MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR MARKERS FOR THE GENETIC ANALYSIS OF CASHEW *(Anacardium occidentale* L.*)*

By

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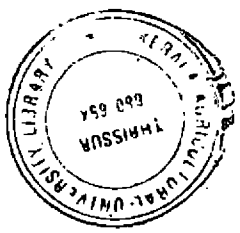

THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Morphological, biochemical and molecular markers for the genetic analysis of cashew (*Anacardium occidentale* L.)"** is a bona fide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara
19.04 -2003

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CERTIFICATE

Certified that the thesis, entitled **"Morphological, biochemical and molecular markers for the genetic analysis of cashew (*Anacardium occidentale* L.)"** is a record of research work done independently by **Miss.Usha Vani, D.,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the Advisory Committee of **Miss.Usha Vani, D.,** a candidate for the degree of **Master of Science in Agriculture** with major in **Plant Breeding & Genetics**, agree that the thesis entitled **"Morphological, biochemical and molecular markers for the genetic analysis of cashew (*Anacardium occidentale* L.)"** may be submitted by Miss.UshaVani, D., in partial fulfilment of the requirement for the degree.

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D. Usha Vani

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Dedicated to
my Family

CONTENTS

Introduction

1. INTRODUCTION

Cashew (*Anacardium occidentale* L.), a native of tropical America, was one of the first fruit trees from the New World to be widely distributed throughout the tropics by the early Portuguese and Spanish adventurers. Since then, it has acclimatized to Indian conditions and now, India is the largest producer and exporter of raw kernels. Being a dollar-earning crop, it supports the economy of not only the farming community but also the industry and the export entrepreneurs.

Being a highly cross-pollinated heterozygous polyploid crop, cashew shows enormous variability in different morphological, physiological and anatomical characters. Considerable seggregation due to heterozygosity release lot of variation among the accessions (Rao and Swamy, 1994; Rao and Bhatt, 1996). Eventhough wide variation exists in cashew, it is difficult to obtain a single tree possessing all the desirable characters. Northwood (1966) reported that even though the yields of the best tree were more than twice that of the mean, the percentage of such trees were very low and it is necessary to consider quality.

Crop improvement through conventional breeding methods in cashew involves collection, conservation, cataloging and evaluation of germplasm, clonal and pedigree methods of selection and hybridisation. Depending on the extent of natural variability among the genotypes and the association between yield and component characters, trees with desirable traits such as compact canopy, short-medium flowering phase, bold nuts, early and cluster-bearing habits etc. are identified and used for breeding programmes. Once the high-yielding trees are identified, commercial exploitation through suitable methods of vegetative propagation can be adopted in cashew.

Variability existing in the crop forms the basis for any conventional breeding programme. In cashew, high variation exists but a systematic evaluation of the germplasm needs to be done to exploit it well. Evaluation of the germplasm requires proper characterisation of the collections already made. At present, morphological characters are used for the purpose which are mostly polygenic in inheritance and are

2. REVIEW OF LITERATURE

Cashew belongs to the family Anacardiaceae and genus *Anacardium*, 20 species of which are known to exist (Rao *et al.*,1998). The commonly cultivated cashew (*Anacardium occidentale* L.) was first reported by the French, Portuguese and Dutch observers in Brazil. The French naturalist and monk, A. Thevet was the first to describe in 1558 the cashew tree and its fruit. Through natural modes of dispersal, cashew spread from its origin in N-E Brazil to South and Central America (Van Eijnatten, 1991). The Portuguese travelers were responsible for spreading it to Africa and India (De Castro, 1994). Though not recorded, the Malabar coast of Kerala is presumed to be the foremost port of entry for cashew in India from where it spread to S-E Asia and both the Indian coasts. Now, it is cultivated in coastal areas of India (686 m ha) for its delicious nuts and is an important source of foreign exchange for Indian economy.

India was the first country to initiate systematic research in the early 1950s which was further strengthened in 1970s with the establishment of Central Plantation Crops Research Institute, Kasargod (Kerala) and an independent National Research Centre on Cashew at Puttur in 1986. Since then, much research has been done to study its floral biology, various agronomic and other cultivation aspects, pollination and hybridization studies in order to improve yield. High variability existing in the crop due to heterozygosity offers great scope for improvement through conventional breeding methods which requires proper collection, evaluation and selection as primary breeding program. Other breeding priorities include investigation in reproductive aspects, influence of environment, compatibility studies, identifying dwarfing genotypes, resistance to tea mosquito bug and lastly, understanding yield through experimental approaches or statistical models (Rao *et al.*, 1998). The following is an overview of the various studies done in cashew related to variability in morphological characters, statistical studies on yield components and also biochemical and molecular studies in tree crops envisaged to aid the conventional breeding programme.

2.1 MORPHOLOGICAL MARKERS IN CASHEW

Various vegetative, reproductive and yield characters have proved valuable in identifying superior trees for breeding programme and thus, can act as morphological markers.

2.1.1 Vegetative characters

Wide variation has been reported in vegetative characters among cashew genotypes. Observations recorded on height, girth and canopy spread of 100 trees, revealed great variation among the population (Nayar *et al.*, 1981). Falade (1981) had observed varietal differences in tree height in mature trees. Reddy *et al.* (1989) had observed variation in tree height, girth and canopy spread between varieties. Canopy spread ranged from 8.1 m to 25.1 m. Great variability for tree height was seen by Swamy *et al.* (1990) among the germplasm accessions evaluated. Eleven accessions were found to possess compact to medium canopy spread. Fifty-six 10-year old F_1 hybrids were studied by Manoj (1992) wherein maximum extent of variation was observed for tree girth followed by mean canopy spread and leaf area. Among the eighteen cashew varieties tested at Cashew Research Station, Madakkathara, the variety V-3 was reported as tallest and M-44/3, the shortest. The canopy spread varied widely and ranged between 7.97 m and 10.35 m. The variety H-1610 recorded highest trunk girth and M-44/3 recorded the lowest (CRS, 1997).

2.1.2 Reproductive characters

The number of perfect flowers per panicle was found to vary significantly among cashew types by Rao and Hasan (1957). Devi (1981) observed high variability for the percentage of hermaphrodite flowers in the F_1 population of cashew. Sriharibabu (1981) reported that average percentage of hermaphrodite flowers in high-yielding varieties may go up to 45. Under Orissa conditions, the percentage of hermaphrodite flowers was found to vary between 5.94 per cent and 20.69 per cent (Patnaik *et al.*, 1985). Reddy and Rao (1985) reported that Bapatla varieties have hermaphrodite flowers ranging from 8 per cent to 15 per cent. Wide variation in the

number of perfect flowers depending on the climatic factors was reported by Das and Sahoo (1987). Khan and Kumar (1988) reported that selections Ullal-1 and Ullal-2 have 2.27 per cent to 6.66 per cent hermaphrodite flowers under humid conditions of Ullal. Manoj (1992) noticed maximum variability for the percentage of hermaphrodite flowers among the floral traits studied for 56 F_1 hybrids and 12 parental combinations of cashew. Krishnappa *et al.* (1994) studied 16 cashew selections in Eastern tracts of Karnataka and noted that 7 selections had more than 45 per cent hermaphrodite flowers and the rest had 10.3 per cent to 44.7 per cent. Sapkal *et al.* (1994) had also observed that the number of perfect flowers per panicle varied significantly among different cashew types. Sheshagiri (1996) had reported similar results in his study on the flowering period and sex-ratio in cashew selections in the hill zone of Karnataka. Lenka *et al.* (1999) had observed high genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) and genetic advance and heritability for number of perfect flowers per panicle. Genotypic coefficient of variation and phenotypic coefficient of variation values were high and genetic advance was moderate for number of nuts per m^2 and nut yield. Number of nuts per m^2 showed very high heritability. Sankarnarayanan and Ahmad Shah (1999) also found high GCV (genotypic coefficient of variation) estimates for nut yield, number of perfect flowers per panicle and fruit set per panicle. Dorajeerao *et al.* (1999) had studied five-year old trees of 14 *Anacardium occidentale* clones and found wide variation in the numbers of male and perfect flowers, duration of flowering, panicle shape, sex- ratio and fruit set. Studies on the flowering and sex-ratio in cashew germplasm selections by Reddy *et al.* (2001) also showed wide variation for the number of perfect flowers per panicle, flowering phase, flowering period, staminate flowers and sex ratio among 52 cashew types.

Wide variation (9.57-23.25) in the number of panicles recorded from different directions of the tree canopy was reported by Krishnappa *et al.* (1991a). In another study, Krishnappa *et al.* (1991b) reported number of nuts per panicle to vary from 4.5 to 8.0. Floral characters of 17 clonally propagated cashew types were tabulated by Sena *et al.* (1995). They identified V-2 as the variety having highest number of

panicles per m^2. Masawe *et al.* (1996) studied the distribution of cashew flower sex types between clones and sides of tree canopies in Tanzania. The number of male, perfect and abnormal flowers were found to be different on different sides of the canopy, although there were consistently more male flowers than other types. High variability was found for percentage of perfect flowers and number of nuts per panicle by Pushpalatha (2000) in a study involving 27 genotypes. Lenka *et al.* (2001) evaluated 13 cashew genotypes and found high genotypic coefficient of variation for number of staminate flowers per panicle, number of perfect flowers per panicle and number of nuts per panicle. GCV (genotypic coefficient of variation), heritability and genetic advance for nut yield per plant, number of male flowers per panicle, number of perfect flowers per panicle and number of nuts per panicle were found to be high in this study.

2.1.3 Yield characters

Apple Weight

Albuquerque *et al.* (1960) studied the apple characters in cashew and noticed a wide variation in cashew apple weight and size. Damodaran (1977) reported that along with other economic characters, considerable variation existed in mean apple weight, size and other apple characters of the F_1 progenies of four cross combinations. Chandran and Damodaran (1985) opined that cashew apples showed a lot of variation in morphological and qualitative characters. Sawke *et al.* (1986) and Haldankar *et al.* (1986) also noted that apple weight in cashew differs with varieties. Aravindakshan *et al.* (1986) observed significant differences in apple weight in thirteen types evaluated at Cashew Research Station, Madakkathara with the highest apple weight (132.67 g) was observed for the cashew hybrid H-3-13 and the lowest (31.33 g) with K-28-2. Antarkar and Joshi (1987) evaluated five cashew varieties (Vengurla-1, V-2, V-3, V-4 and V-5) and recorded highest apple weight (60.76 g) with the variety V-3 and lowest (28.9 g) with V-5. Ghosh and Kundu (1989) recorded variation in apple weight in 17 cashew types tested at Cashew Research Station, Jhargram. Krishnappa *et al.* (1989) observed apple weight to vary from 33.48 g to 62 g in 5 selections tested at ARS, Chintamani.

Antarkar and Joshi (1987) found nut weight varied from 4.08 g to 7.7 g per nut among the 5 varieties evaluated at Konkan Krishi Vidyapeeth, Dapoli. Ghosh and Chatterjee (1987) recorded nut weight ranging from 4.25 g to 6.73 g at Cashew Research Station, Jhargram. In another study, Mohan *et al.* (1987) found nut size to vary greatly among the cashew genotypes. Faluyi (1987) observed maximum variation for nuts per tree and nut weight per tree in a study of eight nut yield traits. Reddy *et al.* (1989) found nut size to vary from 4 g to 6 g among 6 varieties released from APAU. Swamy *et al.* (1990) observed considerable variation among 292 accessions, obtained from different Cashew Research Stations in the country and primary collections from South India for cashew nut weight, apple weight, colour and shape.

Nalini and Santhakumari (1991) found nut size varied from 5.1 g to 8.9 g with highest of 8.9 g for K-16-1 among the 10 cashew selections evaluated at Cashew Research Station, Anakkayam. Nut weight was found to vary from 5.8 g to 10.85 g in 14 hybrids tested at Cashew Research Station, Madakkathara by Salam *et al.* (1991). The highest nut weight of 10.85 g was noted for Priyanka (H-1591). Kernel weight varied from 1.64 g to 2.76 g with the highest of 2.76 g for the hybrid H-1602. George *et al.* (1991) compared the kernel weight of nine cashew selections BLA-139-1 (AKM-1), BLA-39-4 (Mdk-1), K-22-1, NDR-2-1 (Mdk-2), H-3-17 (Dharasree), H-1598 (Kanaka), H-1608 (Dhana), H-1610 and H-1602 and found that the kernel weight varied from 1.64 g (K-22-1) to 2.76 g (H-1602).

Nut yield and Shelling percentage

Significant differences in nut yield between cashew varieties have been reported by many researchers (Kologi *et al.*, 1977 and Falade, 1981). Shelling percentage of cashewnuts was found to vary in F_1 populations (Damodaran, 1977; Devi, 1981). Nandini and James (1984) evaluated 16 cashew types at Cashew Research Station, Anakkayam and found shelling percentage to vary from 22.37 to 28.71. On comparing nut yield for ten years, it was found to vary from 11.9 kg (UL-21-2) to 34.7 kg (BLA-139-1) per tree per year. Variation in shelling percentage was also observed by Vidyachandran and Hanamshetti (1984) and Nandini and James

(1985). Ghosh and Chatterjee (1987) observed shelling percentage to vary from 18 per cent to 34.7 per cent in 17 cashew types tested at Cashew Research Station, Jhargram. Krishnappa *et al.* (1989) observed differences in shelling percentage while comparing 5 cashew selections at the 6th year of planting at ARS, Chintamani, Karnataka. Nut yield ranged from 1.7 kg to 6.36 kg per tree per year. Reddy *et al.* (1989) found shelling percentage to vary from 23.0 per cent to 28.1 per cent among 6 varieties released from APAU. Yield was reported to range from 5.3 kg to 10.08 kg per tree per year.

Based on the evaluation of 292 accessions collected at Central Plantation Crops Research Institute, Vittal, Swamy *et al.* (1990) identified trees having high yield potential ranging from 4.98 kg to 9.84 kg and good shelling percentage (30-35.1). According to Nalini and Santhakumari (1991), shelling percentage of 10 selected cashew types tested at Cashew Research Station, Anakkayam varied from 25.8 per cent (K-25-2) to 27.99 per cent (BLA-139-1). Average yield varied from 13.22 kg per tree per year (K-16-1) to 29.29 kg per tree per year (BLA-139-1). Salam *et al.* (1991) found that among the hybrids tested at KAU, yield varied from 7.06 kg to 12.83 kg per tree per year. Highest yield and shelling percentage was noted with H-1598. Manoj (1992) reported high variation for nut yield followed by number of nuts per panicle. Four varieties and ten hybrids of cashew were evaluated by Naik *et al.* (1997) at RARS, Pilicode. Hybrid H-1600 (Damodar) recorded the highest nut yield followed by Mdk-1.

Twenty-seven genotypes evaluated by Pushpalatha (2000) showed high variability in yield. Lenka *et al.* (2001) found high variation, heritability and genetic advance for nut yield per plant in a study involving 13 cashew genotypes.

2.1.4 Genetic analysis in cashew

Percentage of hermaphrodite flowers and number of nuts reaching maturity were found to have a strong correlation with yield in cashew (Rao, 1974). Nayar *et al.* (1981) reported that canopy spread had maximum positive correlation with yield

followed by trunk girth and height of the tree. Ramdas and Thatham (1982) studied the variability and correlation of yield and seven nut and kernel traits in cashew and found that yield was the most and shell weight, the least variable trait. They also concluded that individual tree yield offered the best scope for selection for improved yield.

Spread of the plant, number of laterals per shoot, panicles per unit area and fruit set per panicle are highly correlated with yield of cashew (Nawale, 1983). A strong correlation was observed between tree yield with percentage of flowering shoots per unit area of tree canopy followed by total canopy area by Parmeswaran *et al.* (1984). No significant correlation was noted between yield and tree height. A weak positive correlation was found between yield and percentage of open perfect flowers. Correlation coefficient worked out in cashew for 8 characters with yield by Mohan *et al.* (1987) suggested that selection could be based on nut weight per tree since this is highly correlated with yield. Anitha *et al.* (1991) found that nut yield had high significant positive correlation with nuts per panicle and mean number of perfect flowers per panicle but regression studies showed that only number of nuts per panicle that reached to maturity showed significant impact on nut yield per panicle. Mean nut weight and nut length had negative correlation with number of nuts per panicle that reached maturity. Strong correlation of nut yield with girth, canopy spread, number of nuts per panicle, kernel weight, leaf area, height of the tree and individual nut weight was observed by Manoj *et al.* (1994). Path studies showed significant positive and direct effect of number of nuts per panicle on yield. Sena *et al.* (1994) had reported inverse correlation of number of nuts per panicle with apple weight.

Kumar and Uduppa (1996) studied the association between nut yield influenced by genetic factors, cultural practices and climatic factors. Among the 26 characters studied, they calculated that 99.7 per cent to 99.9 per cent of total variability in nut yield was controlled by five characters viz. number of reproductive shoots, number of bisexual flowers per panicle, fruit set, fruit retention and the total number of nuts produced per tree. Total number of nuts produced per tree was found as the most important character correlated with yield. Correlation studies in cashew conducted by Reddy *et al.* (1996) showed that out of 19 characters studied, nut yield

had positive correlation with number of nuts per panicle, height, canopy spread, panicle length and stem girth both at phenotypic and genotypic levels whereas days taken for 50 per cent flowering and percentage of perfect flowers had positive correlation with nut yield only at phenotypic level. Significant negative correlation was observed between nut yield with apple weight both at phenotypic and genotypic levels but apple weight and nut weight had significant positive correlation with each other.

Naik *et al.* (1997) found that the spread of branches, production of secondary branches, height and collar girth were significantly correlated with nut yield. Superior vegetative characters did not always result in high yield. It was concluded that moderately vigorous varieties with highest number of secondary branches are the ideal genotypes for obtaining higher yield. Azevedo *et al.* (1998) evaluated 5 traits for genetic variance and genotypic and phenotypic correlations viz. plant height, N-S and E-W canopy spreads, primary and secondary branch numbers. All the genotypic and phenotypic correlations were positive and significant. Correlation between plant height and secondary branch numbers were low indicating the possibility of obtaining smaller plants without causing drastic reduction in secondary branch number.

Swarnapiria *et al.* (1999) studied the correlation between yield and 10 yield–contributing characters in 6 cashew genotypes. The data obtained indicated that collar girth, leaf length and number of nuts per panicle had positive associations with nut yield per tree and may be used as selection indices in cashew improvement programmes. Nut yield and its 13 component traits in 16 cashew genotypes grown at Bhubaneswar were studied by Lenka *et al.* (1999a). Number of staminate flowers and perfect flowers showed positive association with nut yield at both genotypic and phenotypic levels. Number of flowering panicles per m^2, nut weight and number of nuts per panicle were the best contributors to nut yield. Apple weight and nut weight were found to have significant positive correlation with each other. Sankarnarayanan and Shah (1999) found that nut yield was positively correlated with flowers per panicle, fruit set per panicle and shelling percentage.

