desirable to select the genotypes from the clusters with maximum intercluster distance.

5.7. Tagging genetic determinants controlling the inheritance of five important economic traits using RAPD, ISSR and SSR markers

BSA involves comprising two pooled DNA samples of individuals from a segregating population originating from a single cross (Michelmore *et al.*, 1991). Within each pool or bulk, the individuals are identical for the trait or gene of interest but are arbitrary for all other genes. BSA avoids the need for screening the entire mapping population with every primer. In various molecular marker techniques like RAPD (Quirin *et al.*, 2005), RFLP (Monna *et al.*, 1995) and AFLP (Julio *et al.*, 2006) BSA has been used. The information content in an individual RAPD marker is very low. It is only when many of these anonymous markers are used to define a genome that they begin to have utility (Williams *et al.*, 1990). Therefore BSA is employed for identifying markers using mapping populations for several traits of economic importance in crop improvement programme. In a perennial crop like cashew BSA is of immense value as developing a mapping population is time and labour consuming.

Three hundred and nine RAPD primers in the series from NAPS (Table 16 (a)), C, SB and OPH (Sobir *et al.*, 2007) (Table 16 (b)), fifteen ISSR primers from IS series (Table 16 (c)), eighty seven SSR primers from S [1 to 15 from mango (Schnell *et al.*, 2005), 16 to 50 from almond (Mnejja *et al.*, 2005)], CSSR [51 to 71 from cashew (Croxford *et al.*, 2005)] and LMMA [72 to 87 from mango (Viruel *et al.*, 2005)] series (Table 16 (d)) was screened with DNA of contrasting pools of five important economic characters of cashew (Table 15).

The three hundred and nine RAPD primers belonging to series *viz.*, NAPS, C, SB and OPH used to screen on high and low bulk of germplasm

source determine the polymorphism existing among the germplasm source for five important characters is presented in (Table 16a & 16b). Among three hundred and nine RAPD primers twenty four polymorphic primers showed polymorphism for five important selected characters (Table 18). The identified polymorphic random primers was NAPS 3, NAPS 11, NAPS 27, NAPS 32, NAPS 16, NAPS 22, NAPS 40, NAPS 56, NAPS 31, NAPS 34, NAPS 39, NAPS 68, NAPS 75, NAPS 257, NAPS 24, NAPS 63, NAPS 65, NAPS 252, NAPS 13, NAPS 29, NAPS 66, NAPS 288, C 301 and C 302. None of the 24 polymorphic RAPD primers when validated on individual constituting the bulk for each trait produce consistent banding pattern.

The fifteen ISSR primers belonging to IS series was used to screen on high and low bulk of germplasm source determine the polymorphism existing among the germplasm source for five important characters is presented in (Table 16c). Among fifteen ISSR primers one primer showed polymorphism (Table 19). The selected polymorphic IS series primer was IS 7. This one polymorphic ISSR primer was validated on individual constituting each bulk of the concern trait, they found to be inconsistent.

The eighty seven SSR primers belonging to series *viz*., S, CSSR & LMMA (Table 16d) used to screen on high and low bulk of germplasm source determine the monomorphic loci on 3 per cent agarose gel, therefore to resolve this alleles they was run on 4.5 per cent denaturing PAGE where they showed polymorphism between low and high bulk and found polymorphic is presented in Table 20. The selected polymorphic SSR primers was CSSR 1, CSSR 12, CSSR 13, CSSR 14, and CSSR 10. When CSSR 1, CSSR 13 and CSSR 10 primers. Among this CSSR 12 and CSSR 14 when validated on individuals constituting each bulk of the concern trait they found to be consistent. Primer CSSR 12 gave polymorphic band at 310 and 320 bp in individuals of low bulk and high

bulk for nut weight (g) (mean of 100 nuts) and CSSR 14 primer gave polymorphic band at 280 and 310 bp in individual of low bulk and high bulk for shelling percentage (mean of 10 nuts).

BSA was carried out with individuals of low bulk and high bulk for nut weight (g) (mean of 100 nuts) with selected primer CSSR 12 (Plate 25). One locus of size 310 and 320 bp was polymorphic in 5 individuals of low and high bulk. BSA was also carried out with individuals of low bulk and high bulk for shelling percentage (mean of 10 nuts) with selected primer CSSR 14 (Plate 26). One locus of size 280 and 310 bp was polymorphic in all the 5 individuals of low and high bulk (Table 21).