In another study, Lenka *et al.* (2001) found number of flowers per panicle, nut weight, and number of nuts per panicle were the best contributors to nut yield. Number of staminate flowers and perfect flowers showed high positive association with nut yield both at genotypic and phenotypic levels. In the correlation studies undertaken by Samal *et al.* (2001), yield per plant showed positive and significant correlation with the number of nuts per panicle at the genotypic levels. The phenotypic and genotypic association between nut and apple weight, staminate flowers; number of flowering laterals and nuts per panicle; canopy spread (east-west) and staminate flowers; canopy spread and apple weight and plant height and canopy spread were significantly positive. Negative and significant relationships were observed between number of fruits per panicle and apple weight and between staminate flowers and apple weight. Path analysis revealed that the number of nuts per panicle exhibited the highest positive direct effect on yield at genotypic and phenotypic levels. The number of nuts per panicle and apple weight showed positive direct effect on yield and high negative indirect effect on yield through each other. Similar relationship was observed between the number of nuts per panicle and perfect flowers. It was concluded that the number of nuts per panicle should be considered independently for improving cashew yield.

Genetic Divergence

Grouping of cashew types based on similarity in characters had been attempted by many scientists. Cashew germplasm, consisting of 292 accessions collected by the Central Plantation Crops Research Institute, was categorised into different groups by Swamy *et al.* (1990) based on characters such as yield, nut size, shelling percentage, apple size, maturity, bearing and plant habit. Nalini *et al.* (1994) selected and grouped 36 F_1 Hybrids from a population of 216 F_1 hybrids for having nut size above 8g. Sheshagiri (1996) grouped 15 cashew types growing in RARS, Mudigiree on the basis of variation in percentage of hermaphrodite flowers. At National Research Centre on Cashew, 153 clonal accessions were grouped to 18 clusters based on certain strongly inherited or key characters by Swamy *et al.* (1998). Lingaiah *et al.* (1998) grouped 25 entries of 8-year old cashew trees and found that plant height, stem girth, canopy

spread, flowering shoots per m^2, percentage of flowering per m^2 contributed most to genetic divergence. Sardinha *et al.* (1998) had physically characterised 42 cashew accessions in Guinea-Bissau into 7 groups based on colour, shape and size of cashew apples. Between groups, there were differences in nut size, nut components, flowering date and yield potential.

Lenka *et al.*(1999b) had assessed genetic divergence in 13 cultivars of cashewnut evaluated for vegetative, flowering and fruit characters at Ranasinghpur during 1992. Varieties were grouped into 5 clusters using Mahalanobis' D^2 analysis and Tocher's method. Apple weight and nut weight contributed for more than 36 per cent and 17 per cent respectively to genetic divergence. Rao (1999) had done cluster analysis in cashew along with molecular studies and found that BPP-5 and VRI-2; BLA-139-1(AKM-1) and BLA-39-4 (Mdk-1) were grouped based on flowering and fruiting habit. Hybrids V-5, Priyanka, Kanaka, V-7, V-6, Dhana, V-3 and V-4 were grouped into one cluster as were BPP-1 and BPP-2 in one and Vengurla hybrids V-3, V-4, V-5, V-6 and V-7 into another cluster. Fifty promising types of cashew at Cashew Research Station, Vridhachalam were grouped into 10 clusters by D^2 analysis by Sankarnarayanan and Shah (1999) based on number of flowers per panicle, number of perfect flowers per panicle, number of branches per panicle, number of fruit set per panicle, apple weight, 100-nut weight, nut length, nut width, shelling percentage, 100-kernel weight etc.

2.2 BIOCHEMICAL MARKERS IN CASHEW

2.2.1 Isozymes

Isozymes, discovered by Hunter and Markert in 1957, are the multiple molecular forms of enzymes, separable by electrophoretic procedures, occuring

within the same organism and having same catalytic activities (Market and Moller, 1959). As enzymes are the primary products of a gene, variation in their structure will give reliable information about the variability in the genotype itself and is less susceptible to environmental influence at the population level. There are numerous reports in which a number of cultivars within a species have been examined for isozyme polymorphism. Other studies have determined the genetic origin of seedlings, distinguished progeny from selfing Vs crossing, documented the parentage of cultivars (Pierce and Brewbaker, 1973).

Anont *et al.* (1995) studied 54 varieties of *Tamarindus indica* using vertical PAGE and found that sour and sweet tamarinds showed three different band colours of which the dark brown band was useful for varietal classification. Jayalekshmy (1996) found that peroxidase isozyme analysis detected two isozymes PRX 7 and PRX 42 only in tall varieties and hybrids of coconut. In Chinese jujube, Zhang Hui Mei *et al.* (1997) were able to classify 64 varieties of Chinese jujube with the help of peroxidase banding patterns. Electrophoretic patterns of leaf peroxidase among four cultivars and 2 hybrids of coconut showed polymorphism and were found useful for characterising genotypes (Cardena *et al.*, 1998). Nineteen lychee cultivars were classified into 14 groups based on peroxidase banding patterns which were further grouped based on that for acid phosphatase by Yapwattanaphun *et al.* (1999). Chikkaswamy *et al.* (2001) had studied some of the morphological variations and variations in peroxidase isoenzyme banding pattern in mulberry cultivars taken from the germplasm.

On studying the leaf peroxidase and esterase banding pattern in 10 sandal (*Santalum album* L.) types, Parthsarthy *et al.* (1985) found that the sandal plants with normal, ovate wavy and normal, ovate, non-wavy leaves were found to be close genetically. Satrabandhu *et al.* (1996) had done electrophoretic analysis of leaf extracts to identify lime (*Citrus aurantifolia*) cultivars and found that esterase isozymes could be used to discriminate cultivars. Protopapadakis and Papanikolaou (1999) had used four isozymes to detect genetic diversity in lemon and lemon-like cultivars and found that zymograms of esterase were useful as a diagnostic tool for cultivar identification in view of the extensive polymorphism of this enzyme. Leaf

extracts of 12 peach cultivars were characterised by Nath *et al.* (2001) using 6 enzyme systems: esterase, malate dehydrogenase, isocitrate dehydrogenase, acid phosphatase, alkaline phosphatase and peroxidase of which the latter four showed variation between cultivars and the cultivars were grouped based on genetic distance.

Jayalekshmy (1996) had detected one isozyme PPO 43 present only in the dwarf varieties with high intensity which can be a suitable marker for differentiating the tall and dwarf varieties of coconut. Santalla *et al.* (2000) characterised 24 crab apple (*Malus* sps.) clones using isozyme patterns of different isozymes including catechol oxidase and esterase.

2.2.2 Storage Proteins

Proteins can be used to provide varietal profiles. These markers are ubiquitous and the variation can be understood in genetic terms. Hence, they are used and widely accepted as a source of reliable data in evolution, taxonomy and genetics (Tanksley and Orton, 1983; Crawford, 1990). Proteins are molecules with net electrical charges that are affected by pH and hence can be separated by electrophoresis. There is abundant evidence showing that protein profiles can be obtained for all major crop sps. and that they are independent of environmental or storage conditions. They are, thus, reflective of the genotype. It is imperative that data having a quantitative component like 2-dimensional electrophoresis can be carefully examined in order to be able to evaluate their degree of significance in discriminating between qualitatively similar profiles (Smith and Smith, 1992).

Studies on storage proteins of palms was initiated by Chandrashekhar and Demason (1988) by electrophoresing the seed proteins from two date palm species. which revealed heterogenity. Canto *et al.* (1992) reported biochemical markers in *Cocos nucifera* L. which revealed variation between tall and dwarf varieties. Biochemical changes during storage of cashewnuts were studied by Nagaraja (1996). Changes in kernel proteins, lysine and albumin to globulin ratio during storage were not uniform. Total storage protein profile of coconut were studied by Jayalekshmy and Sree Rangaswamy (1998) which revealed less variation between cultivars. However,

the smaller fractions of storage protein i.e. globulins, albumins and glutelin showed polymorphic bands in the study.

Electrophoresis (SDS-PAGE) of cotyledon storage proteins was done for 17 cashew varieties by Samanta *et al.* (1990) for varietal identification. The band patterns were compared with each other and distinct differences were observed in the presence or absence of some bands of different molecular weights.

2.2.3 Phenols and tannins

The term phenolics embrace a wide range of plant substances, which possess a common aromatic ring bearing one or more hydroxyl substituents. Phenolics play an important role in plant's hormonal balance, disease resistance and protection of injured tissues from infection. Phenolics in high concentration are toxic to plant cells themselves (Tepper and Anderson, 1984). Hence, phenolics will be present in plants in small quantities.

In cashew apple, leucodelphinidins were identified as the major polyphenolic constituents by Sastry *et al.* (1962). Variability in terms of different apple characters including proteins and tannins were reported by Chandran and Damodaran (1985). They also found that there was a sharp decline in tannin and progressive increase in ascorbic acid as the fruits ripened. Phenolic content of the apples were estimated by Sapkal *et al.* (1992). Giridharan (1993) observed that the total phenol content varied from 2.9 mg g^{-1} to 5.03 mg g^{-1} among the grape cultivars. Fechtal *et al.* (1996) evaluated the bark tannin contents of two *Pinus pinaster* bioclimatic types. The effect of tree age on soluble extract yield and tannins was found to be distinct with younger trees yielding more than older trees. Daggade (1999) reported the total phenol content in pepper genotypes and found that the genotype Kalluvally had higher phenolic content (3.802 mg/g) compared to Panniyur I (2.483 mg/g). Content of phenolic substances in cashew apple ranged from 0.25 in variety M-44/3 to 0.79 in variety H-3-17 in a study done by Narayanankutty (2000) involving different varieties.

In a study involving 27 genotypes, Pushpalatha (2000) found that the phenol content in the vegetative flushes varied significantly. Content was maximum in early

high yielding variety Mdk-1 (2.05%) and minimum in early low yielding variety BRZ-2 (1.25%) and H-718 (1.27%).

2.3 RAPD AS MOLECULAR MARKERS

Molecular markers have lately become fundamental tools for fingerprinting varieties, establishing phylogenetics, tagging desirable genes, determining similarities among inbreds (to maximise heterosis in hybrids) and mapping plant genomes (Kang Fu Yu *et al.*, 1993). In perennial plants, such as most fruit crops, the use of marker genes may have the most practical value because breeding and genetic studies in these species are difficult using conventional techniques due to the long juvenile period.

RAPD markers had been successfully utilised for varietal characterisation in oil palm (Shah *et al.*, 1994), coconut (Jayalekshmy, 1996; Ashburner *et al.*, 1997; Sawazaki *et al.*, 1998; Upadhyay *et al.*, 2002) and apple (Koller *et al.*, 1993).

Identification of cultivars and validation of genetic relationships in *Mangifera indica* L. using RAPD was done by Schnell *et al.* (1995). Twenty five accessions of mango were examined for RAPD genetic markers with 80 decamer random primers. Shin *et al.* (1995) had carried out RAPD analyses to identify 16 *Citrus* sps, and cultivars of Cheju island. Six cultivars or species could be identified using two primers. The genetic diversity of seed-propagated Kesington Pride mango was investigated using RAPD analysis by Bally *et al.* (1996). Out of the 30 selections, 15 were found identical for all markers. Only 2 selections differed by more than 5 per cent. Eighteen commercial mango cultivars grown in India were assessed for genetic relatedness by Ravishankar *et al.* (2000) through RAPD analysis using 30 primers. Twenty-seven primers were found to amplify the genomic DNA. Based on cluster analysis, Western, Northern and Eastern mango cultivars were grouped together and southern cultivars in another. It was concluded that majority of the mango cultivars originated from a local mango gene pool and were domesticated later. Fifty South Indian mango cultivars representing wide geographical sources were screened using RAPD markers with 80 decamer primers by Kumar *et al.* (2001). Ten primers, which

gave 139 clear and bright fragments, were selected for analysis. Cluster analysis showed that alternate bearers and regular bearers formed separate groups.

Molecular markers in Cashew

Neto *et al.* (1995) had evaluated seedlings of 4 dwarf cashew clones (CP06, CP09, CP76 and CP1001) through RAPD using 6 decamer arbitrary primers. The size of amplicons ranged from 240-1780 bp. The number of band in the profiles varied from 0-4 depending on the primer and seedling tested. A total of 27 amplicons suitable for DNA fingerprinting were disclosed. Of the 6 primers, one was sufficient to distinguish each of the four clone seedlings investigated. Two primers were selected for further identification.

Mneney *et al.* (1997, 2001) extracted high molecular weight DNA from a selection of cashew varieties obtained from geographically diverse locations around the world and a pool of elite Tanzanian lines. RAPD profiling showed a high degree of DNA level similarity between Tanzanian accessions. They had noted that accessions from India, Mozambique and Tanzania showed the closest relationship with Brazil accession.

Rao (1999) had estimated genetic diversity in cashew cultivars using morphological characters and molecular markers i.e. RAPD and found varieties to be more diverse than hybrids. Among the different regions, he found that varieties and hybrids released from Kerala were much more diverse than other regions with least being among the varieties released from Maharashtra.

Dhanraj *et al.* (2002) had used RAPD markers to estimate the diversity among 90 accessions from the National Cashew Gene Bank at National Research Centre on Cashew, Puttur. A dendrogram, using Ward's method, squared Euclidean distance confirmed that the diversity of Indian cashew collections can be considered to be moderate to high. A core collection was identified based on the study which represents the same diversity as the entire population maintained at the centre. Seven decamer primers of the Operon series: OPB-17, OPB-20, OPC-20, OPD-2, OPD-20, OPE-7 and OPF-3 were used for the study. The results showed that among the 90 accessions, Mdk-1 and Mdk-2 were grouped together with varieties from Anakkayyam and

Bapatla. AKM-1 was grouped with accession from Bapatla (NRC 140) and Vridhachalam (NRC 141) and also with accessions NRC 121, NRC 126 and NRC 127 from Puthur, Kundapur and Gujjadi areas of Karnataka. Mdk-2 was also grouped with varieties from Goa and West Bengal.

Materials and Methods

3. MATERIALS AND METHODS

The present study envisaged to characterise different cashew genotypes using morphological, biochemical and molecular markers was carried out at the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during the years 2000-2002. The experimental field is located at Cashew Research Station, Madakkathara, at an altitude of 22.25 m above mean sea level and is between 103° 2′ N latitude and 76°13′ E longitude.

The experimental material comprised of 12 genotypes maintained at the Demonstration Plot, Cashew Research Station Madakkathara (planted in 1993). The list of genotypes selected for the study are given in the Table 1 and Plates 1a and 1b. The biochemical studies were carried out in the Biochemistry laboratory, College of Horticulture, Vellanikkara and at Cashew Research Station, Madakkathara. The facilities available at the Centre for Plant Biotechnology and Molecular Biology, Vellanikkara, were utilised for molecular studies. All the studies were undertaken for five trees in each genotype.

The study included three aspects:

Morphological markers.
Biochemical markers.
Molecular markers i.e., using RAPD.

3.1 MORPHOLOGICAL MARKERS FOR CHARACTERISATION

Sixty trees, five for each genotype, planted during the year 1993 and at a spacing of 7.5 m x 7.5 m in the Demonstration Block of Cashew Research Station, Madakathara, were selected for the study. The selection was based on the uniformity in the morphology of trees within each genotype. The trees were critically observed during the vegetative and reproductive phases for the following morphological characters.

Table 1. List of the genotypes selected for the study

Name of the genotype selected for the study	Year of release	Parentage
AKM-1 (BLA-139-1)	1982	T.No. 139 of Bapatla
Mdk-1 (BLA-39-4)	1990	T.No. 39 of Bapatla
Mdk-2 (NDR-2-1)	1990	Neduvellur Material
Kanaka (H-1598)	1993	BLA-139-1 X H-3-13
Damodar (H-1600)	2002	BLA-139-1 X H-3-13
Priyanka (H-1591)	1995	BLA-139-1 X K-30-1
H-1593	Yet to be released	BLA-139-1 X K-30-1
K-22-1	1987	Selection
Sulabha (K-10-2)	1996	Selection
Dhana (H-1608)	1993	ALGD-1 X K-30-1
Dharasree (H-3-17)	1996	T 30 X Brazil-18
P-3-2	Not released	Exotic Genotype

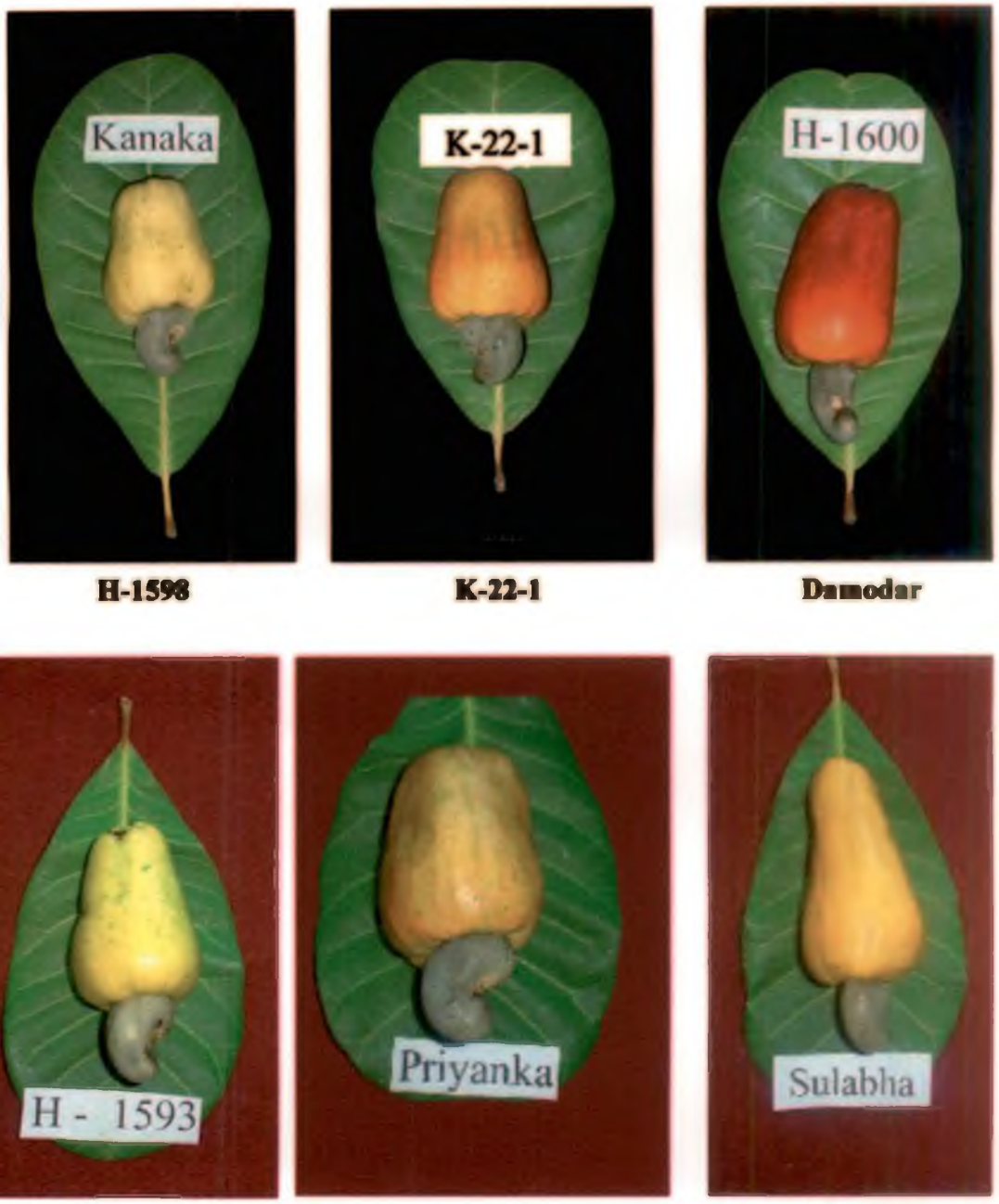
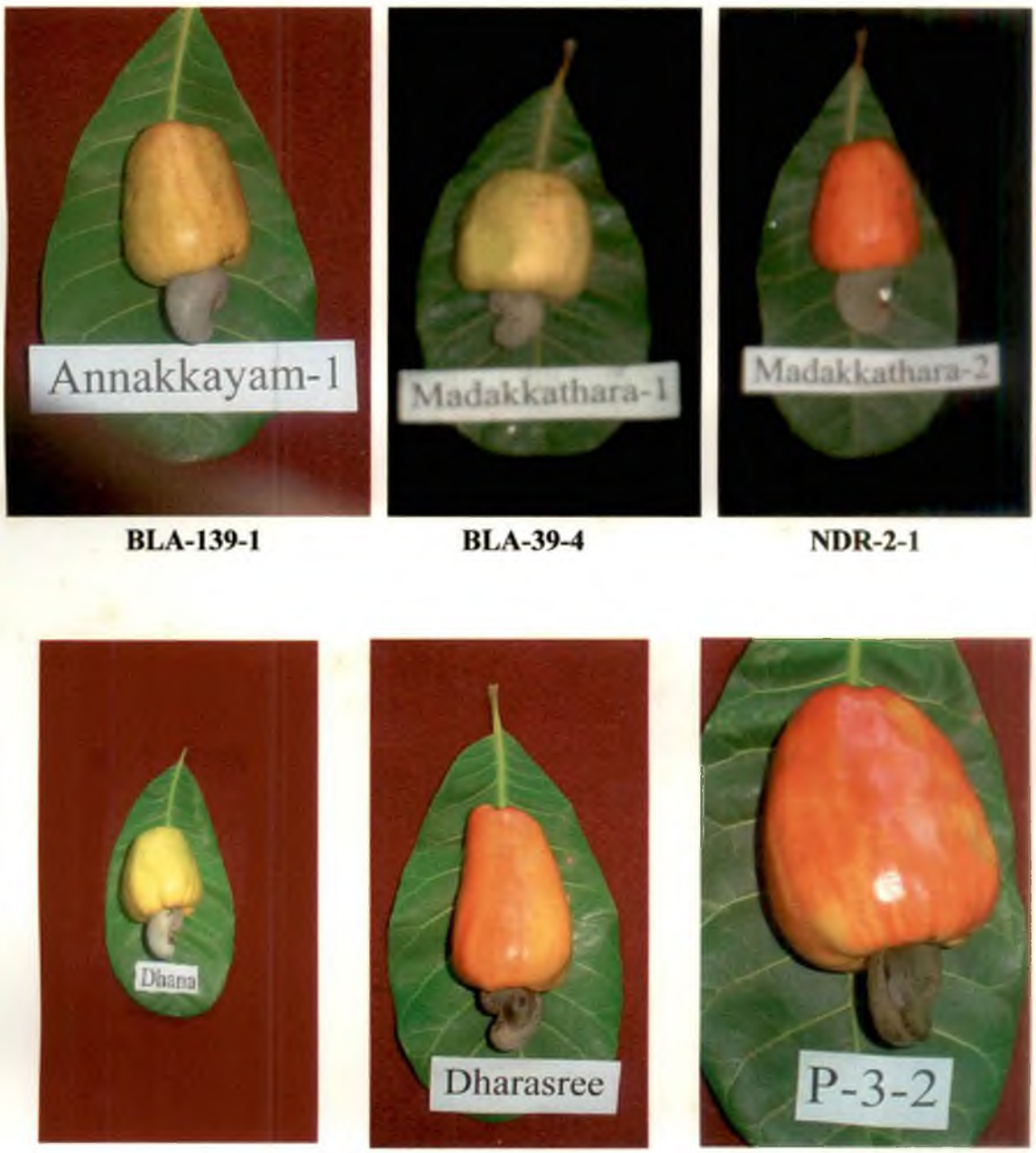


Plate 1a. Cashew genotypes chosen for the study



BLA-139-1 **BLA-39-4** **NDR-2-1**

H-1608 **H-3-17** **P-3-2**

Plate 1b. Cashew genotypes chosen for the study

3.1.1 Vegetative Characters

- *Height of the tree*: Measured from the ground level to the tip of the topmost leaf expressed in metre (m).
- *Trunk girth*: Measured at 1.5 m above the ground level and expressed in centimetres (cms).
- *Canopy spread*: Average of the East-West and North-South spread expressed in metre (m).
- *No. of primary and secondary branches* : Visually recorded.
- *Leaf area:* Average of 3rd, 4th and 5th central leaves using Leaf Area Meter, LI-3000 A, Li-Cor. Inc., Nebraska, USA [Bhagwan and Mohan, 1983].

3.1.2 Flowering characters

- *Number of Panicles per m^2*: Average of the number of panicles observed at 10 randomly selected quadrants of 1 m^2 in the canopy.
- *Number of Hermaphrodite flowers per m^2*: Recorded in the western direction throughout the flowering season.
- *Number of mature nuts per m^2*: Recorded in all the four directions i.e. North, South, East and West.

3.1.3 Yield characters

- *Apple weight*: Average weight of 10 apples expressed in grams (g).
- *Nut weight*: Average weight of 100 nuts expressed in grams (g).
- *Shelling percentage*: Worked out as the ratio of weight of kernels to the weight of raw nuts expressed as percentage.
- *Kernel weight:* Average weight of 100 kernels was recorded as kernel weight and expressed in grams (g).

3.1.4 Analysis of growth and yield components

The data generated on morphological characters of the 12 varieties were analysed using SPAR1 package developed at IASRI, New Delhi available at CCF,

College of Horticulture, Vellanikkara. Analysis of variance was performed on morphological characters. Genotypic and phenotypic coefficient of variation, heritability and genetic advance were worked out for each character separately. Morphological characters associated with yield were identified through genotypic and phenotypic correlation coefficient and path coefficient analysis. D^2 statistics was performed to detect the degree of genetic divergence between the genotypes.

Phenotypic and genotypic variance

Variance components were estimated using the formula suggested by Burton (1952).

Genotypic variance (Vg) = VT-VE/N where VT= MSS due to treatments
VE= Error mean sum of squares
N = Number of replications.

Phenotypic variance (Vp) = Vg + Ve where Vg = Genotypic variance.
Vp = Phenotypic variance.
Ve = Environmental variance.

Phenotypic and genotypic coefficients of variation

The coefficients of variation were calculated as follows:

$PCV = (Vp^{1/2} / X) \times 100$

$GCV = (Vg^{1/2} / X) \times 100$ where PCV = Phenotypic coefficient of variation.
GCV = Genotypic coefficient of variation.
X = Overall mean.

Heritability

Heritability(H^2) in broad sense was estimated as follows :

$H^2 = Vg / Vp \times 100$

Expected genetic advance

Genetic advance (GA) under 5 per cent selection pressure was calculated using the formula suggested by Lush (1949) and Johnson *et al.* (1955).

$EGA = K \times (Vg / Vp) \times Vp^{1/2}$

where EGA = Expected genetic advance.
Vg = Genotypic variance.
Vp = Phenotypic variance.
K = Selection intensity
(which is 2.06 for selection of 5% individuals)

Phenotypic and genotypic correlation coefficients

The correlation coefficients were worked out to study the extent of association between characters. The phenotypic and genotypic covariances were worked out for calculating the variance.

Path coefficient analysis

The principles and techniques suggested by Wright (1921) and Li (1955) were employed for the analysis. In path coefficient analysis, the correlation among the causes and effects were partitioned into direct and indirect effects of causal factors on effect factors.

D^2 Analysis

Replication mean for each character of each genotype was used for analysis of variance. After testing the differences, a simultaneous test of significance of difference with regard to the pooled effects of the 14 characters under study was carried out using Wilk's criterion (Rao, 1952).

Original mean values were, then, transformed into uncorrelated mean using pivotal condensation of common dispersion matrix. From the uncorrelated variables, the actual values of D^2 between any two varieties based on 14 characters were, then, calculated. In order to determine the population constellation, all the varieties were grouped into a number of clusters on the basis of D^2 values (Rao, 1952). A dendrogram was constructed using the D^2 totals using the procedure suggested by Sneath and Sokal (1973).

Cluster Analysis

Non-hierarchal Euclidean cluster analysis was done using SPAR1 package following the method suggested by Spark (1973).

3.2 BIOCHEMICAL MARKERS

3.2.1 Storage protein studies

Cashew kernels were crushed, wrapped in a filter paper and dipped in ethyl alcohol for four days. The defattened kernels were, then, used for protein extraction.

Extraction of kernel protein

From each of the sixty kernel samples, 1g was weighed and ground with 1ml of 0.1M Sodium phosphate buffer (pH 7.0) in a mortar and pestle. The paste was transferred into an eppendorf tube and centrifuged at 15,000 rpm for 15 minutes at 25°C. The supernatant was saved and stored in the refrigerator and used for electrophoretic studies.

Estimation of protein by Lowry's method (Sadashivam and Manickam, 1996)

Reagents used

Reagent A 2% Sodium carbonate in 0.1N Sodium Hydroxide

Reagent B 0.5% Copper Sulphate ($CuSO_4$. 5 H_2O) in 1% Potassium Sodium Tartarate

Reagent C Alkaline Copper Solution (50 ml of A & 1 ml of B were mixed prior to use).

Reagent D Folin-Ciocalteau's Reagent.

Protein Stock Solution (Bovine Serum Albumen)

Procedure

- 20 µl of each of the 12 kernel protein samples extracted were taken and made up to 1 ml in different test tubes.

- 5 ml of reagent C was added to each of them and also to another test tube with 1 ml distilled water serving as a blank.
- The solutions were mixed well and allowed to stand for 10 minutes.
- 0.5 ml of reagent D was, then, added, mixed and incubated in dark for 30 minutes.
- The intensity of the blue colour developed was measured at 660 nm in Spectronic R Genesys 20.
- The amount of protein was estimated against a standard curve prepared using Bovine serum albumen at different concentrations and equal amounts of each kernel protein sample was used for leading in the gel.

Electrophoresis

Electrophoretic separation of proteins was performed with SDS-PAGE as described by Laemmli (1970).

Preparation of Stock Solutions

The following stock solutions were prepared as per Sadashivam and Manickam (1996):

I. **Stock Acrylamide Solution**

Acrylamide (30%)	:	30 g
Bisacrylamide (0.8%)	:	0.8 g
Distilled water to	:	100 ml

II. **Separating gel Buffer (1.875 M Tris-HCl pH: 8.8) :**

22.7 g of Tris base was dissolved in small quantity of water and the pH was adjusted to 8.8 with 1N HCl. The volume was, then, made up to 100ml.

III. **Stacking gel buffer (0.6 M Tris-HCl pH: 6.8)**

7.26 g of Tris base was dissolved in small quantity of water and the pH was adjusted to 6.8 using 1N HCl. Then, the volume was made up to 100 ml.

IV. **Polymerising agents:**

a) Ammonium persulphate : 0.1 g dissolved in 2ml distilled water in an eppendorf tube (prepared fresh each time).

b) TEMED fresh from the refrigerator.

V. **Electrode buffer (0.05 M Tris , 0.192 M Glycine , 0.1% SDS)**

Tris base	-	6.0 g
Glycine	-	14.4 g
SDS	-	1.0 g
Distilled water	-	1000 ml
pH	-	8.2-8.4 [No adjustment required]

The solution was used 2-3 times.

VI. **Sample buffer (5X concentration)**

Tris-HCl buffer (0.6 M, pH 6.8)	5 ml
SDS	0.5 g
Sucrose	5 g
Mercaptoethanol	0.25 ml
Bromophenol blue	1.0 ml (0.5% W/V solution in water)
Distilled water to	10 ml

VII. SDS (10%)

5 g SDS in 50 ml distilled water was prepared and stored at room temperature.

VIII. **Standard marker protein [Bovine Serum Albumen]**

IX. **Sodium Phosphate buffer (0.1 M, pH 7.0)**

Solution A: 0.2M Sodium monobasic hydrogen phosphate

Solution B: 0.2M Sodium dibasic hydrogen phosphate

39 ml of solution A was mixed with 61 ml of solution B. The pH was adjusted to 7.0 and the volume was, then, made up to 100 ml (0.4M). This was diluted three times to get 400 ml of 0.1M solution.

X. Staining solution

Coomassie Brilliant Blue	0.1g
Methanol	40 ml
Acetic acid	10 ml
Distilled water	50 ml

XI. Destaining solution

Methanol	40 ml
Acetic acid	10 ml
Distilled water	50 ml

Procedure

Resolving gel composition	**12.5%**	**10%**	**7%**
Monomer	8.3 ml	6.64 ml	4.1 ml
Tris-HCl 1.875 M	4.0 ml	4.0 ml	4.0 ml
Water	7.4 ml	9.06 ml	11.6 ml
SDS 10%	0.2 ml	0.2 ml	0.2 ml
APS 5%	0.1 ml	0.1 ml	0.1 ml
TEMED	20 µl	20 µl	20 µl

Stacking gel composition

Monomer	1.35 ml
Distilled water	7.5 ml
Stacking gel buffer	1.0 ml
SDS (10%)	0.05 ml
APS (5%)	0.05 ml
TEMED	20 µl

Experiment

a) The glass plates, spacers and comb were cleaned thoroughly and wiped. The spacers were placed on the edges, in between the plates and clamped the clips tightly.
b) Molten agar (0.5%) was poured along the sides and was allowed to solidify to seal.
c) 20ml of the separating gel mixture (12.5%) was prepared and poured carefully between the glass plates. A layer of distilled water was added above the gel layer and was allowed to polymerise for 30 minutes.
d) Stacking gel mix (4%) was prepared and poured over the separating gel after removing the layer of water.
e) The comb was placed in the gel and allowed to set for 30 minutes.
f) After polymerising, the gel was installed in the electrophoresing apparatus.
g) The electrode buffer was poured slowly and air bubbles removed.
h) Equal quantity of protein (200 μg) was loaded in each well by adjusting the protein content with sample buffer to have equal quantity of protein per unit volume.
i) Before loading, the sample solutions were heated in boiling water for 3 minutes to ensure complete reaction between proteins and SDS for effective denaturing.

The electrophoresis was conducted at a constant current of 20 mA till the protein moved over stacking gel and then, at 30 mA for 2.5-3 hours. The gel was removed and kept in the staining solution overnight. It was, then, destained using destaining solution to visualize protein bands.

3.2.2 Isozyme analysis

Native PAGE was done for which reagents were prepared as for kernel protein studies excluding SDS (wherever it was used) for all the three isozymes.

Peroxidase (PRX)

Extraction buffer (0.1M Tris-HCl)

Tris base	1.2114 g
Cysteine-HCl	0.1g
Ascorbic Acid	0.1g
Distilled water to	100 ml

0.4 g leaf tissue was weighed into a pre-cooled mortar and pestle and ground into a paste with 0.4 ml of the extraction buffer. The sample was centrifuged at 14,000 rpm and at 4°C for 4 minutes. It was mixed with the treatment buffer in 10:1 and 15:1 ratios. 0.017 g of sucrose was also added to the supernatant and then, loaded immediately in 10 per cent gel.

Staining solution for peroxidase

0.2M Sodium Acetate buffer (pH 5.6)	200 ml
Benzidine	0.2 g
H_2O_2 (3%)	0.8 ml

Acetate buffer and benzidine were mixed, heated to boil, cooled and filtered. Hydrogen peroxide (30%) was diluted to 3 per cent and 0.8 ml of it was added to the solution at the time of staining. Fresh staining solution was prepared each time.

Electrophoresis

The gel was run at 5 mA for 4 hrs. and put in the staining solution immediately. It was kept overnight and then, destained with 7 per cent acetic acid.

Polyphenol oxidase (PPO)

Extraction buffer:

1% Tween-80

1 g of Tween-80 was dissolved in 10 ml of 0.1M potassium phosphate buffer (pH 7.0) and the volume was made up to 100 ml with the buffer.

Potassium Phosphate Buffer (0.1M, pH 7.0)

Solution A : 0.2M Potassium Hydroxide

Solution B : 0.2M Potassium dihydrogen Phosphate

50 ml of solution B was mixed with 30 ml of solution A. The pH was adjusted to 7.0 and the volume was made up to 100 ml. This was diluted to 400 ml to get 0.1M buffer.

To 0.5 g of the sample, 0.5 ml buffer, 20 µl β-mercaptoethanol and a pinch of insoluble PVP were ground in a pre-cooled mortar and pestle and centrifuged at 15,000 rpm and at 4°C for 15 minutes.

Staining solution for PPO

0.1M Potassium Phosphate buffer (pH 7.0)	100 ml
p-phenylenediamine	0.1 g
Catechol	0.11 g

Electrophoresis was done on 7 per cent gel at 20 mA constant current till the tracking dye reached the resolving gel and the at 30 mA for 3 hours. The gel was kept in the freshly prepared staining solution for 30 minutes only and then destained with 7 per cent acetic acid.

Esterase (EST)

Extraction buffer

0.1 M Sodium Phosphate buffer, pH 8.0	100 ml
1 mM EDTA	0.342 g

Extraction was done as for PPO.

Staining solution for EST :

Sodium dihydrogen phosphate	1.4 g
Disodium hydrogen phosphate	0.55 g
Fast Blue RR salt	0.1 g
α-naphthyl acetate	0.015 g
Distilled water to	100 ml

The gels were stained in the freshly prepared staining solution for 10-30 minutes and then, destained with methanol: acetic acid: water in 10:10:1 ratio.

3.2.3 Phenol Estimation

Total phenol content in the leaves was estimated using Folin-Ciocalteau method (Sadasivam and Manickam, 1996).

Reagents used

20 per cent Sodium Carbonate

Catechol stock solution

Procedure

- 0.5 g of the mature leaf tissue was ground in a mortar and pestle with 5 ml ethanol.
- The ground sample was transferred to a test tube with 5 ml ethanol.
- The sample was allowed to settle for half an hour and then filtered into a conical flask.
- The filtrate was dried, and then, dissolved in 100 ml of distilled water.
- 1ml of the solution was made up to 3 ml. 0.5 ml of Folin and Ciocalteau reagent was added.
- After 3 minutes, 2 ml of 20 per cent sodium carbonate was added.
- The intensity of blue colour developed was read at 660 nm in a spectrophotometer.
- Total phenol was calculated from a standard curve of catechol and expressed as mg of phenol per gram of plant sample.