5.8. Validation of identified markers for nut weight and shelling percentage

Validation of identified marker was done using National Cashew Gene Bank (NCGB) germplasm source maintained at DCR, Puttur.

Validation of identified marker, CSSR 12 for LB and HB at 310 and 320 bp respectively for nut weight (g) (mean of 100 nuts) was done by taking five germplasm source for low bulk and five for high bulk (Table 22). Ten PCR reactions was setup (five for low bulk and five for high bulk) with CSSR 12 primer and run on 4.5 per cent denaturing PAGE. Out of five, NRC 152 and NRC 168 for low bulk and NRC 278 and NRC 194 for high bulk (Plate 25) was found to be consistent with marker identified using Chintamani germplasm source.

Validation of identified marker, CSSR 14 for LB and HB at 280 and 310 bp for shelling percentage (mean of 10 nuts) was done by taking five germplasm source for low bulk and five for high bulk (Table 22). Ten PCR reactions was setup (five for low bulk and five for high bulk) with

CSSR 14 primer and run on 4.5 per cent denaturing PAGE. Out of five, NRC 156 and NRC 107 for low bulk and NRC 318 for high bulk (Plate 26) was found to be consistent with marker identified using Chintamani germplasm source.

Inconsistency in validation of nut weight and shelling percentage in NCGB, germplasm source might be due to misclassification of these sources during phenotyping, or due to difference in fertility status of soil.

The close segregation pattern of these markers with economic characters helps in selection of desirable lines in large breeding population.

Summary

6. SUMMARY

An investigation entitled "Tagging genetic determinants for nut weight and shelling percentage in cashew (*Anacardium occidentale* L.)" was carried out at Department of Genetics and Plant Breeding, University of Agricultural Sciences, GKVK Campus, Bangalore – 560 065 during the period from 2007-2009. The main objective of the study was to identify molecular markers linked to economic characters in cashew and to validate molecular markers for use in breeding programmes. The results of the present study are summarized hereunder.

The salient findings of present investigation are summarized below :

- Estimates of PCV and GCV values was high for tree spread, size of cashew apple, weight of cashew apple, nut dimension, nut weight, shell (pericarp) thickness, kernel weight and cumulative yield.

- Narrow difference between PCV and GCV has reflected in higher heritability and high genetic advance was recorded for kernel weight and cumulative yield.

- Size of cashew apple had positive significant correlation with nut dimension.

- Weight of cashew apple had positive significant correlation with nut dimension.

- Nut weight had positive significant correlation with nut dimension and nut weight has positive significance relation with shell (pericarp) thickness.

- Shelling percentage shown highest positive direct effect on yield, whereas nut dimension shown lowest positive direct effect on yield, while kernel weight shown highest negative direct effect on yield, whereas size of cashew apple had lowest negative direct effect on yield.

- Kernel weight shown highest positive indirect effect on yield, whereas shell (pericarp) thickness shown lowest positive indirect effect on yield, while nut weight shown highest negative indirect effect on yield, whereas kernel weight had lowest negative indirect effect on yield.

- Leptokurtic and positively skewed distribution suggested the involvement of relatively fewer number of segregating genes with majority of them had decreasing effects with complementary type of interaction in the inheritance of plant height, size of cashew apple, weight of cashew apple, cashew nut dimension, shelling percentage, shell (pericarp) thickness, kernel weight and cumulative yield.

- Platykurtic and positively skewed distribution suggested the involvement of very large numbers of genes with majority of them had increasing effects with complementary type of interaction in the inheritance of tree spread and nut weight.

- Genetic diversity studies using Mahalanobis D^2 statistics revealed highest contribution of cumulative yield, followed by tree spread, nut weight, tree height, shelling percentage, kernel weight, weight of cashew apple, shell (pericarp) thickness, nut dimension lowest contribution was by size of cashew apple towards total divergence.