3.2.4 Total Tannin Estimation

Total tannins in the mature leaves was estimated using Folin-Denis method (Sadasivam and Manickam, 1996).

Reagents

35 per cent Sodium Carbonate

Folin-Dennis reagent

Standard tannic acid Solution

Procedure

- 0.5 g of the mature leaf tissue was ground in 5 ml of distilled water and then, made up to 100 ml.
- The mixture was allowed to settle.
- One ml of the sample was taken in a volumetric flask.
- 10 ml of Sodium carbonate solution and 5 ml of Folin-Denis reagent were added, and then, made up to 100 ml.

- The intensity of the blue colour developed was read at 700 nm using Spectronic R Genesys 20.
- Total tannin content was calculated from a standard curve of tannic acid and expressed as mg/g of plant sample.

3.3 RAPD ASSAY

3.3.1 DNA Extraction

A modified CTAB extraction procedure by Doyle and Doyle (1987) was followed in the present study.

Reagents used

I. 4X Extraction Buffer

25.6 g	Sorbitol / Sucrose
4.8 g	Tris-base
0.74 g	EDTA
100 ml	Milli Q water
pH	7.5

The above chemicals were dissolved in about 70ml of distilled water and the pH was adjusted with 6N HCl. The volume was, then, made up to 100 ml.

II. Lysis buffer

1M	Tris base	2.4228 g
0.25M	EDTA	1.8612 g
5M	NaCl	11.7 g
2 g	Cetyl Trimethyl Ammonium Bromide (CTAB)	
100 ml	Milli Q water	
pH	8.0	

Tris base and EDTA were weighed and dissolved in 40 ml of Milli Q water. Sodium chloride was dissolved in another 40 ml of Milli Q water. Both were mixed

and the pH was adjusted to 8.0. The volume was, then, made up to 100 ml. CTAB was then added. The mixture was warmed to dissolve.

III. **5 per cent Sarcosin**

5 g of Sarcosin was dissolved in 100 ml of Milli Q water.

IV. **Chloroform : Isoamyl alcohol (24:1, V/V)**

V. **70 per cent Ethanol**

VI. **Isopropanol**

VII. **TE Buffer**

1 mM	Tris base	1.211 g
0.1 mM	EDTA	0.372 g
Water		1 litre
pH		7.5 (with Acetic acid)

Extraction procedure

- 7.5 ml lysis buffer and 2.5 ml Sarcosin solution were taken in a clean, sterile centrifuge tube and kept in a beaker of water at 60° C.
- 1 g of tender leaf was taken in a clean, autoclaved mortar.
- 50 µl β-mercaptoethanol, a pinch (1mg) of sodium metabisulphite and insoluble PVP each were added to prevent phenolic interference. Extraction buffer (6 ml) was, then, added leaf sample and ground in the mortar using a pestle.
- Extract was ground into a paste and transferred to the tube containing lysis buffer and sarcosin solution.1.5 ml of extraction buffer was used to wash the mortar and pestle and transferred the whole content to the centrifuge tube.
- The mixture was, then, incubated for 10 minutes at 60°C.
- 18 ml of chloroform: isoamyl alcohol was added to and mixed by gentle inversion.
- The mixture was centrifuged at 10,000 rpm at 4°C for 10 minutes.
- Supernatant was transferred to a fresh tube and kept in the refrigerator for 5 minutes.
- 0.6 volume isopropanol was added to it and recentrifuged at 10,000 rpm at 4°C for 10 minutes.

- The supernatant was thrown off.
- The DNA precipitate was washed using ethanol and recentrifuged at 10,000 rpm at 4°C for 10 minutes.
- The DNA pellet was dried, dissolved in 200 µl sterile water and stored in the refrigerator at -4°C.

3.3.2 Purification of DNA

RNase preparation

Pancreatic RNase		RNase A
0.01M	Sodium acetate	pH 5.2 (solvent)
1M	Tris-Cl	pH 7.4

A stock of RNase (10mg/ml) was prepared in 0.01M sodium acetate. The solution was heated at 100°C for 15 minutes and allowed to cool slowly to room temperature.0.1 volume of Tris-Cl was added and stored at -20°C.

Incubation of DNA with RNase

100 µl of DNA sample was made up to 500 µl with milli Q water and 5 µl RNase was added. It was incubated for 1hr at 37°C.

- Equal volume of phenol-chloroform-isoamyl alcohol mixture (25: 24:1, v/v/v) was added and mixed gently in an eppendorf tube.
- The mixture was centrifuged at 10,000 rpm for 10 minutes.
- The aqueous layer was transferred to a fresh tube and mixed with 0.6 volume chilled isopropanol and kept at -20°C for half an hour.
- The mixture was centrifuged at 10,000 rpm for 15 minutes.
- The supernatant was poured off and the pellet air-dried on a tissue paper.
- It was dissolved in 50 µl TE buffer and stored at -20°C.
- 2 µl of the RNase treated DNA sample was run on agarose gel to check for complete removal of RNA.

Agarose gel electrophoresis

Reagents used

I. TAE buffer 50X

Tris base 242 g

Glacial Acetic Acid - 57.1 ml

0.5M EDTA - 100 ml

pH - 8.0

Made up with distilled water to 1 litre.

II. Gel Loading Dye

Glycerol - 60%

TAE buffer - 30%

1% Bromophenol blue - 10%

Preparation of the agarose gel

- Gel casting tray was sealed with cellotape to form a mould and set on a horizontal section of the bench after checking the level. The comb was adjusted in such a way that the teeth were 0.5 mm - 1 mm above the plate
- 1.5 per cent agarose in 1X TAE buffer was prepared. The solution was boiled and allowed to cool to 42°C - 45°C
- Ethidium bromide was added to a final concentration of 0.5 µg/ml from a stock of 10 mg/ml water to the agarose solution
- The agarose solution was, then, poured on to the gel casting tray to a height of 3 mm - 5 mm with comb in position
- The gel was allowed to solidify for 15-20 minutes
- The tape and comb was removed and the tray was mounted in the electrophoresis tank
- Electrophoresis buffer (1X TAE) was added to the tank to cover the gel to a depth of 1 mm
- DNA samples were prepared by mixing DNA with gel loading dye in the ratio of 1:1. 20 µl was loaded into the slots of gel using a micropipette near the negative terminal

- λDNA digested with EcoRI and Hind III was loaded into the slots on the gel so as to determine the size of the unknown DNA samples
- The gel tank was closed. The cathode and anode of the electrophoresis unit were attached to the power supply and a constant voltage of 120 V was used for the run
- The power supply was turned off when the loading dye moved about 2/3rd of the gel
- The gel was taken from the electrophoresis unit and viewed under UV light in a UV transilluminator
- Then, the gel was documented using Alpha Imager 1200 (Alpha Innotech Corporation, USA)

3.3.3 Quantification of DNA

After ensuring the quality of DNA in the sample by electrophoresis, 10 µl of it was diluted to 1.5 ml with sterile water and absorbance at 260 nm and 280 nm read against distilled water blank using UV visible spectrophotometer (Spectronic R Genesys 5). The purity of DNA was assessed from the ratio of OD 260/OD 280. A ratio of 1.8-2.0 indicates pure DNA. The quantity of DNA in the pure sample was calculated using the formula

OD_{260} = 1 is equivalent to 50 µg double stranded DNA.

Therefore, OD_{260} x 50 gives the quantity of DNA in µg/ml.

3.3.4 Random Amplified Polymorphic DNA

The procedure of Demeke *et al.* (1992) was modified and used for the amplification of plant DNA.

One cycle included :

(a) DNA denaturation at 94°C for 1minute.

(b) Annealing of the primer to the template DNA at 37.5°C for 1 minute.

(c) Primer extension at 72°C for 2 minutes.

The PCR was programmed for 45 cycles to get a proper amplification.

The reaction mixture (25 µl) consisted of :

1. 10X Assay buffer 2.5 µl (with 15mM $MgCl_2$)
2. dNTP Mix 1.0 µl
3. Taq DNA polymerase 2.0 µl (0.6 units)
4. Primer 2.0 µl (10pmoles)
5. Template DNA 1.0 µl (50 ng)
6. Milli Q water 16.5 µl

- A master mix without the template was prepared using the reaction mixture for the required number of reactions.
- From this master mix, 24 µl was pipetted into each PCR tube and 1 µl of the template DNA were added.
- The reaction mix was overlaid with 25 µl of sterile mineral oil.
- The PCR tubes were loaded in the Thermal Cycler (MJ Research, USA) and the programme was run.
- The programme was completed in 5 hours.
- The amplified products were electrophoresised on 1.5 per cent agarose gel.
- The gel was viewed under UV light in transilluminator and then documented using the alpha imager.

3.3.5 Screening of random primers for RAPD

A total of 10 decamer primers under different Operon series viz., OPE, OPF, OPP etc. were screened for amplification of genomic DNA extracted from the cashew variety, using the Thermal Cycler mentioned under RAPD. From these, 4 primers that gave good amplification (3-10 bands) were selected and utilized for further characterisation of 12 isolates. The total number of bands along with the number of polymorphic bands obtained in all 12 isolates with each of the 4 primers tried were recorded.

3.3.6 Data Analysis

The pattern of DNA amplification for the 4 primers were scored as 1 or 0 by the presence or absence of bands respectively and the data was fed to the NTSYS PC 2.0 software package. Similarity indices were computed as JACCARD's coefficient through 'Simqual' routine and clustering was done using 'SAHN' routine of the package. A dendrogram was constructed for the 12 genotypes based on the clustering.

Results

4. RESULTS

In the present investigation, twelve cashew (*Anacardium occidentale* L.) genotypes were subjected to genetic analysis based on morphological, molecular and biochemical markers. The data recorded were analysed statistically and the results for the different studies are presented in this chapter as follows :

- Morphological markers.
- Biochemical markers
- Molecular markers

4.1 MORPHOLOGICAL MARKERS

4.1.1 Variability Studies

Fourteen morphological traits were studied for the twelve cashew genotypes. Analysis of variance showed significant F value for all the variables except for the number of primary branches suggesting significant variation among the genotypes for these parameters. F-values ranged from 1.12 to 38.82 for the different traits. Mean, SE and coefficient of variation for the 14 variables are given in Table 2. Genotypic and phenotypic variances, heritability and genetic advance for different characters are given in Table 3. and the comparison of the same in Fig.1.

Tree Height

The mean tree height was found to be 5.81 m with H-1600 having the highest (6.31 m) and P-3-2 having the lowest tree height (4.5 m). Genotypic coefficient of variation (GCV) for this character was found to be 8.28 per cent; phenotypic coefficient of variation (PCV) was 10.27 per cent. Heritability was 0.65 indicating moderate genotypic influence for this character. A genetic advance of 0.8 was indicated for selection.

Tree girth

The mean tree girth for the twelve genotypes was 66.72 cm. Dhana had the highest tree girth (79.94 cm) and P-3-2, the only exotic variety included in the study,

Table 2. Mean values of different characters for the genotypes studied

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
V1	5.81	54.08	**2.25**	**8.00**	7.04	**81.14**	18.50	15.75	1099.25	**84.58**	9.05	2.58	27.90	8.28
V2	6.30	71.94	2.75	**8.25**	7.89	67.68	20.50	25.75	926.75	**85.77**	7.62	2.45	29.01	4.73
V3	5.46	67.41	2.75	**7.25**	7.82	69.15	21.00	**37.50**	1833.75	**74.15**	8.36	1.83	27.65	**10.00**
V4	6.12	79.75	3.00	9.75	7.90	64.59	**15.00**	15.50	**568.00**	**46.71**	8.52	1.76	25.96	7.94
V5	**6.31**	68.00	**2.25**	9.00	7.94	71.56	21.50	14.25	939.75	**70.20**	8.41	1.77	25.83	6.76
V6	5.38	72.88	3.25	10.25	**6.09**	**45.38**	24.50	22.00	1191.00	**42.44**	6.25	1.54	24.61	7.16
V7	5.69	59.04	3.25	**13.50**	7.55	59.83	**26.50**	22.50	1091.50	**47.76**	**6.04**	1.58	27.25	4.42
V8	6.03	60.50	2.75	**10.00**	6.36	57.87	22.25	22.50	1016.50	**76.67**	6.69	**1.45**	**23.85**	7.84
V9	5.94	59.50	3.00	**10.25**	7.87	79.99	17.25	20.75	**2802.00**	**82.91**	**11.87**	**2.65**	25.27	5.35
V10	6.04	78.75	**3.75**	**9.75**	**9.19**	58.35	25.00	21.25	1070.50	**81.67**	6.84	1.98	**29.04**	5.97
V11	6.19	**79.94**	2.75	**11.75**	8.31	58.83	20.25	17.50	1720.25	**55.23**	7.95	2.28	28.57	7.44
V12	**4.50**	**48.86**	3.00	**7.00**	6.18	68.27	**15.00**	**10.50**	1744.25	**103.56**	**7.81**	1.88	24.08	**2.77**
GM	**5.81**	**66.72**	**2.90**	**9.56**	**7.51**	**65.22**	**20.60**	**20.48**	**1333.63**	**70.97**	**7.95**	**1.98**	**26.58**	**6.55**
SEM	0.25	5.00	0.56	1.31	0.45	4.39	1.58	1.75	135.75	4.96	**0.39**	0.14	1.45	1.62
CD	0.72	14.35	1.61	3.77	1.28	12.60	4.54	5.02	389.71	14.25	**1.11**	0.40	4.16	4.65
F	8.43	8.60	1.12	3.99	8.71	10.45	11.00	31.07	38.83	29.21	32.27	22.09	3.41	3.03

V1 : Sulabha (K-10-2)
V2 : Dharasree (H-3-17)
V3 : H-1593
V4 : Mdk-2 (NDR-2-1)
V5 : Damodar (H-1600)
V6 : Mdk-1 (BLA-39-4)
V7 : AKM-1 (BLA-139-1)
V8 : K-22-1
V9 : Priyanka (H-1591)
V10: Kanaka (H-1598)
V11: Dhana (H-1608)
V12: P-3-2
GM : Grand Mean
SEM : Standard error of Means
CD : Critical Difference
F : F value

X1 : Tree height
X2 : Tree girth
X3 : Number of primary branches
X4 : Number of secondary branches
X5 : Canopy spread
X6 : Leaf area
X7 : Number of panicles m^{-2}
X8 : Number of nuts m^{-2}
X9 : Number of perfect flowers m^{-2}
X10: Apple weight
X11: Nut weight
X12: Kernel weight
X13: Shelling percentage
X14: Yield

Bold figures show range and GM.

had the lowest (48.86 cm). This character showed a moderately high GCV (14.62%), PCV (18.07%) and a heritability of 0.66. A genetic advance of 16.27 was indicated for selection.

Number of primary branches

The genotypes did not differ significantly both at 5 per cent and 1 per cent level for this character. The mean was found to be 2.90 with a range of 2.25 to 3.75. The GCV (4.75%) was the least for this character but PCV was high (27.75%). Heritability was very low (0.03) as was genetic advance for selection (0.05) indicating low genotypic influence for this character.

Number of secondary branches.

The range for this character varied from 7 (P-3-2) to 13.50 (AKM-1). The mean was found to be 9.56. A high GCV (16.8%) and PCV (25.69%) was noted. Heritability was found to be moderate (0.43) indicating a moderate genotypic influence for this character. Genetic advance for selection was found to be 2.16.

Canopy spread

Canopy spread was highest for Kanaka (9.19 m) and least for Mdk-1 (6.09 m) with a mean of 7.51 m.This character showed a GCV of 11.66 per cent, PCV of 14.37 per cent and a moderate heritability of 0.66 indicating moderate genotypic influence on the character. Genetic advance of 1.46 was indicated for selection.

Leaf area

The highest leaf area (81.14 cm^2) was noted for the genotype Sulabha (K-10-2) and the lowest (45.38 cm^2) for Mdk-1 with a mean of 65.22 cm^2. A medium GCV (14.63%), PCV (17.45%) and a high heritability of 0.70 was noted which indicated high genotypic influence for this character. Genetic advance of 16.48 was noted for selection.

Table 3. Genotypic and phenotypic variances, heritability and genetic advance for the characters studied

Character Studied	**GCV	*PCV	Heritability	Genetic Advance
Tree Height	8.28	10.27	0.65	0.80
Tree Girth	14.62	18.07	0.66	16.27
Number of primary branches	4.75	27.75	0.03	0.05
Number of secondary branches	16.80	25.69	0.43	2.16
Canopy Spread	11.66	14.37	0.66	1.46
Leaf Area	14.63	17.45	0.70	16.48
Number of panicles m^{-2}	17.18	20.32	0.71	6.16
Number of nuts m^{-2}	33.13	35.26	0.88	13.13
Number of perfect flowers m^{-2}	44.27	46.55	0.90	1156.49
Apple weight	26.26	28.06	0.88	35.93
Nut weight	19.30	20.50	0.89	2.98
Kernel weight	23.34	25.45	0.84	0.85
Shelling percentage	5.99	9.77	0.38	2.01
Yield	24.86	42.88	0.34	1.95

*Phenotypic Coefficient of variation
**Genotypic Coefficient of Variation

Number of panicles m^{-2}

The number of panicles m^{-2} ranged from 26.5 (AKM-1) to 15.0 (P-3-2) and Mdk-2 with a mean of 20.6. A medium GCV (17.18%), PCV (20.32%) and a high heritability (0.71) was noted indicating high genotypic influence for this character. A genetic advance of 6.16 was indicated for selection.

Number of nut m^{-2}

The mean number of nuts m^{-2} was found to be 20.48 with a highest of 37.5 (H-1593) and a lowest of 10.5 (P-3-2). A very high GCV (33.13%), PCV (35.26%) and a high heritability of 0.88 indicated a high genotypic influence for this character. A genetic advance of 13.13 was indicated for selection.

Number of perfect flowers m^{-2}

The number of perfect flowers m^{-2} was highest for Priyanka (2802) and lowest for Mdk-2 (568) with a mean of 1333.62. The character showed the highest GCV (44.27%), PCV (46.55%) and a very high heritability of 0.90 indicating a predominant genotypic influence on it. A genetic advance of 1156.49 for selection was noted.

Apple weight

The exotic variety, P-3-2, showed the highest apple weight (103.56 g) and Mdk-1, the lowest (42.44 g) with a mean of 70.97 g for apple weight. The character showed a high GCV (26.26%), PCV (28.06%) and a high heritability of 0.88 indicating high genotypic influence on it. A genetic advance of 35.93 g for selection was indicated.

Nut weight

The mean for this character was 7.95 g with highest (11.87 g) for Priyanka and lowest for AKM-1 (6.04 g). A medium GCV (19.3%), PCV (20.5%) and a high heritability (0.89) was noted indicating high genotypic influence for this character. A genetic advance of 2.98 was noted for selection.

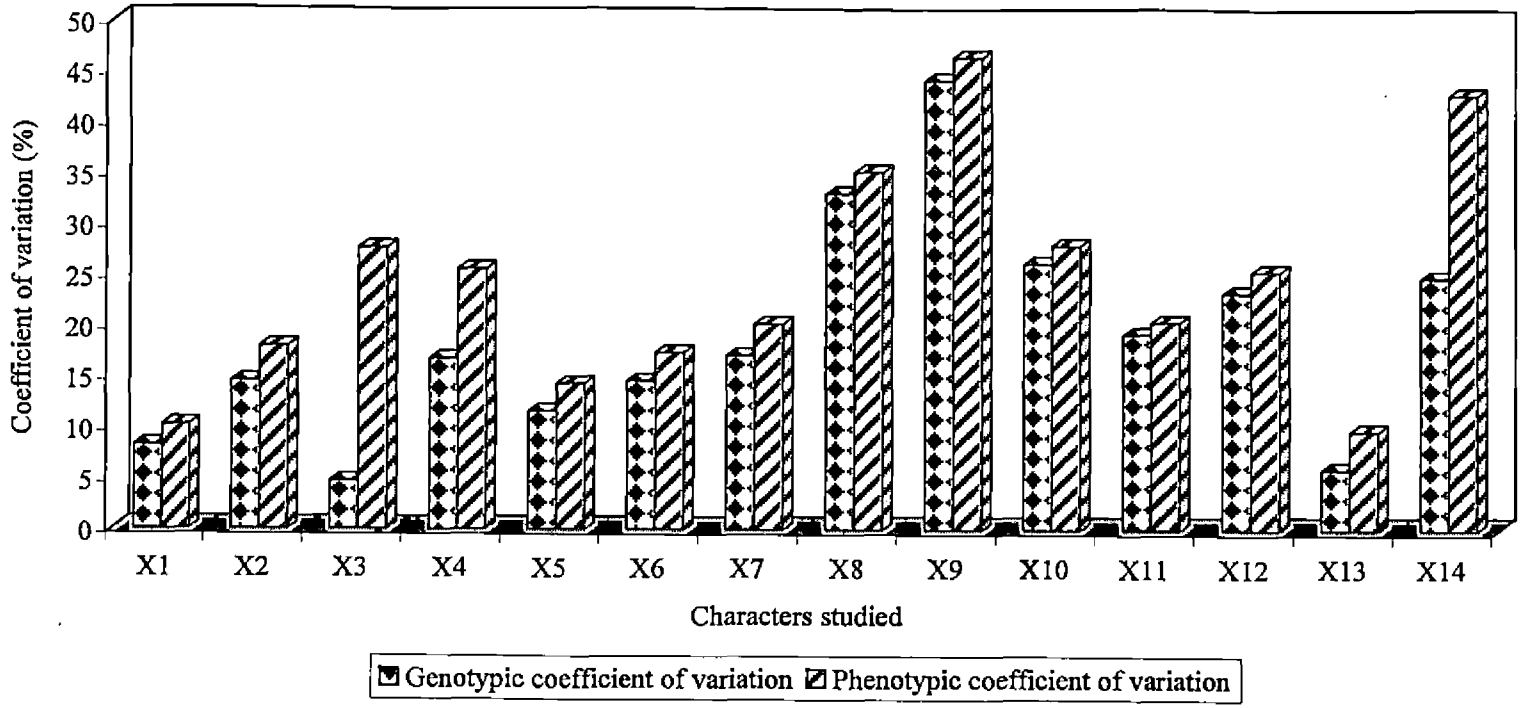


Fig. 1. Phenotypic and genotypic coefficient of variation for the 14 characters studied in cashew

X1 : Tree height
X2 : Tree girth
X3 : Number of primary branches
X4:Number of secondary branches
X5 : Canopy spread
X6 : Leaf area
X7 : Number of panicles m^{-2}
X8 : Number of nuts m^{-2}
X9 : Number of perfect flowers m^{-2}
X10: Apple weight
X11: Nut weight
X12: Kernel weight
X13: Shelling percentage
X14: Yield

Kernel weight

The mean for this character was 1.98 g with highest (2.65 g) for Priyanka and the lowest (1.45) for K-22-1. A high GCV (23.34%), PCV (24.45%) and a high heritability (0.84) was noted for this character indicating high genotypic influence. A genetic advance of 0.85 was noted for selection.

Shelling Percentage

The highest shelling percentage was noted for Kanaka (29.04%) and the lowest for K-22-1 (23.85%) with a mean of 26.58 per cent. A GCV (5.99%), PCV (9.77%) and a medium heritability (0.38) was noted indicating moderate genotypic influence for this character. A genetic advance of 2.01 was indicated for selection.

Yield

The hybrid H-1593 gave the highest yield (10 kg) and P-3-2, the lowest (2.77 kg) with a mean yield of 6.55 kg. A high GCV (24.86%), PCV (42.88%) and a medium heritability (0.34) was noted indicating moderate genotypic influence for this character. A genetic advance of 1.95 was indicated for selection.

4.1.2 Correlation studies

Correlation between the fourteen variables were estimated and presented in the Table 4. The correlation matrix is given in Fig.2.

Tree height showed significant positive correlation at 1 per cent level with canopy spread, tree girth, shelling percentage, kernel weight, yield and number of secondary branches at 1 per cent level. It showed negative correlation with number of primary branches at 1 per cent level and with perfect flowers m^2 and apple weight at 5 per cent level.

Tree girth also showed significant positive correlation with canopy spread, number of primary branches, shelling percentage and yield. It was found to be negatively correlated with apple weight and leaf area at 1 per cent level and with perfect flowers m^2 at 5 per cent level.

Table 4. Genotypic Correlations between the different characters

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
X1	1.000	0.665**	-0.463**	0.402**	0.668**	0.069	0.184	0.119	-0.334*	-0.304*	0.158	0.559**	0.566**	0.403**
X2		1.000	0.523**	0.276	0.688**	-0.512**	0.258	0.214	-0.359*	-0.596**	-0.224	0.142	0.474**	0.469**
X3			1.000	0.480**	0.648**	-1.426**	1.190**	0.243	0.095	-0.569**	-1.234**	-0.807**	-0.105	-1.508**
X4				1.000	0.225	-0.532**	0.655**	-0.065	-0.055	-0.775**	-0.310*	-0.049	0.073	-0.176
X5					1.000	0.180	0.178	0.204	0.014	-0.069	0.244	0.526**	0.963**	0.117
X6						1.000	-0.658**	-0.120	0.378*	0.621**	0.865**	0.592**	0.179	0.003
X7							1.000	0.466**	-0.280	-0.425**	-0.702**	-0.200	0.365*	0.108
X8								1.000	0.170	-0.104	-0.121	0.107	0.416**	0.564**
X9									1.000	0.343*	0.655**	0.336*	-0.196	-0.163
X10										1.000	0.346*	0.206	-0.009	-0.429**
X11											1.000	0.668**	-0.099	0.106
X12												1.000	0.674**	0.151
X13													1.000	0.072
X14														1.000

X1: Tree height
X2: Tree girth
X3: Number of primary branches
X4: Number of secondary branches
X5: Canopy spread
X6: Leaf area
X7: Number of panicles m^{-2}
X8: Number of nuts m^{-2}
X9: Number of perfect flowers m^{-2}
X10: Apple weight
X11: Nut weight
X12: Kernel weight
X13: Shelling percentage
X14: Nut yield
** Significant at 1% level
* Significant at 5% level

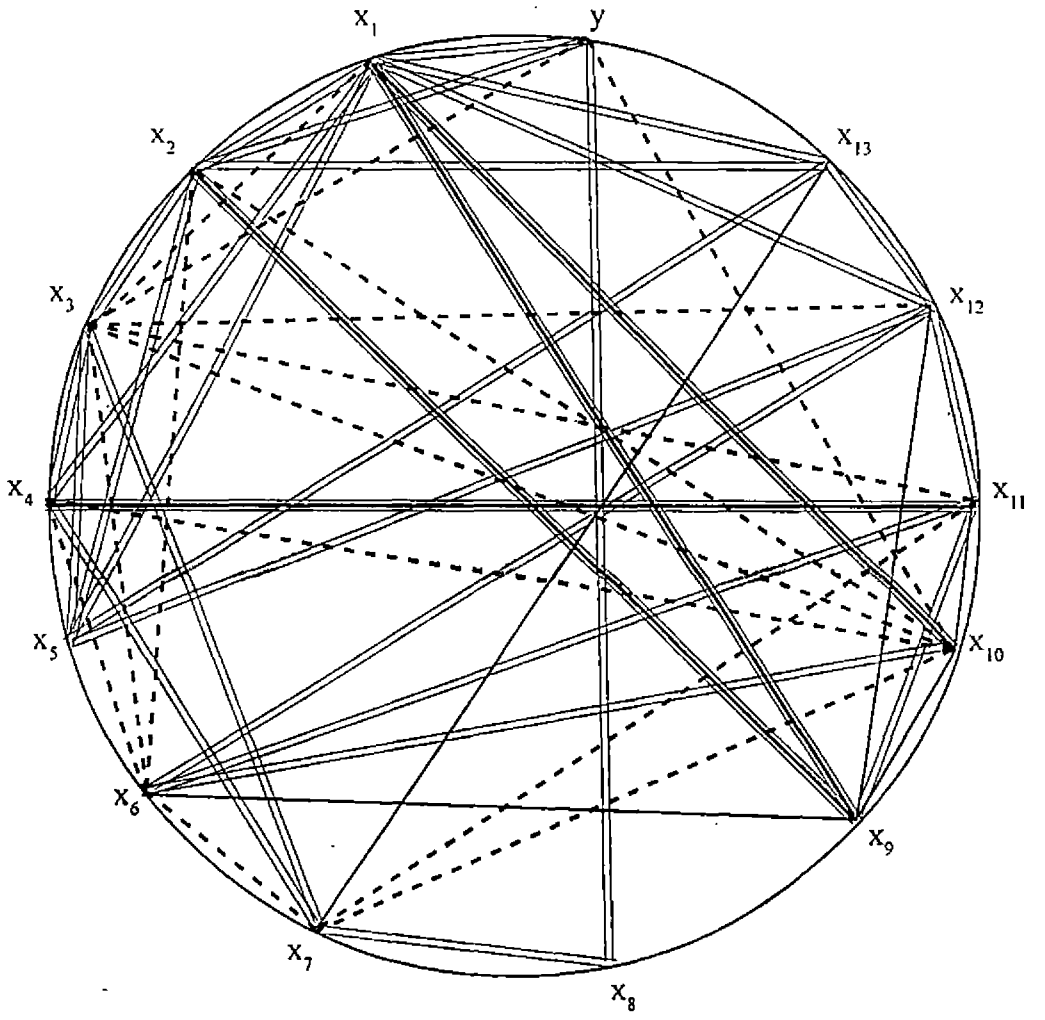


Fig. 2. Genotypic correlations among different characters in cashew

- - - - - Significant negative correlation at 1 per cent level
═══ Significant positive correlation at 1 per cent level
——— Positive correlation at 5 per cent level
═══ Negative correlation at 5 per cent level

x_1 - Tree height
x_2 - Tree girth
x_3 - No. of primary branches
x_4 - No. of secondary branches
x_5 - Canopy spread
x_6 - Leaf area
x_7 - No. of panicles/m^2
x_8 - No. of nuts/m^2
x_9 - No. of perfect flowers/m^2
x_{10} - Apple weight
x_{11} - Nut weight
x_{12} - Kernel weight
x_{13} - Shelling percentage
Y - Nut yield

Significant positive genotypic correlation of number of primary branches was noted with number of panicles m^{-2}, canopy spread and number of secondary branches at 1 per cent level. Number of primary branches was negatively correlated with leaf area, nut weight, kernel weight, apple weight and yield at the same level.

Number of secondary branches showed significant positive correlation with number of panicles per m^2 at 1 per cent level and negative correlation with apple weight and leaf area at 1 per cent level and nut weight at 5 per cent level.

Canopy spread showed significant positive correlation with shellling percentage and kernel weight at 1 per cent level. Leaf area showed significant positive correlation with nut weight, apple weight, kernel weight at 1 per cent level and with number of perfect flowers at 5 per cent level. It showed significant negative correlation with number of panicles m^{-2} at 1 per cent level.

Number of panicles per m^2 showed significant positive correlation with number of nuts per m^2 at 1 per cent level and with shelling percentage at 5 per cent level. It was found to be negatively correlated with nut weight and apple weight at 1 per cent level.

Number of nuts per m^2 showed significant positive correlation with yield and shelling percentage at 1 per cent level. Number of perfect flowers per m^2 showed significant positive correlation with nut weight at 1 per cent level and with apple weight and kernel weight at 5 per cent level. Apple weight was significantly correlated positively with nut weight at 5 per cent level but was negatively correlated with yield at 1 per cent level. Nut weight, in turn, was significantly positively correlated with kernel weight at 1 per cent level. Kernel weight was significantly positively correlated with shelling percentage at 1 per cent level.

4.1.3 Path analysis

In order to study the direct and indirect effects of the 13 variables considered for the estimation of genotypic correlation coefficients, path coefficient analysis was

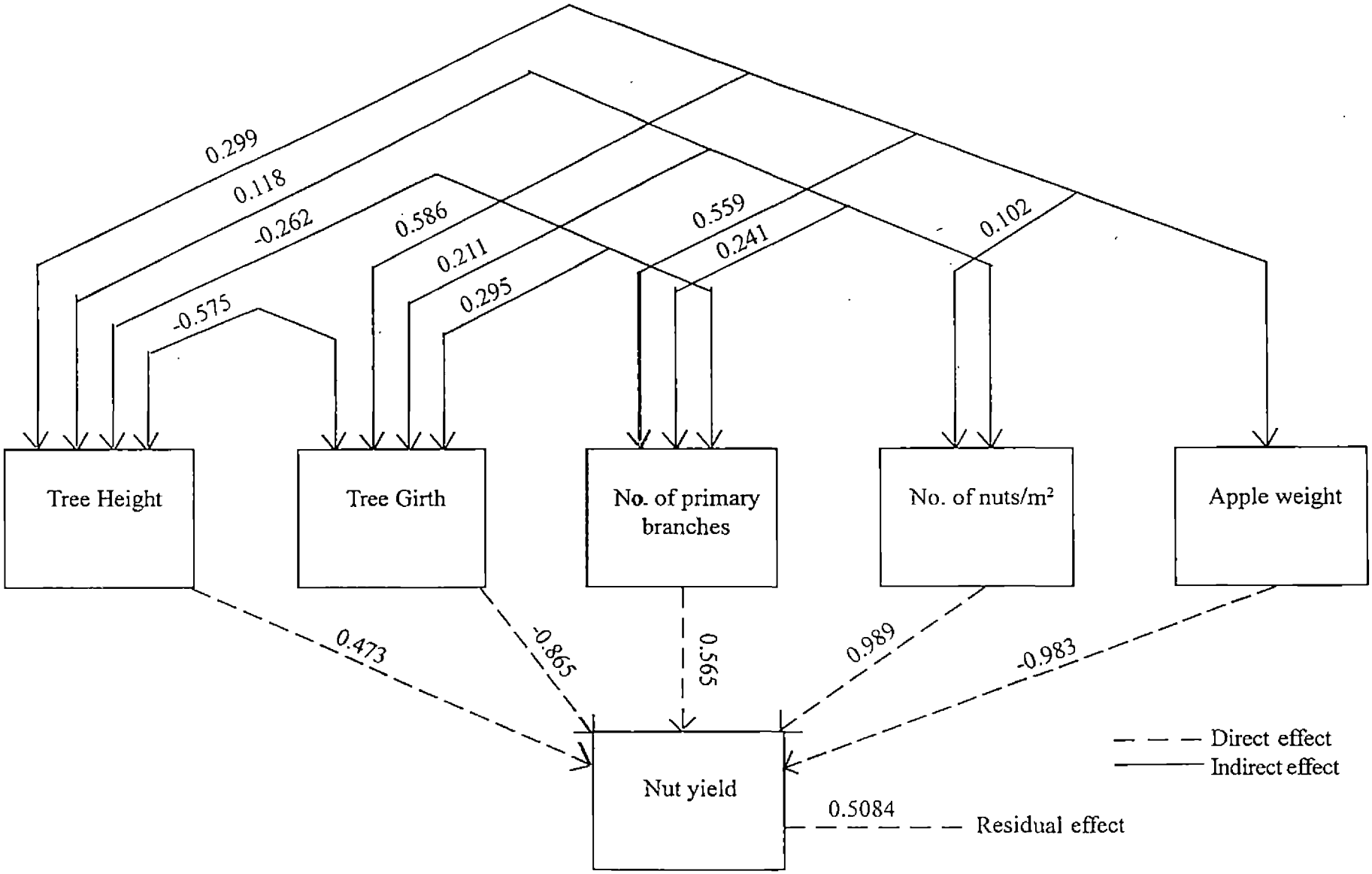


Fig. 3. Path diagram indicating direct and indirect effects of the characters correlated to yield

done, the results of which are tabulated in Table 5. A path diagram was constructed with characters showing significant correlation with yield (Fig.3).

The result showed that about 50 per cent of the variability in the nut yield was contributed by the 13 characters recorded in the study. Residual effect was 0.5084. It is seen from the table that maximum positive direct effect on nut yield was by nut weight (1.214) followed by number of nuts per m^2 (0.989), kernel weight (0.867), number of primary branches (0.565) and tree height (0.473). Significant negative direct effect was by number of perfect flowers per m^2 (-1.381), apple weight (-0.983), tree girth (-0.865), number of secondary branches (-0.853), shelling percentage (-0.803) and leaf area (-0.326). The least positive direct effect on nut yield was by canopy spread (0.059) whereas the least negative direct effect was by number of panicles per m^2 (-0.042).

Tree height showed significant positive indirect effect through kernel weight (0.485) and number of perfect flowers per m^2 (0.462). Tree girth showed significant positive indirect effect through apple weight (0.586), number of perfect flowers per m^2 (0.495) and tree height (0.314).

Number of primary branches showed significant positve indirect effect on nut yield through apple weight (0.559) and leaf area (0.464) and significant negative indirect effect of nut weight (-1.498), kernel weight (-0.700), tree girth (-0.452) and number of secondary branches (-0.409). Number of secondary branches showed significant negative indirect effect of nut weight (-0.377) and significant positive indirect effect of apple weight (0.762).

Canopy spread showed a weak positive correlation with yield through the significant positive indirect effects of tree height (0.316), number of secondary branches (0.366) and kernel weight (0.456). Leaf area showed significant positive indirect effects through tree girth (0.443), nut weight (1.050), kernel weight (0.513) and number of secondary branches (0.454) and significant negative indirect effects through number of primary branches (-0.806), number of perfect flowers per m^2 (-0.522) and apple weight (-0.611).