- Among 104 genotypes studied, Veng-3 exhibited higher mean performance for size of cashew apple, weight of cashew apple and kernel weight whereas the genotype CKD-5 exhibited higher mean performance for weight of cashew apple, nut weight and kernel weight and the genotype K-2B exhibited higher mean performance for nut weight and kernel weight.

- The three hundred and nine RAPD primers belonging to series *viz.*, NAPS, C, SB and OPH used to screen on high and low bulk of germplasm collection determine the polymorphism existing among the germplasm collection for five important characters found twenty four polymorphic RAPD primers. None of the 24 polymorphic RAPD primers when validated on individual constituting the bulk for each trait produce consistent banding pattern.

- The fifteen ISSR primers belonging to IS series used to screen on high and low bulk of germplasm collection determine the polymorphism existing among the germplasm collection for important characters found one polymorphic ISSR primer. This one polymorphic primer when validated on individuals constituting the bulk of the concern trait, they found to be inconsistent.

- The eighty seven SSR primers belonging to series *viz.*, S, CSSR & LMMA used to screen on high and low bulk of germplasm collection determine the polymorphism existing among the germplasm collection for important characters found ten polymorphic SSR primers on 4.5 per cent denaturing PAGE. Among this CSSR 12 and CSSR 14 primer when validated on individual constituting each bulk of the concern trait they found to be consistent.

- The primer CSSR 12 gave polymorphic loci at 310 and 320 bp in individuals of low bulk and high bulk respectively for nut weight (g) (mean of 100 nuts) and the difference between the two bulks is 10 bp.

- When CSSR 12 primer was validated on DCR, Puttur germplasm collection, NRC 152 and NRC 168 for low bulk and NRC 278 and NRC 194 for high bulk (Plate 25) was found to be consistent with marker identified using Chintamani germplasm collection.

- The primer CSSR 14 gave polymorphic loci at 280 and 310 bp in individuals of low bulk and high bulk respectively for shelling percentage (mean of 10 nuts) and the difference between the two bulks is 30 bp.

- When CSSR 14 primer was validated on DCR, Puttur germplasm collection, NRC 156 and NRC 107 for low bulk and NRC 318 for high bulk (Plate 26) was found to be consistent with marker identified using Chintamani germplasm collection.

- Inconsistency in validation of nut weight and shelling percentage in NCGB, DCR, germplasm collection might be due to misclassification of these collections during phenotyping, or due to difference in fertility status of soil.

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(* Originals not seen)

Appendix

8. APPENDIX – I

List of laboratory equipments used for the study

Balance	: ACCULAB sartorious group
Centrifuge	: REMI cooling
Electrophoresis power pack	: Spectrum of innovation
Gel documentation system	: Molecular imager ^{R} Gel Doc ^{TM} XR Imaging System from Bio-RAD
Hot air oven	: KEMI HOT AIR OVEN
Icemaker	: Icematic
Micropipette	: Gilson
Microwave oven	: Intello wave LG Anti Bacteria
Minispin	: Eppendorf
PAGE unit	: Life technologies gibco BRL sequencing system
Power pack for PAGE	: Chromous Biotech PVT. LTD.
Shaker	: Remi rotary shaker
Thermal cycler	: Corbett Research & Applied Biosystems
Vortex	: ROTEK CYCLO (VORTEX) MIXER
Refrigerator	: VESTFROST
Waterbath	: EQUITRON

APPENDIX – II

Composition of reagents used for DNA isolation

Buffers	Composition	Concentrations	200 ml
Extraction buffer	Tris HCl NaCl EDTA CTAB	100 mM 1.4 M 20 mM 3 %	2.42 g 16.32 g 1.488 g 6 g
Tris-EDTA buffer (TE)	Tris Base EDTA	10 mM (pH 8) 1 mM (pH 8)	0.24 g 0.07 g

APPENDIX – III

Composition of buffers and dyes used for gel electrophoresis

1. TAE (50 X)

Tris base	: 242 g
Glacial acetic acid	: 57.1 ml
EDTA (0.5 M, pH 8.0)	: 200 ml
Distilled water to make 1L	

2. Ethidium Bromide

Ethidium bromide	: 10 mg
Distilled water	: 1.0 ml

3. Gel loading dye (6 X)

Bromophenol Blue	: 1.0 %
Sucrose in water	: 40 %

APPENDIX IV : Estimation of DNA quantity isolated from germplasm source by flourometer and normalisation of DNA content