Table 5. Direct and indirect effects of thirteen characters on nut yield of cashew

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
X1	**0.473***	-0.575*	-0.262	-0.343*	0.039	-0.023	-0.008	0.118	0.462*	0.299	0.192	0.485*	-0.454*	0.403**
X2	0.314*	**-0.865***	0.295	-0.235	0.041	0.167	-0.011	0.211	0.495*	0.586*	-0.272	0.123	-0.381*	0.469**
X3	-0.219	-0.452*	**0.565***	-0.409*	0.038	0.464*	-0.050	0.241	-0.132	0.559*	-1.498*	-0.700*	0.085	-1.508**
X4	0.190	-0.238	0.271	**-0.853***	0.013	0.173	-0.027	-0.065	0.076	0.762*	-0.377*	-0.043	-0.059	-0.176
X5	0.316*	-0.596*	0.366*	-0.192	**0.059**	-0.059	-0.007	0.202	-0.020	0.068	0.296	0.456*	-0.773*	0.117
X6	0.033	0.443*	-0.806*	0.454*	0.011	**-0.326***	0.027	-0.119	-0.522*	-0.611*	1.050*	0.513*	-0.144	0.003
X7	0.087	-0.223	0.672*	-0.558*	0.011	0.214	**-0.042**	0.461*	0.387*	0.418*	-0.852*	-0.174	-0.293	0.108
X8	0.056	-0.185	0.138	0.056	0.012	0.039	-0.019	**0.989***	-0.235	0.102	-0.147	0.093	-0.334*	0.564**
X9	-0.158	0.310*	0.054	0.047	0.001	-0.123	0.012	0.168	**-1.381***	-0.337*	0.795*	0.291	0.158	-0.163
X10	-0.144	0.516*	-0.322*	0.661*	-0.004	-0.202	0.018	-0.103	-0.473*	**-0.983***	0.420*	0.179	0.008	-0.429**
X11	0.075	0.194	-0.697*	0.265	0.014	-0.282	0.029	-0.120	-0.904*	-0.340*	**1.214***	0.579*	0.079	0.106
X12	0.264	-0.123	-0.456*	0.042	0.013	-0.193	0.008	-0.106	-0.464*	-0.202	0.810*	**0.867***	-0.542*	0.151
X13	0.267	-0.410*	-0.060	-0.063	0.057	-0.058	-0.015	0.411*	0.271	0.009	-0.120	0.585*	**-0.803***	0.072

Residual effect : 0.5084

X1: Tree height
X2: Tree girth
X3: Number of primary branches
X4: Number of secondary branches
X5: Canopy spread
.X6: Leaf area
X7: Number of panicles m^{-2}
X8: Number of nuts m^{-2}
X9: Number of perfect flowers m^{-2}
X10: Apple weight
X11: Nut weight
X12: Kernel weight
X13: Shelling percentage
X14: Correlation with nut yield
*Significant direct or indirect effects
**Significant at 1% level. (correlation coefficients)
Bold figures indicate direct effects.

Number of panicles per m^2 showed significant positive indirect effect through number of primary branches (0.672), apple weight (0.418), number of nuts per m^2 (0.461) and number of perfect flowers per m^2 (0.387) and significant negative indirect effect through nut weight (-0.852) and number of secondary branches (-0.558).

Apple weight showed significant indirect effect of number of perfect flowers per m^2 (-0.473) and number of primary branches (-0.322).

Nut weight showed significant negative indirect effect through apple weight (-0.340), number of primary branches (-0.697) and number of perfect flowers/m^2 (-0.904). Similarly, kernel weight showed a significant negative indirect effect through shelling percentage (-0.542), number of primary branches (-0.456) and number of perfect flowers m^{-2} (-0.464).

Shelling percentage showed significant negative indirect effect through tree girth (-0.410) and positive indirect effect through kernel weight (0.585) and number of nuts m^{-2} (0.411).

4.1.4 Genetic Divergence

To assess the degree of divergence between the 12 genotypes, D^2 analysis and cluster analysis were conducted for the data using 'SPAR1' computer programme. Based on the D^2 totals obtained (Table 6.), grouping of genotypes was done and a dendrogram was constructed as in Fig.8 (Sneath and Sokal, 1973). The dendrogram shows that the pairs Dharasree and Kanaka, Mdk-1 and AKM-1 and Sulabha (K-10-2) and H-1600 (Damodar) are tied together. H-1591 was placed close to H-1593. AKM-1 and Dhana were also placed close to each other. H-1593 was found to be the most divergent genotype in the studies.

Non-heirarchial Euclidean cluster analysis grouped that the twelve cashew genotypes into 4 clusters (Fig. 4). Cluster I was found to consist of 3 genotypes (Sulabha, Priyanka and P-3-2), Cluster II-3 genotypes (Mdk-1, AKM-1 and K-22-1), Cluster III, a single genotype (H-1593) and Cluster IV consisted of 5 genotypes (Dharasree, Mdk-2, H-1600, Kanaka and Dhana).

Table 6. D^2 totals for the twelve cashew genotypes

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
V1	0											
V2	94.34	0										
V3	250.32	119.57	0									
V4	120.4	299.7	467.28	0								
V5	41.46	142.47	292.73	43.59	0							
V6	121.95	118.9	194.89	160.34	90.02	0						
V7	123.98	89.18	155.68	215.06	105.82	40.32	0					
V8	74.53	575.6	156.33	196.1	81.43	66.06	61.22	0				
V9	176.87	325.32	318.16	320.37	211.03	288.02	330.37	288.53	0			
V10	115.87	**34.13**	126.24	244.74	117.39	73.32	45.38	61.31	317.12	0		
V11	94.18	126.68	197.24	152.7	76.34	53.85	91.61	111.28	134.94	84.93	0	
V12	127.19	274.78	444.33	244.55	146.21	256.41	265.17	146.33	295.81	268.66	257	0

Bold figures indicate the lowest D^2 total

V1 : Sulabha (K-10-2)
V2 : Dharasree (H-3-17)
V3 : H-1593
V4 : Mdk-2 (NDR-2-1)
V5 : Damodar (H-1600)
V6 : Mdk-1 (BLA-39-4)
V7 : AKM-1 (BLA-139-1)
V8 : K-22-1
V9 : Priyanka (H-1591)
V10: Kanaka (H-1598)
V11: Dhana (H-1608)
V12: P-3-2

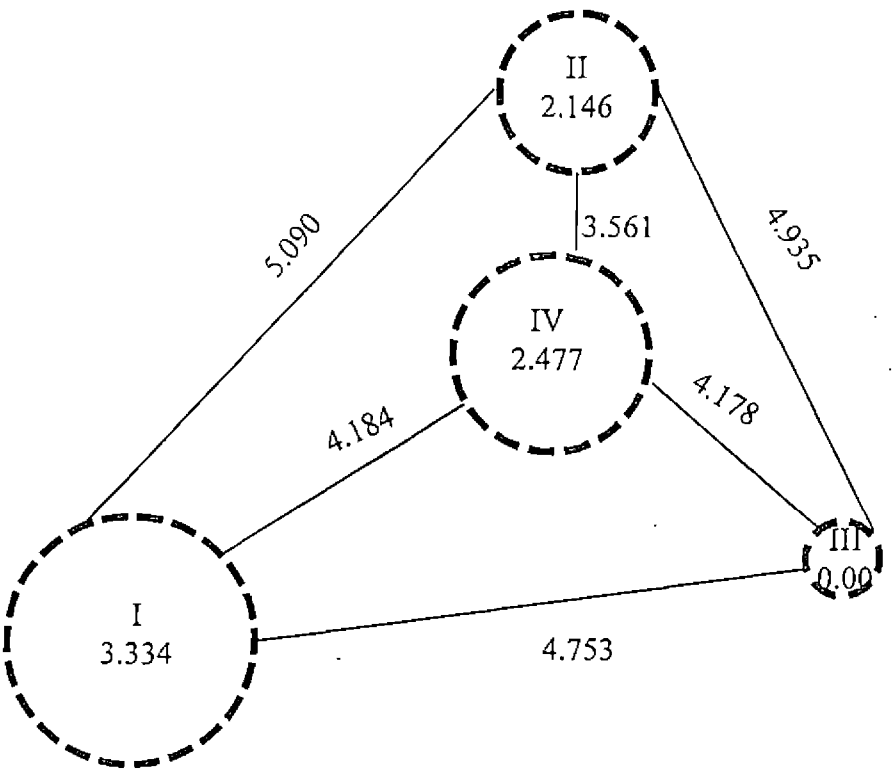


Fig. 4. Cluster diagram of 12 cashew genotypes

—— Inter cluster distance

- - - Intra cluster distance

Cluster I - Sulabha (K-10-2), Priyanka (H-1591) & P-3-2
Cluster II - Mdk-1 (BLA-39-4), AKM-1 (BLA-139-1) & K-22-1
Cluster III - H-1593
Cluster IV - Dharasree (H-3-17), Mdk-2 (NDR-2-1),
H-1600 (Damodar), Kanaka (H-1598) & Dhana (H-1608)

Table 7. Cluster means for different characters

Sl. No.	Characters	Cluster I	Cluster II	Cluster III	Cluster IV
1.	Tree Height	5.42	5.7	5.46	**6.19**
2.	Tree Girth	54.15	64.14	67.41	**75.68**
3.	Number of Primary Branches	2.75	**3.08**	2.75	2.90
4.	Number of Secondary Branches	8.42	**11.25**	7.25	9.70
5.	Canopy Spread	7.03	6.67	7.82	**8.25**
6.	Leaf Area	**76.47**	54.36	69.15	64.20
7.	Number of panicles m^{-2}	16.92	**24.42**	21.0	20.45
8.	Number of nuts m^{-2}	15.67	22.33	**37.5**	18.85
9.	Number of perfect flowers m^{-2}	**1881.83**	1099.67	1833.75	1045.05
10.	Apple weight	**90.35**	55.62	74.15	67.92
11.	Nut weight	**9.57**	6.33	8.36	7.87
12.	Kernel weight	**2.17**	1.52	1.83	2.05
13.	Shelling percentage	25.75	25.24	27.65	**27.68**
14.	Nut yield	5.47	6.47	**10.0**	6.57

Bold Figures indicate highest means among the clusters

Table 8. Average inter and intracluster distances

Clusters	I	II	III	IV
I	**3.334**			
II	5.090	**2.146**		
III	4.753	4.935	**0.00**	
IV	4.184	3.561	4.178	**2.477**

Bold figures indicate intracluster distances.

The means of characters for the genotypes belonging to different clusters are presented in Table 7. Cluster I showed the highest mean for leaf area (76.47 m^2), number of perfect flowers per m^2 (1881.83), apple weight (90.35 g), nut weight (9.57 g) and kernel weight (2.17 g). Cluster II showed the highest mean for number of primary (3.08) and secondary branches (11.25) and number of panicles/m^2 (24.42). Cluster III showed the highest mean for nut yield (10 kg) and number of nuts/m^2 (37.5). Cluster IV showed the highest mean for tree height (6.19 m), tree girth (75.68 cm), canopy spread (8.25 m) and shelling percentage (27.68%).

The inter and intracluster distances of the three clusters worked out have been presented in Table 8. The average intracluster distances in the three clusters ranged from 0 (Cluster III) to 3.334 (Cluster I) with Cluster II having a value of 2.146 and Cluster IV, 2.477. The intercluster distances show that Cluster I and II were the farthest (5.090) and the Clusters II and IV were the closest (3.561).

4.2 BIOCHEMICAL MARKERS

4.2.1 Storage protein studies

SDS-PAGE for kernel protein indicated the presence of 19 bands depending on the variety. Three major band zones were noticed apart from other minor bands (Fig.5a & 5b; Plates 2a & 2b) at distances 5 cm, 7.3 cm and 9.5 cm approximately with molecular weights 66 KDa, 30 KDa, and 24 KDa. Minor bands were also distributed within the range 66 KDa and 20 KDa. Only a single polypeptide was seen above 66 KDa and below 20 KDa. Out of the 19 bands, 13 were found to be polymorphic. Band number 12 was uniquely found in the genotype K-22-1.The dendrogram constructed using NTSYS PC 2.0 software (Table 9 and Fig.9) showed the pairs H-1600 and Dharasree; P-3-2 and Kanaka to be closer. Based on the similarity indices, AKM-1 and Dhana were found to be close. Priyanka was found to be the most divergent genotype based on these studies.

4.2.2 Isozyme studies

Seperation of the isozymes by the proposed protocol were not satisfactory. Hence, different extraction procedures and gel per cent were tried for standardising the protocol for isozyme analysis.

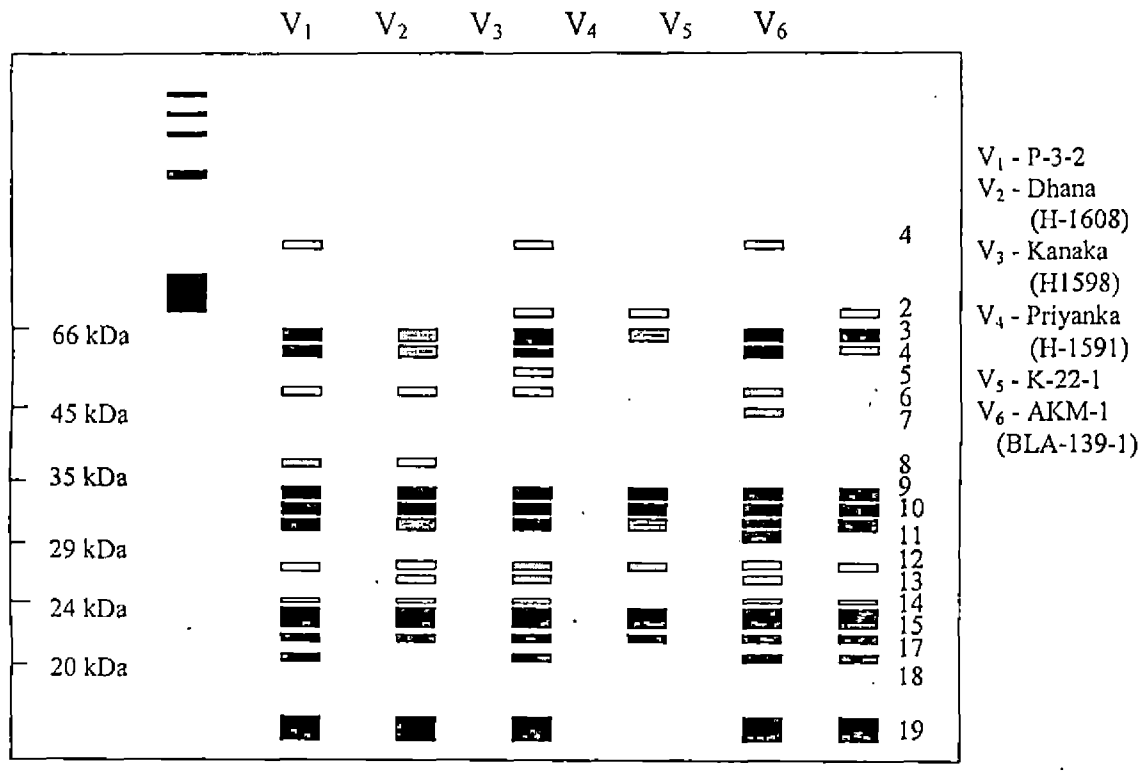


Fig. 5a. Zymogram for SDS-PAGE of kernel protein for six cashew genotypes

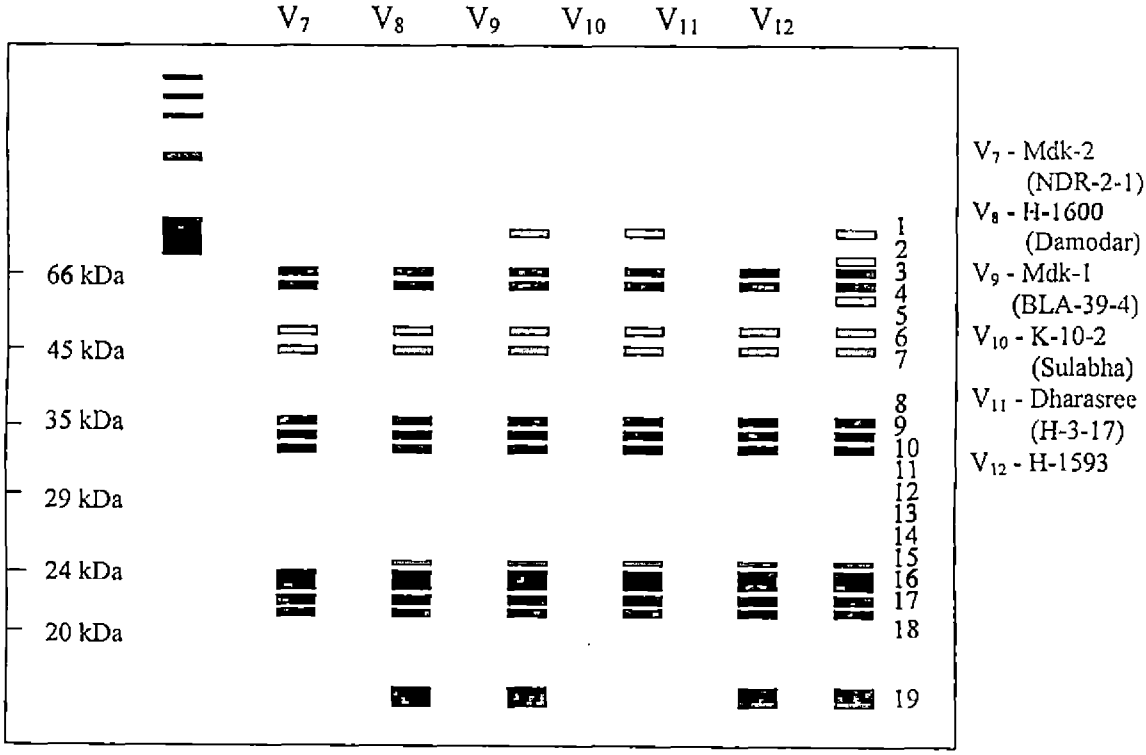


Fig. 5b. Zymogram for SDS-PAGE of kernel protein for six cashew genotypes

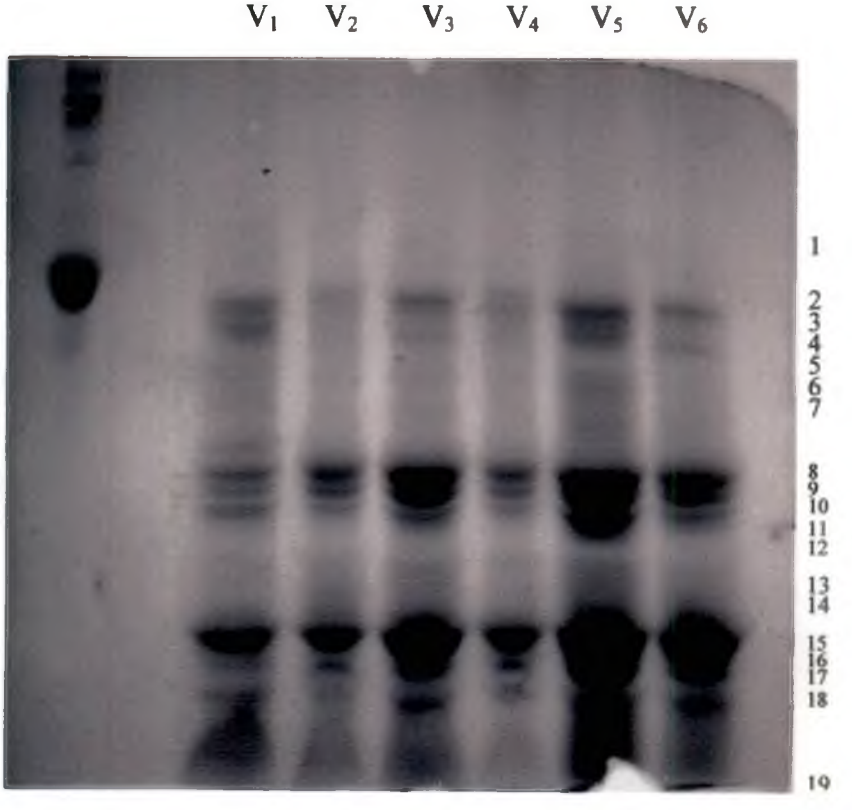


Plate 2a. SDS - PAGE pattern for kernel protein of six cashew genotypes

V_1 - P-3-2 V_2 - Dhana (H-1608) V_3 - Kanaka (H-1598)

V_4 - Priyanka (H-1591) V_5 - K-22-1 V_6 - AKM-1(BLA-139-1)

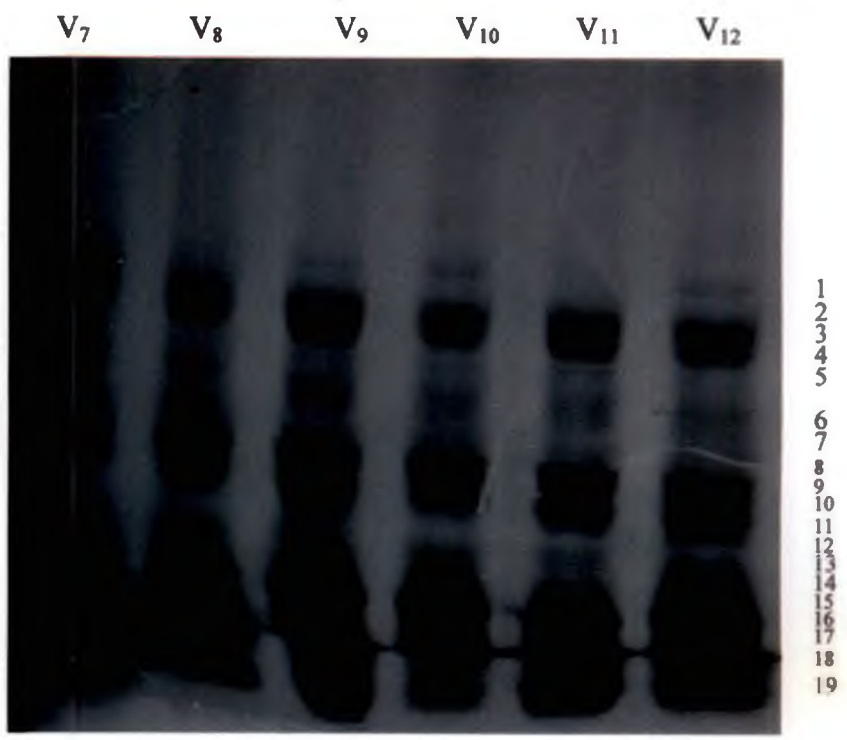


Plate 2b. SDS - PAGE pattern for kernel protein of six cashew genotypes

V_7 - Mdk-2 (NDR-2-1) V_8 - H-1600 (Damodar) V_9 - Mdk-1 (BLA-39-4)

V_{10} - K-10-2 (Sulabha) V_{11} - Dharasree (H-3-17) V_{12} - H-1593

Table 9. Similarity indices for SDS-PAGE of Kernel protein in Cashew

	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	V_{12}
V_1	1.00											
V_2	0.80	1.00										
V_3	0.81	0.75	1.00									
V_4	0.53	0.57	0.50	1.00								
V_5	0.72	0.67	0.78	0.53	1.00							
V_6	0.79	0.71	0.73	0.67	0.65	1.00						
V_7	0.56	0.50	0.53	0.67	0.65	0.57	1.00					
V_8	0.73	0.67	0.69	0.50	0.71	0.77	0.77	1.00				
V_9	0.80	0.63	0.75	0.47	0.76	0.71	0.71	0.92	1.00			
V_{10}	0.73	0.56	0.69	0.50	0.71	0.64	0.77	0.85	0.92	1.00		
V_{11}	0.73	0.67	0.69	0.50	0.71	0.77	0.77	1.00	0.92	0.85	1.00	
V_{12}	0.71	0.56	0.76	0.50	0.78	0.63	0.73	0.80	0.87	0.80	0.80	1.00

V_1 - P-3-2
V_2 - Dhana (H-1608)
V_3 - Kanaka (H-1598)
V_4 - Priyanka (H-1591)
V_5 - K-22-1
V_6 - AKM-1 (BLA-139-1)
V_7 - Mdk-2 (NDR-2-1)
V_8 - H-1600 (Damodar)
V_9 - Mdk-1 (BLA-39-4)
V_{10} - K-10-2 (Sulabha)
V_{11} - Dharasree (H-3-17)
V_{12} - H-1593

Plate 3a. Peroxidase pattern in the leaf samples of cashew

Plate 3b. Peroxidase pattern in the axillary bud samples of cashew

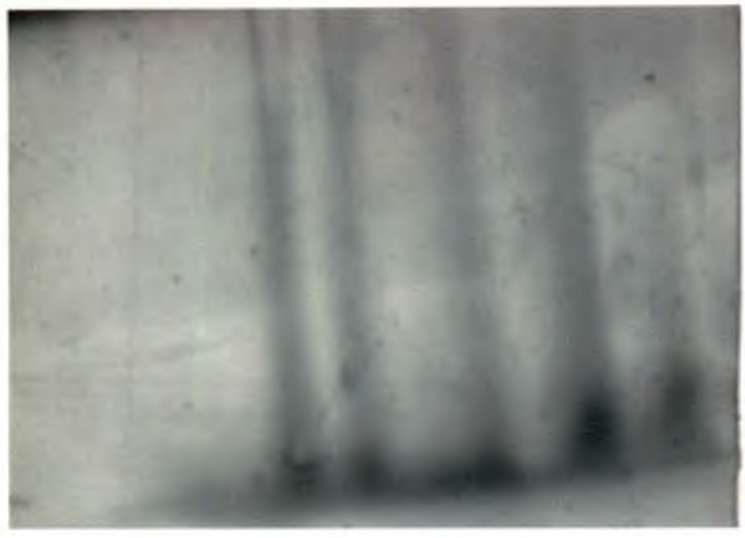


Plate 4a. Polyphenol oxidase pattern in the axillary bud samples of cashew

Plate 4b. Esterase pattern in the axillary bud samples of cashew

I. Different explants were tried i.e., young leaves, flower buds and leaf buds. Of all these, leaf buds were found to give better results.

II. As suggested by Wendel (1989), the extraction buffer for plants with high phenolic contents were tried with certain modifications. Use of Tris-HCl buffer with sodium metabisulphite, sucrose, insoluble PVP, β-mercaptoethanol, ascorbic acid and 1 per cent cysteine-HCl in suitable concentrations (Wendel, 1989) were found suitable for extraction. Staining procedure using sodium acetate buffer was found suitable for peroxidase. On staining the gel, streaks were seen wherein degradation of protein was noticed in case of leaf samples (Plates 3a). Axillary buds extract showed a tendency for banding after travelling for some distance on the gel (Plates 3b, 4a & 4b). No improvement was observed beyond this and the procedure for isozyme analysis in cashew is yet to be standardised.

4.2.3 Phenols and tannins

The mean values of leaf phenols and tannins in the different genotypes are given in Table 10 (Fig 6.).

The genotype, K-10-2 (Sulabha) was found to have the highest total leaf phenolic content during the off-season. The phenolic content of the genotypes Dharasree, Mdk-2, Priyanka (H-1591), Kanaka and H-1600 were found to be statistically on par with K-10-2. The least phenolic content was found to be in the genotype H-1593 (2.048 mg per g). The other genotypes Mdk-1, K-22-1, Dhana, AKM-1 and P-3-2 were found to be on par with H-1593.

In case of tannins estimation, the genotypes: H-1593, Priyanka (H-1591), Kanaka, Mdk-1, Dhana, K-22-1 and P-3-2 were on par with the genotype Dharasree which is having the highest tannin content. The remaining genotypes: Sulabha (K-10-2), Mdk-2, H-1600 and AKM-1 were found to have low tannin content. The genotype H-1600 was found to have the lowest tannin content (6.187 mg per g).

Table 10. Mean values of leaf phenols and tannins in cashew genotypes

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	CD	GM	CV
Phenols	5.608	4.781	2.048	3.857	4.238	2.485	4.006	2.748	4.524	4.066	2.657	2.908	2.12	3.660	3.43
Tannins	8.410	11.329	9.960	8.355	6.187	10.888	7.610	9.519	8.973	9.605	10.843	9.960	2.505	9.303	1.59

V1: Sulabha (K-10-2)
V2: Dharasree (H-3-17)
V3: H-1593
V4: Madakkathara-2 (NDR-2-1)
V5: Damodar (H-1600)
V6: Madakkathara-1 (BLA-39-4)
V7: Anakkayam-1 (BLA-139-1)
V8: K-22-1
V9: Priyanka (H-1591)
V10: Kanaka (H-1598)
V11: Dhana (H-1608)
V12: P-3-2
CD: Critical difference
GM: Grand mean
CV: Coefficient of variation

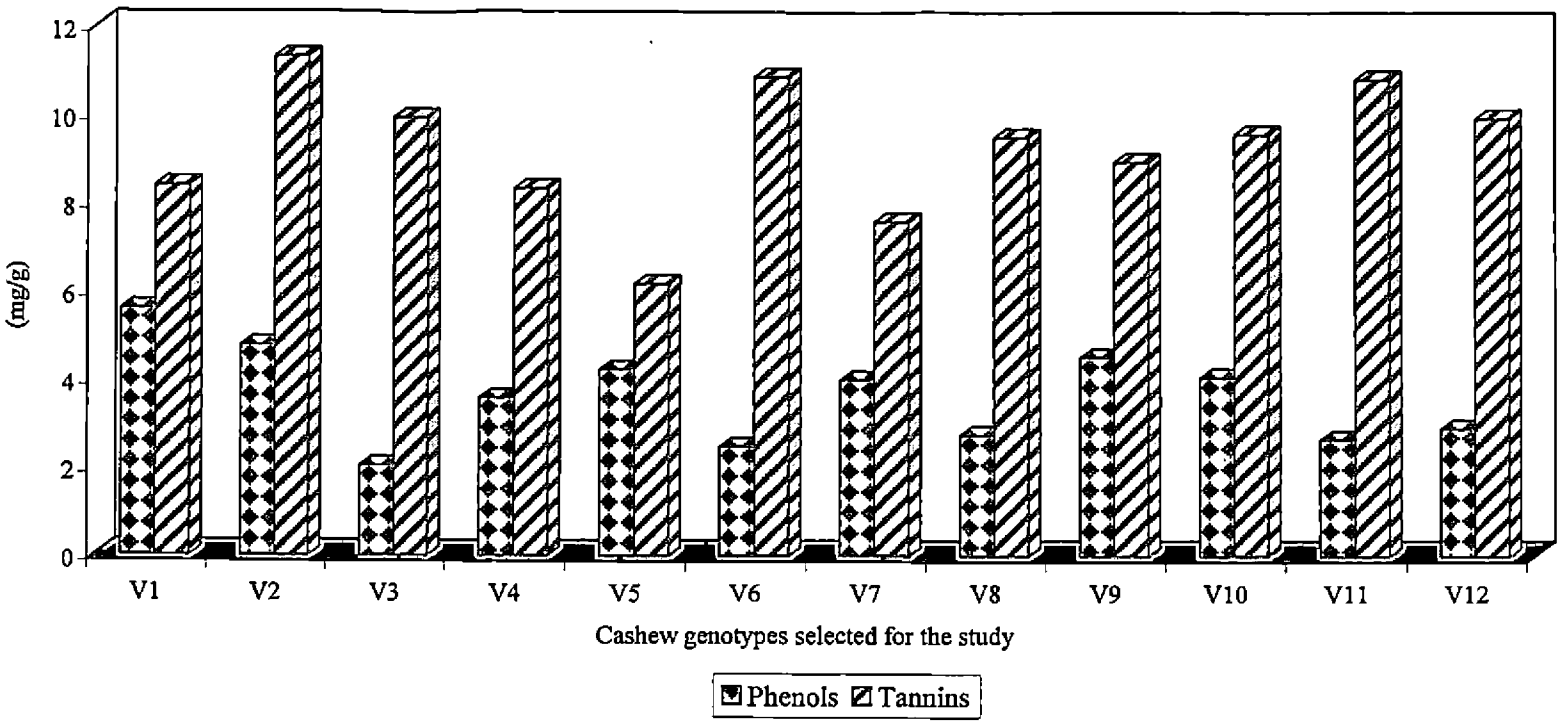


Fig. 6. Variation in leaf phenols and tannins among the cashew genotypes

V1 - Sulabha (K-10-2) V3 - H-1593 V5 - Damodar (H-1600) V7 - AKM-1 (BLA-139-1) V9 - Priyanka (H-1591) V11 - Dhana (H-1608)
V2 - Dharasree (H-3-17) V4 - Mdk-2 (NDR-2-1) V6 - Mdk-1 (BLA-39-4) V8 - K-22-1 V10 - Kanaka (H-1598) V12 - P-3-2

4.3 RAPD ANALYSIS

Ten random primers from Operon series: OPP and OPF series were tried for producing amplification products in cashew varieties. Out of these, four primers which gave polymorphism were selected after repeating three times. Primer number, its sequence and the number of amplification products produced are given in Table 11. A total number of 44 amplified products were produced of which 30 (68.18%) were polymorphic products and 14 were monomorphic products. Amplification products are numbered serially starting with the first product of OPF-3. The percentage polymorphism computed for each primer ranged from 77.77 per cent to 50 per cent. Primer OPF-3 showed the highest per cent of polymorphism. Least polymorphism was shown by OPP-15.

OPF-3

The pattern of DNA amplification of the 12 varieties is shown in Plate 5a. Altogether 9 amplified products were found out of which 7 were polymorphic. Bands 3 and 9 were found to be monomorphic. The first band was present only in varieties H-1600 and P-3-2; the second was present in H-1593, H-1600 and P-3-2. The fourth band was absent in Priyanka (H-1591) and K-22-1. The fifth band was absent in Priyanka (H-1591). The sixth band was present in varieties Dhana, Mdk-1, Mdk-2 and P-3-2. Band 7 was absent in Dhana, H-1600 and P-3-2. Band 8 was present in varieties AKM-1, Dhana, Mdk-1, Mdk-2, H-1593 and P-3-2.

OPP-5

Amplification pattern of this primer for all the twelve varieties is shown in Plate 5b. OPP-5 primer produced 13 amplification products out of which 10 were found to be polymorphic. Bands 18, 20 and 21 were monomorphic. Mdk-2 was the only variety showing band 22. Band 10 was present in varieties Dhana, H-1600 and P-3-2. Band 11 was present in Kanaka and Dharasree. Band 12 was present in varieties Mdk-2, H-1593, H-1600 and P-3-2. Band no. 13 was present in AKM-1 and Dharasree. Band 14 was present in Kanaka, Dhana, Mdk-1, Mdk-2 and Sulabha. Band 15 was present in AKM-1, Dhana, Dharasree and P-3-2. Band 16 was present Mdk-1, Mdk-2, K-22-1, H-1593, P-3-2 and Sulabha. Band 17 was present in AKM-1, and H-1593. Band 19 was absent in K-22-1.