Sl. No.	Germplasm source	DNA quantity (ng/µl)	DNA content (25 ng/µl)	SDW for 100 µl
1	9/2 Ullal	142	17.6	82.4
2	3/108 Gubbi	33	75.8	24.3
3	5/23 Kundapura	30	83.3	16.7
4	1/26 Neeleshwar	45	55.6	44.5
5	8/46 Taliparamba (Chintamani)	10	49.0	51.0
6	9/66 Chirala	93	26.9	73.1
7	2/77 Tuni	122	20.5	79.5
8	2/97 Kottarakara	42	59.5	40.5
9	1/11 Ullal	22	39.1	60.9
10	6/21 Mudbidri	51	49.0	51.0
11	4/43 Wyanadu	44	56.8	43.2
12	1/61 Aalangudi	96	26.1	74.0
13	4/62 Aalangudi	80	31.3	68.8
14	1/63 Chrompet	47	53.2	46.8
15	1/64 Madhuranthakam	52	48.1	51.9
16	5/11 Ullal	81	30.9	69.1
17	3/3 Madhuranthakam	63	39.7	60.3
18	6/91 Kanhargad	64	39.1	60.9
19	2/61 Aalangudi	58	43.1	56.9
20	5/61 Aalangudi	10	49.0	51.0
21	8/61 Aalangudi	95	26.3	73.7
22	3/67 Chrompet	46	54.4	45.7
23	4/63 Guntur	48	52.1	47.9
24	T-56	174	14.4	85.6

Sl. No.	Germplasm source	DNA quantity (ng/µl)	DNA content (25 ng/µl)	SDW for 100 µl
25	3/3 Simhachalam	100	25.0	75.0
26	Hyb-2/11	77	32.5	67.5
27	9/8 EPM	63	39.7	60.3
28	Hyb-2/10	54	46.3	53.7
29	Tree No.-1	127	19.7	80.3
30	H-3-17	96	26.1	74.0
31	NDR-2-1	44	56.8	43.2
32	BCA-139-1	72	34.7	65.3
33	H-3-13	112	22.3	77.7
34	K-25-2	59	42.4	57.6
35	BCA-273-1	24	26.1	74.0
36	T-56 Vittal	105	23.8	76.2
37	M-44/2	104	24.1	76.0
38	Vetori-56	58	43.1	56.9
39	Ansuri-1	46	54.4	45.7
40	M-6/1	86	29.1	70.9
41	A-18-4	130	19.2	80.8
42	13/5 Kodur	43	58.2	41.9
43	M-16/4	94	26.6	73.4
44	2/9 Dicherla	47	53.2	46.8
45	2/4 Baruva	47	53.2	46.8
46	1/11 Dicherla	72	34.7	65.3
47	Tree No. 121	71	35.2	64.8
48	8/1 Kodur	74	33.8	66.2
49	Tree No. 274	31	80.7	19.4
50	M-54/4	79	31.7	68.4
51	H-2/3	104	24.1	76.0
52	8/7 Sompet	66	37.9	62.1

Sl. No.	Germplasm source	DNA quantity (ng/µl)	DNA content (25 ng/µl)	SDW for 100 µl
53	ME-5/3	41	61.0	39.0
54	ME-4/4 (Chintamani 2)	161	15.5	84.5
55	ME-6/1	101	24.8	75.3
56	M-1/3	72	34.7	65.3
57	M-6/1	100	25.0	75.0
58	Tree No. 40	173	14.5	85.6
59	Tree No. 129	191	13.1	86.9
60	2/15	179	14.0	86.0
61	2/16	75	33.3	66.7
62	9/88	80	31.3	68.8
63	1/40	175	14.3	85.7
64	6/86	49	51.0	49.0
65	4/48	125	20.0	80.0
66	5/37 Manjery	71	35.2	64.8
67	1/84	68	36.8	63.2
68	V-1	32	78.1	21.9
69	V-2	70	35.7	64.3
70	V-3	56	44.7	55.4
71	V-4	162	15.4	84.6
72	V-5	24	104.2	4.2
73	H-19	17	14.3	85.7
74	Veng-1	76	32.9	67.1
75	Veng-2	70	35.7	64.3
76	Veng-3	56	44.7	55.4
77	Veng-4	162	15.4	84.6
78	Veng-5	24	104.2	4.2
79	BPP-1	62	40.3	59.7
80	BPP-2	26	96.2	3.8

Sl. No.	Germplasm source	DNA quantity (ng/µl)	DNA content (25 ng/µl)	SDW for 100 µl
81	BPP-3	34	73.5	26.5
82	BPP-4	33	75.8	24.3
83	BPP-5	17	14.3	85.7
84	BPP-6	29	86.2	13.8
85	VRI-1	52	48.1	51.9
86	VRI-2	28	89.3	10.7
87	Vetori-56	162	15.4	84.6
88	39/14	174	14.4	85.6
89	Kankadi	67	37.3	62.7
90	NDR	34	73.5	26.5
91	A-1	65	38.5	61.6
92	G-1C	33	75.8	24.3
93	H-1B (Hebbari 1) Bold	13	33.3	66.7
94	Kottarakara-4B Bold	41	61.0	39.0
95	K-7B	47	53.2	46.8
96	K-3C (Cluster)	173	14.5	85.6
97	K-2B	45	55.6	44.5
98	K-6BC	132	19.0	81.1
99	Kottarakara-5B	39	64.1	35.9
100	CKD-1 (Chikka Dasara Halli)	37	67.6	32.4
101	CKD-2	24	36.8	63.2
102	CKD-3	19	75.8	24.3
103	CKD-4	49	51.0	49.0
104	CKD-5	41	61.0	39.0

APPENDIX V : Mean performance of 104 cashew germplasm source across 10 quantitative traits

Sl. No.	Germplasm source	Tree height (m)	Tree spread (m)	Size of apple (cm^3)	Weight of apple (g)	Nut dimension (cm^3)	Nut weight (g)	Shelling percentage	Shell thickness (mm)	Kernel weight (g)	Cumulative yield (kg)
1	9/2 Ullal	5.30	93.96	11	37.50	0.38	4.58	23.88	0.25	1.19	6.91
2	3/108 Gubbi	5.00	127.5	13.52	52.50	1.34	5.43	24.34	0.25	1.33	10.2
3	5/23 Kundapura	5.00	109.9	14.23	56.50	3.06	5.79	31.60	0.35	1.58	5.71
4	1/26 Neeleshwar	7.30	149.9	17.37	65.00	0.71	6.48	41.15	0.30	1.76	2.97
5	8/46 Taliparamba	4.80	95.42	11.88	44.00	1.30	6.53	29.89	0.30	2.04	9.42
6	9/66 Chirala	4.65	127.1	8.08	19.00	0.91	3.36	30.60	0.35	1.68	2.90
7	2/77 Tuni	5.90	112.9	15.58	64.75	4.09	8.48	30.82	0.30	2.30	3.40
8	2/97 Kottarakara	6.30	116.5	9.175	33.50	1.55	6.74	23.99	0.25	1.28	4.27
9	1/11 Ullal	5.90	181.5	12.62	58.75	4.96	6.05	24.41	0.25	1.61	2.28
10	6/21 Mudbidri	4.65	60.19	15.56	33.50	2.68	8.70	23.63	0.35	1.89	1.38
11	4/43 Wyanadu	5.65	124.3	16.52	66.25	0.52	3.99	17.31	0.15	0.71	3.06
12	1/61 Aalangudi	4.50	92.06	10.41	32.75	0.29	3.74	25.39	0.25	0.98	5.32
13	4/62 Aalangudi	5.00	113.2	16.8	49.73	0.40	4.44	13.41	0.35	0.54	6.60
14	1/63 Chrompet	4.65	104.5	12.94	59.55	1.96	5.72	28.15	0.35	1.93	7.20
15	1/64 Madhuranthakam	4.80	73.97	20.88	63.90	0.93	5.27	38.76	0.20	2.07	7.79
16	5/11 Ullal	5.70	149.2	16.69	96.50	1.90	8.33	24.19	0.35	2.01	2.47
17	3/3 Madhuranthakam	3.40	46.22	9.37	70.00	1.21	5.69	31.36	0.30	1.80	2.77

Sl. No.	Germplasm source	Tree height (m)	Tree spread (m)	Size of apple (cm^3)	Weight of apple (g)	Nut dimension (cm^3)	Nut weight (g)	Shelling percentage	Shell thickness (mm)	Kernel weight (g)	Cumulative yield (kg)
18	6/91 Kanhargad	5.40	120.5	11.05	50.63	0.25	3.65	31.69	0.35	1.13	0.65
19	2/61 Aalangudi	3.60	68.06	13.31	41.00	1.70	6.65	35.75	0.15	2.31	2.72
20	5/61 Aalangudi	4.95	43.94	15.94	51.50	1.60	5.64	31.36	0.35	1.93	2.77
21	8/61 Aalangudi	4.85	116.6	12.77	43.25	1.30	5.70	27.64	1.45	2.19	5.25
22	3/67 Chrompet	4.85	111.3	9.01	22.50	2.20	5.81	31.33	0.30	1.82	7.25
23	4/63 Guntur	5.00	89.29	10.52	38.12	0.30	4.67	33.04	0.15	1.49	2.70
24	T-56	5.00	88.33	9.83	38.25	2.29	6.78	28.31	0.35	1.93	3.29
25	3/3 Simhachalam	5.25	133.4	12.51	47.10	1.26	6.44	25.22	0.35	1.68	2.80
26	Hyb-2/11	5.70	115.3	13.76	51.00	0.76	4.17	23.28	0.25	0.87	2.11
27	9/8 EPM	4.70	129.7	13.04	45.00	1.75	7.19	26.18	0.35	1.97	2.61
28	Hyb-2/10	6.05	105.2	16.4	74.00	1.56	3.68	31.54	0.25	1.89	2.16
29	Tree No.-1	4.70	89.26	15.92	36.00	7.19	6.95	28.31	0.40	1.63	1.88
30	H-3-17	4.05	67.83	26.61	32.55	3.15	7.80	21.80	0.30	1.74	2.32
31	NDR-2-1	4.50	98.01	15.31	57.75	3.13	7.52	20.63	0.20	1.67	1.74
32	BCA-139-1	3.80	54.75	15.27	37.20	1.42	7.47	29.00	0.25	2.27	3.64
33	H-3-13	4.70	115.6	16.39	71.00	1.02	5.36	32.02	0.25	1.80	3.63
34	K-25-2	4.25	67.57	10.45	41.00	2.49	5.87	20.34	0.30	1.51	2.69
35	BCA-273-1	4.95	78.7	6.735	37.30	1.04	4.99	24.90	0.35	1.31	3.10

Sl. No.	Germplasm source	Tree height (m)	Tree spread (m)	Size of apple (cm^3)	Weight of apple (g)	Nut dimension (cm^3)	Nut weight (g)	Shelling percentage	Shell thickness (mm)	Kernel weight (g)	Cumulative yield (kg)
36	T-56 Vittal	5.45	69.67	6.29	18.51	0.40	4.84	28.65	0.25	1.36	2.34
37	M-44/2	4.70	80.04	9.34	33.36	1.44	4.86	34.38	0.20	1.70	3.25
38	Vetori-56	5.60	111.60	13.75	40.70	1.79	6.96	29.23	0.35	2.04	3.74
39	Ansuri-1	5.60	111.20	14.05	40.51	1.42	6.17	28.63	0.35	1.82	2.25
40	M-6/1	3.95	54.020	26.26	86.02	1.75	6.38	24.61	0.35	1.93	4.19
41	A-18-4	3.95	75.20	26.31	100.80	2.65	6.44	23.98	0.35	1.90	2.44
42	13/5 Kodur	5.55	151.80	12.27	21.76	1.04	6.67	30.95	0.35	2.08	3.73
43	M-16/4	4.40	80.05	15.40	66.50	3.04	8.48	25.54	0.33	2.10	3.72
44	2/9 Dicherla	4.35	46.73	14.38	50.50	3.18	6.48	27.01	0.25	2.01	2.73
45	2/4 Baruva	4.75	85.27	38.40	64.00	2.27	7.38	24.91	0.35	1.86	4.46
46	1/11 Dicherla	4.40	89.73	16.19	45.50	1.06	4.74	31.22	0.20	1.46	2.31
47	Tree No. 121	5.40	102.40	15.84	44.50	1.55	4.58	21.24	0.20	1.78	4.69
48	8/1 Kodur	6.30	84.61	12.76	48.51	0.48	3.81	26.87	0.30	1.05	1.77
49	Tree No. 274	5.75	132.50	10.35	29.00	0.68	3.82	30.43	0.20	1.58	6.34
50	M-54/4	5.05	87.38	10.28	47.68	0.36	4.39	32.87	0.25	1.47	2.65
51	H-2/3	6.30	208.00	20.22	85.17	3.40	4.89	24.12	0.30	1.88	1.55
52	8/7 Sompet	7.15	130.50	13.24	56.65	1.49	7.00	33.13	0.25	2.21	3.33
53	ME-5/3	4.35	105.80	8.845	32.00	0.96	5.03	26.03	0.35	1.33	2.87

Sl. No.	Germplasm source	Tree height (m)	Tree spread (m)	Size of apple (cm^3)	Weight of apple (g)	Nut dimension (cm^3)	Nut weight (g)	Shelling percentage	Shell thickness (mm)	Kernel weight (g)	Cumulative yield (kg)
54	ME-4/4	7.45	136.70	11.29	51.50	1.85	7.42	28.94	0.35	2.38	12.85
55	ME-6/1	5.95	85.98	16.14	56.00	1.55	5.84	28.63	0.35	1.61	1.09
56	M-1/3	6.15	134.70	12.00	48.49	3.53	5.02	31.10	0.35	2.38	6.67
57	M-6/1	7.05	181.70	16.15	58.25	2.10	6.80	25.53	0.20	1.74	5.92
58	Tree No. 40	5.20	137.70	15.58	48.05	0.94	5.26	37.26	0.25	2.60	4.95
59	Tree No. 129	5.35	100.60	4.51	37.00	1.61	5.08	34.06	0.15	1.77	7.70
60	2/15	5.05	92.64	26.92	38.25	3.21	8.35	32.16	0.35	2.38	5.24
61	2/16	4.90	117.90	21.16	48.05	2.25	7.06	34.96	0.25	2.14	4.70
62	9/88	5.50	151.70	15.81	46.50	1.65	5.70	34.09	0.20	1.83	6.53
63	1/40	6.00	162.50	12.43	55.15	0.77	5.24	23.20	0.25	1.78	6.09
64	6/86	4.70	187.50	15.03	67.15	1.76	6.99	24.50	0.30	2.25	9.15
65	4/48	6.20	141.80	15.53	68.50	1.60	5.51	20.96	0.30	1.61	3.56
66	5/37 Manjery	5.10	184.80	15.76	72.38	2.89	8.16	24.94	0.35	2.59	13.9
67	1/84	5.90	105.50	17.90	63.50	2.09	8.55	22.51	0.20	1.64	2.48
68	V-1	5.60	184.90	16.26	53.25	2.81	6.84	23.72	0.30	1.56	4.70
69	V-2	5.10	97.19	16.90	46.83	1.06	5.88	27.73	0.25	1.37	8.75
70	V-3	5.65	158.50	17.09	70.50	1.42	7.10	19.49	0.25	1.66	5.03
71	V-4	5.00	126.90	16.60	59.50	1.96	6.17	25.09	0.15	1.75	5.68

Sl. No.	Germplasm source	Tree height (m)	Tree spread (m)	Size of apple (cm^3)	Weight of apple (g)	Nut dimension (cm^3)	Nut weight (g)	Shelling percentage	Shell thickness (mm)	Kernel weight (g)	Cumulative yield (kg)
72	V-5	5.00	154.40	9.965	44.00	2.11	6.89	29.14	0.35	2.04	15.2
73	H-19	5.35	175.60	15.97	54.15	4.07	8.81	21.45	0.35	1.94	5.43
74	Veng-1	5.05	100.70	16.04	64.75	1.10	5.28	26.83	0.15	1.28	6.87
75	Veng-2	4.80	105.70	14.03	58.50	0.91	4.35	25.57	0.25	1.85	9.42
76	Veng-3	6.00	170.90	28.09	97.00	1.95	8.10	31.08	0.35	2.65	7.69
77	Veng-4	4.35	117.20	19.79	72.63	2.61	8.01	21.77	0.45	1.81	5.86
78	Veng-5	5.45	137.40	13.13	32.00	1.10	5.07	29.83	0.35	1.47	5.69
79	BPP-1	5.00	91.24	15.62	44.37	1.51	5.30	30.27	0.35	1.38	4.30
80	BPP-2	5.65	198.10	5.56	19.00	3.13	8.04	30.67	0.35	2.23	3.60
81	BPP-3	4.75	108.00	9.57	53.25	3.24	7.02	39.70	0.25	2.30	2.15
82	BPP-4	4.80	80.95	39.51	49.00	10.24	5.57	30.74	0.30	1.93	6.05
83	BPP-5	4.70	76.55	14.59	52.50	1.40	3.94	31.01	0.25	1.27	4.50
84	BPP-6	5.80	176.80	9.25	61.50	2.26	6.05	22.95	0.30	1.68	4.20
85	VRI-1	4.35	62.17	12.19	36.50	2.05	7.25	24.53	0.20	1.62	4.76
86	VRI-2	4.75	91.68	16.75	10.00	1.49	5.09	25.00	0.35	1.28	8.87
87	Vetori-56	3.75	19.25	18.10	53.00	3.34	6.15	24.05	0.35	1.85	4.08
88	39/14	4.05	44.89	14.28	62.58	1.18	5.81	33.80	0.15	1.61	4.41
89	Kankadi	4.50	120.90	17.33	99.50	14.27	7.88	24.09	0.15	1.96	7.75

Sl. No.	Germplasm source	Tree height (m)	Tree spread (m)	Size of apple (cm^3)	Weight of apple (g)	Nut dimension (cm^3)	Nut weight (g)	Shelling percentage	Shell thickness (mm)	Kernel weight (g)	Cumulative yield (kg)
90	NDR	5.05	58.13	22.10	85.50	1.75	7.49	38.77	0.25	2.05	6.07
91	A-1	5.75	95.94	14.56	56.00	1.41	7.05	33.38	0.20	2.00	9.83
92	G-1C	4.65	102.50	7.40	27.00	1.54	4.87	25.70	0.30	1.64	5.73
93	H-1B (Hebbari 1) Bold	4.50	70.16	16.55	52.00	1.55	4.25	30.15	0.25	2.62	7.35
94	Kottarakara-4B Bold	4.80	100.50	23.44	78.80	1.08	6.95	22.74	0.35	1.46	4.77
95	K-7B	4.70	112.40	17.69	77.00	1.51	7.28	25.85	0.25	1.36	2.24
96	K-3C (Cluster)	4.50	53.98	13.58	62.5	1.66	5.79	31.07	0.30	1.79	9.90
97	K-2B	4.80	57.34	17.35	98.35	6.18	9.65	29.01	0.45	3.03	3.19
98	K-6BC	5.40	95.65	19.19	66.50	2.61	9.1	21.08	0.35	1.88	5.58
99	Kottarakara-5B	5.30	93.97	18.13	75.15	3.32	8.34	19.70	0.35	1.94	3.93
100	CKD-1	6.00	176.90	15.27	65.64	1.07	8.89	33.35	0.35	2.87	6.75
101	CKD-2	6.00	144.10	19.95	78.50	11.8	7.32	31.47	0.30	2.08	5.93
102	CKD-3	5.60	87.67	15.09	75.15	0.71	5.73	23.65	0.35	1.49	10.21
103	CKD-4	3.95	52.68	7.95	37.50	0.47	6.24	31.50	0.30	1.66	8.28
104	CKD-5	4.80	86.44	18.37	99.00	2.91	9.85	37.36	0.35	3.42	6.08
Min	-	3.40	19.25	4.51	10.00	0.25	3.36	13.41	0.15	0.54	0.65
Max	-	7.45	208.00	39.51	100.80	14.27	9.85	41.15	1.45	3.42	15.2
MEAN	-	5.13	108.60	15.33	54.05	2.25	6.24	27.92	0.309	1.81	5.03