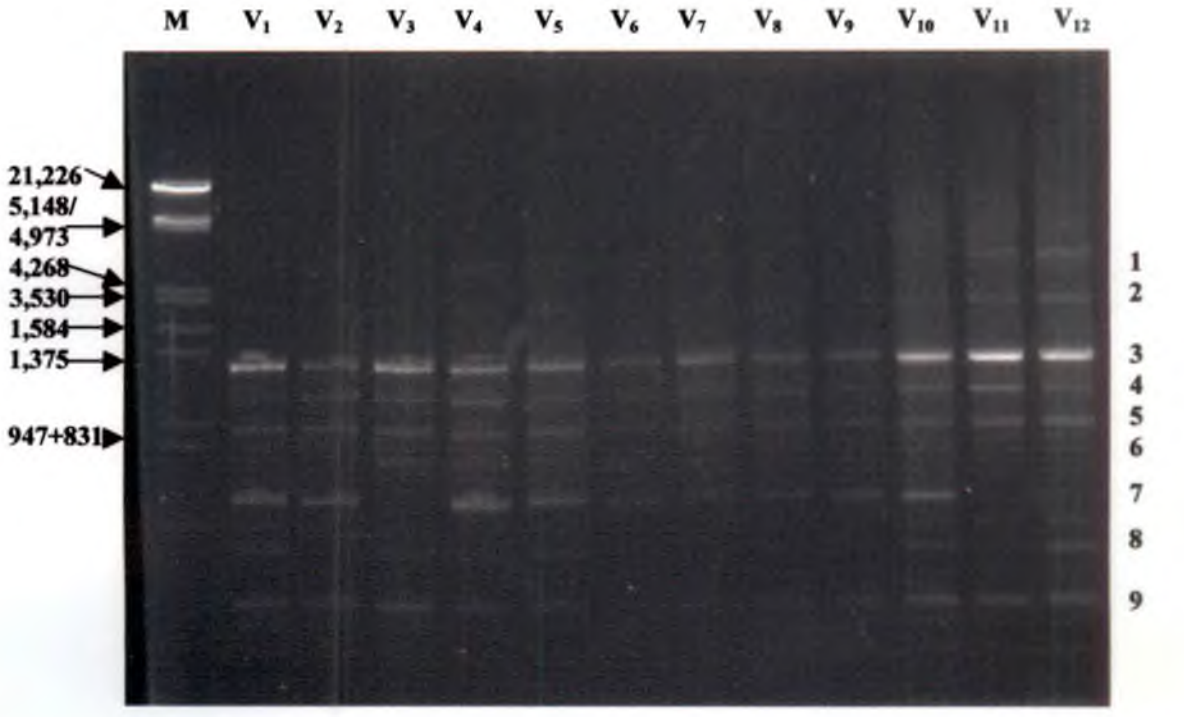


Plate 5a. RAPD profile for the 12 cashew genotypes using primer OPF-3

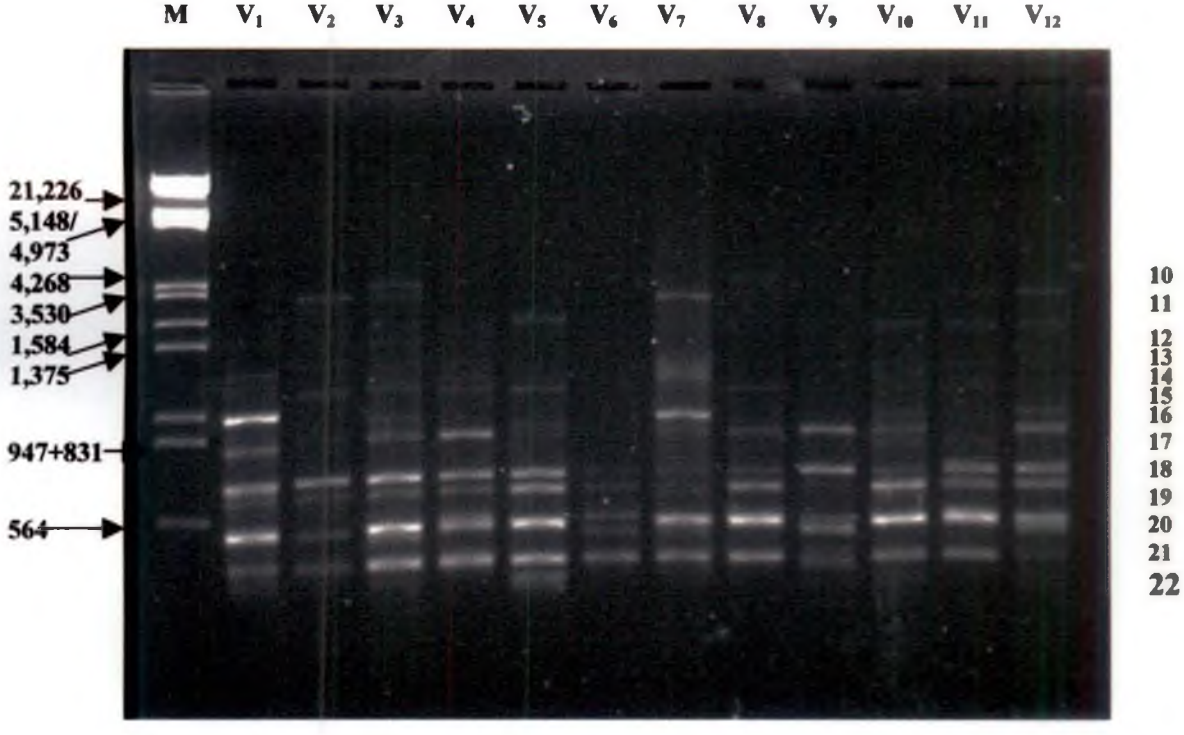


Plate 5b. RAPD profile for the 12 cashew genotypes using primer OPP-5

V1 - AKM-1 (BLA-139-1) V2 - Kanaka (H-1598) V3 - Dhana (H-1608) V4 - Mdk-1 (BLA-39-4)
V5 - Mdk-2 (NDR-2-1) V6 - Priyanka (H-1591) V7 - Dharasree (H-3-17) V8 - Sulabha (K-10-2)
V9 - K-22-1 V10 - H-1593 V11 - Damodar (H-1600) V12 - P-3-2

Table 11. Base sequence of primers, number of amplicons and percent of polymorphism in cashew genomic DNA

Primer Name	Sequence	Number of amplicons	Number of polymorphic amplicons	Number of monomorphic amplicons	Percentage of polymorphism (%)
OPF-3	CCTGATCACC	9	7	2	77.77
OPP-5	CCCCGGTAAC	13	10	3	76.92
OPP-10	TCCCGCCTAC	12	8	4	66.66
OPP-15	GGAAGCCAAC	10	5	5	50.00
Total number of products		44	30	14	68.18

OPP-10

Pattern of amplification for DNA of 12 varieties is shown in Plate 6a. A total of 12 amplified products were produced out of which 8 were found to be polymorphic. Band 29, 30, 32 and 33 were monomorphic. Band 25 was present only in Mdk-1. Band 23 was absent in Sulabha, K-22-1, H-1593, H-1600 and P-3-2. Band 24 was present in Kanaka and Mdk-1. Band 26 was present in AKM-1, Dhana and Mdk-1. Band 27 was present in AKM-1, Kanaka and Sulabha. Band 28 was present in AKM-1, Dhana, Mdk-1, Mdk-2, H-1600 and P-3-2. Band 31 was absent in Mdk-1 and K-22-1. Band 34 was absent in H-1593.

OPP-15

Amplification pattern for this primer is shown in Plate 6b. A total of 10 amplified products were produced out of which 5 were polymorphic. Bands 39, 41, 42, 43 and 44 were monomorphic. Band 35 and 36 were absent in Mdk-2, Priyanka (H-1591), Sulabha and K-22-1. Bands 37 and 38 were absent in K-22-1. Band 40 was absent in AKM-1, Dhana, K-22-1, Sulabha and H-1600.

Genetic similarity computed from the RAPD profiles (Table 12) ranged from a minimum of 0.48 to a maximum of 0.85 for the different genotypes. Highest genetic similarity was observed for H-1600 and P-3-2.

The dendrogram constructed from the pooled data (Fig.10) had five clusters. AKM-1 and Dhana were found to be genotypically closer. Similarly, Mdk-1 and Mdk-2 were found to be genotypically closer. Kanaka and Dharasree were the closest next to H-1600 and P-3-2. H-1593 was found to be closer to H-1600 and P-3-2. AKM-1 and Mdk-1 were also placed close to each other. Similarity indices show H-1591 to be close to H-1593. Similarly, Priyanka and Sulabha were found to be closer to Kanaka and Dharasree. K-22-1 was the only genotype found to be genetically diverse.

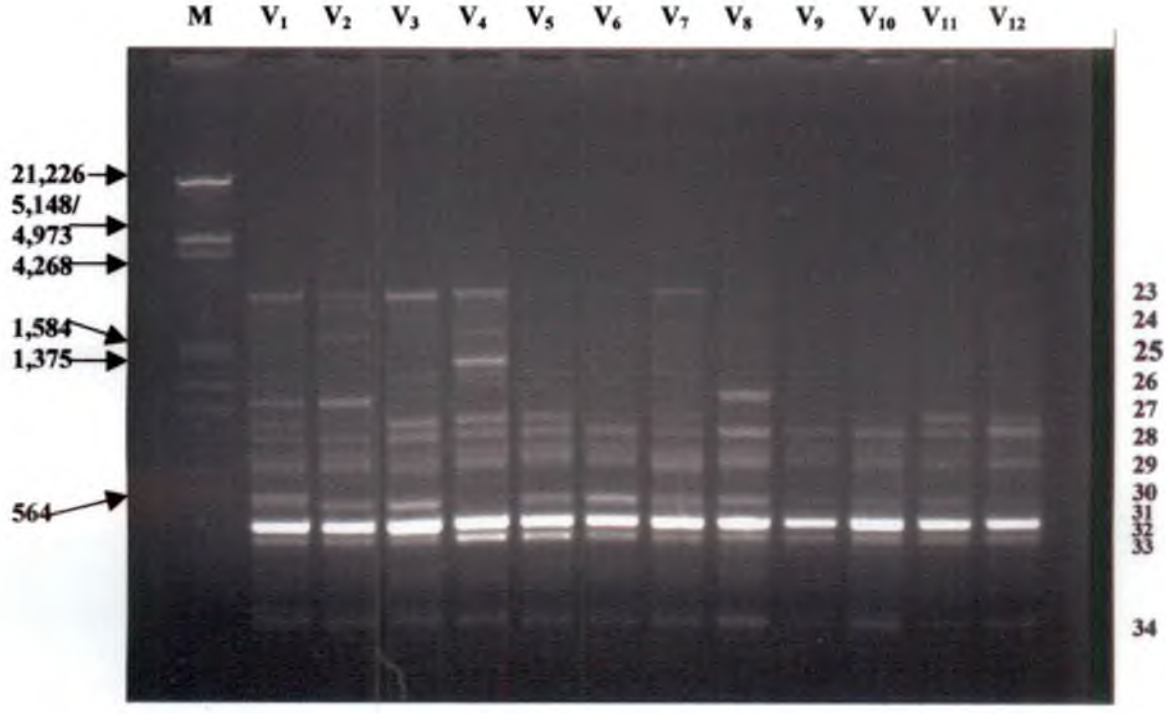


Plate 6a. RAPD profile for the 12 cashew genotypes using primer OPP-10

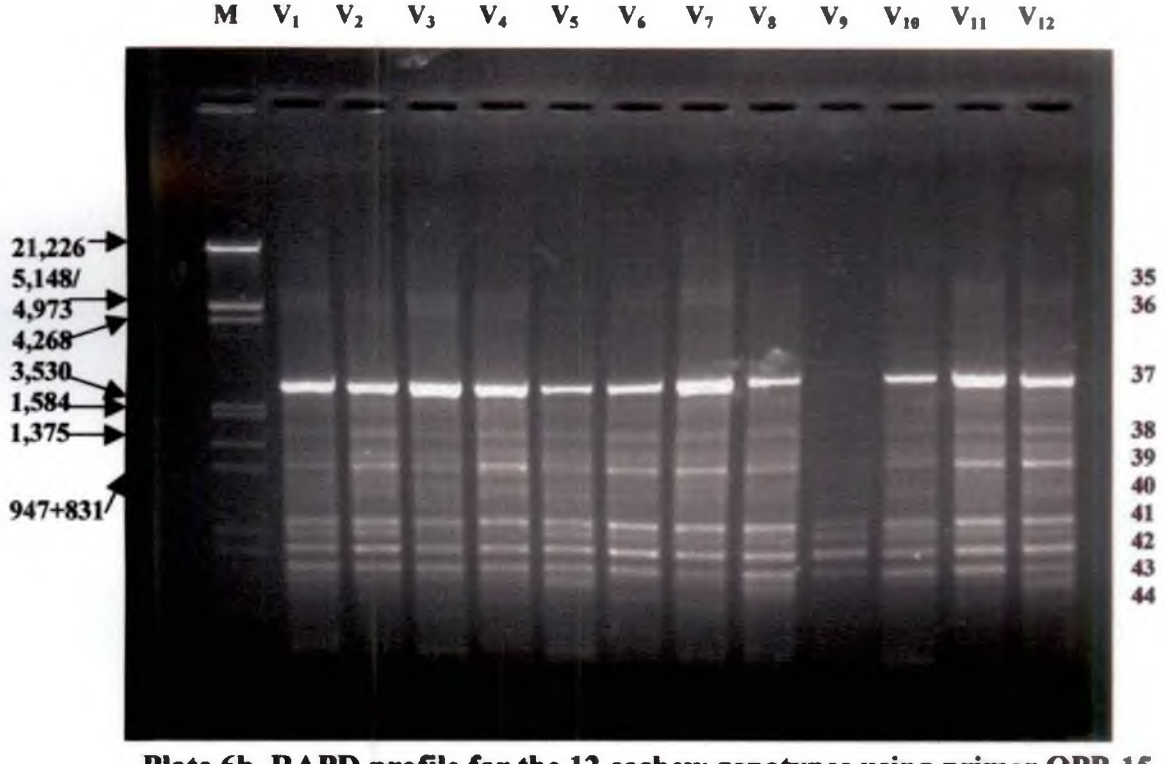


Plate 6b. RAPD profile for the 12 cashew genotypes using primer OPP-15

V1 - AKM-1 (BLA-139-1) V2 - Kanaka (H-1598) V3 - Dhana (H-1608) V4 - Mdk-1 (BLA-39-4)
V5 - Mdk-2 (NDR-2-1) V6 - Priyanka (H-1591) V7 - Dharasree (H-3-17) V8 - Sulabha (K-10-2)
V9 - K-22-1 V10 - H-1593 V11 - Damodar (H-1600) V12 - P-3-2

Table 12. Similarity indices for the DNA amplicons in cashew genotypes

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
V1	1.00											
V2	0.72	1.00										
V3	0.80	0.69	1.00									
V4	0.71	0.75	0.78	1.00								
V5	0.66	0.69	0.72	0.78	1.00							
V6	0.64	0.73	0.61	0.62	0.71	1.00						
V7	0.79	0.84	0.71	0.68	0.67	0.76	1.00					
V8	0.68	0.77	0.65	0.66	0.75	0.74	0.69	1.00				
V9	0.52	0.55	**0.48**	0.55	0.58	0.67	0.57	0.72	1.00			
V10	0.69	0.69	0.62	0.68	0.71	0.65	0.71	0.69	0.57	1.00		
V11	0.67	0.66	0.79	0.61	0.63	0.61	0.68	0.66	0.53	0.73	1.00	
V12	0.67	0.62	0.78	0.69	0.73	0.57	0.68	0.61	0.50	0.77	**0.85**	1.00

Bold figures indicate highest and lowest similarity indices

V1: Anakkayam -1 (BLA-139-1)
V2: Kanaka (H-1598)
V3: Dhana (H-1608)
V4: Mdk-1 (BLA-39-4)
V5: Mdk-2 (NDR-2-1)
V6: Priyanka (H-1591)
V7: Dharasree (H-3-17)
V8: Sulabha (K-10-2)
V9: K-22-1
V10: H-1593
V11: Damodar (H-1600)
V12: P-3-2

Discussion

5. DISCUSSION

Conventional breeding methods like selection and hybridization, usually practiced in cashew, relies on the wide variability existing in the germplasm. The most genetically diverse plants are selected for hybridization experiments. Superior trees are being identified based on morphological descriptors alone. Selection of suitable genotypes from a highly heterogenous mass population based on morphological characters requires an indepth study as these characters are highly influenced by environment. Many scientists have assessed the genetic diversity in cashew phenotypically, but works at biochemical and molecular levels are few. An attempt was made in the present study to characterize 12 genotypes based on morphological, biochemical and molecular markers and to analyse the genetic divergence based on these markers. The results obtained are discussed elaborately in this chapter.

5.1 MORPHOLOGICAL MARKERS

Cashew is an evergreen tree having a resinous bark, leathery leaves and polygamous flowers borne on panicles. Variability exists among the cashew genotypes for many characters. To get a general awareness of the variability existing among the genotypes and to have a better understanding of cashew varieties, morphological data on 14 variables in 12 genotypes were analysed statistically.

5.1.1 Distribution of the characters observed

All the variables studied, except the variable number of primary branches, can be used to distinguish the genotypes from each other. The high F values for the characters: number of perfect flowers m^{-2}, nut weight, number of nuts m^{-2}, apple weight and kernel weight provide a clear separation of the genotypes. The same was reported by many workers. The number of perfect flowers per panicle were found to vary significantly among cashew types by Rao and Hasan (1957), Das and Sahoo (1987), Sapkal *et al.* (1994), Sheshagiri (1996) and Reddy *et al.* (2001). Similarly, Sena *et al.* (1995) and Lenka *et al.* (1998) had found significant variation in apple

weight among the various apple characters studied. Nut weight and kernel weight were also found to show significant variation (Salam *et al.*, 1991; George *et al.*, 1991). Number of nuts per panicle was found to vary significantly by Pushpalatha (2000) and Lenka *et al.* (2001).

High GCV, PCV, heritability and genetic advance were found for the number of perfect flowers per m^2 indicating the reliability of this trait for selection for high nut yield. Lenka *et al.* (1999a) had observed similar results for number of perfect flowers per panicle. In this study, GCV and PCV values were high and genetic advance was moderate for number of nuts per m^2 and nut yield. Number of nuts per m^2 showed very high heritability. Sankarnarayanan and Ahmad Shah (1999) also found high GCV estimate for nut yield, number of perfect flowers per panicle and fruit set per panicle. Lenka *et al.* (2001) found high GCV, heritability and genetic advance for nut yield per plant, number of male flowers per panicle, number of perfect flowers per panicle and number of nuts per panicle.

5.1.2 Correlation and path studies

Yield in any crop is a complex character determined by a number of genetic factors and environmental conditions occurring at various growth stages of the plant. In cashew, it becomes more complicated due to high heterogeneity and heterzygosity being a cross-pollinated polyploid showing polygenic inheritance for morphological traits (Rao *et al.*, 1998). Knowledge of the relationship of yield with other characters becomes essential for effective screening of genotypes for selection. Path coefficient analysis developed by Wright (1923) furnishes a means for finding out the direct and indirect effects of individual components on yield. Thus, it supplements the studies revealing correlations between the characters so that the breeder may be able to select superior trees based of few important characters.

In this study, the results of the correlations between yield and 13 other characters showed that nut yield was found to be significantly correlated with tree height, tree girth, number of primary branches, number of nuts per m^2 and apple

weight. Among the vegetative traits, tree height and tree girth showed significant positive correlation with yield. The same was reported by Nayar *et al.* (1981), Manoj *et al.* (1994), Reddy *et al.* (1996) and Naik *et al.* (1997). Both tree height and number of primary branches had significant positive direct effect on yield. But, number of primary branches showed significant negative correlation with yield. This may be due to its significant negative indirect effect through tree girth, number of secondary branches, nut weight and kernel weight. In this study, canopy spread showed a weak positive correlation with yield but significant positive correlation with yield was reported by Nayar *et al.* (1981) and Manoj *et al.* (1994).

Number of nuts per m^2 was found to be most significantly and positively correlated with nut yield and its direct effect was significant and positive. Similar results were reported for number of nuts/panicle on nut yield by Anitha *et al.* (1991), Manoj *et al.* (1994), Reddy *et al.* (1996), Swarnapiria *et al.* (1999), Pushpalatha (2000) and Samal *et al.* (2001). Regression studies by Anitha *et al.* (1991) showed that only number of nuts per panicle that reached to maturity showed significant impact on nut yield per panicle. Manoj *et al.* (1994) and Samal *et al.* (2001) have also reported significant positive and direct effect of number of nuts per panicle on yield.

Number of perfect flowers per m^2 showed a significant negative direct effect and a weak negative correlation with yield. Samal *et al.* (2001) found that perfect flowers and number of flowering panicles per m^2 showed non-significant correlation with yield. Anitha *et al.* (1991) found that even though significant correlation existed between nut yield per panicle and number of perfect flowers per panicle, percentage of fruit-set, they did not have significant influence on yield mainly due to severe and premature fruit drop. Contrarily, Kumar and Udupa (1996) observed that number of perfect flowers per panicle was positively correlated with yield and that along with four other characters, significantly influenced yield. Lenka *et al.* (2001) had found that number of flowers per panicle, nut weight and number of nuts per panicle were the best contributors to nut yield and further, number of staminate flowers and perfect flowers showed high positive association with nut yield both at genotypic and phenotypic levels. This may be due to the significant positive indirect effects of

number of perfect fowers m^{-2} on yield through nut weight and tree girth as reported in this study.

Apple weight showed significant negative correlation with yield. Reddy *et al.* (1996) had also observed significant negative correlation of nut yield with apple weight both at phenotypic and genotypic levels.

Significant positive direct effects and weak positive correlations with yield was reported for nut weight and kernel weight. Anitha *et al.* (1991) found significant negative association of yield with mean nut weight and nut length indicating that an increase in the two characters lead to decrease in nut yield.

Among the intercorrelations of the different characters, number of nuts m^{-2} and apple weight had significant negative correlation. Samal *et al.* (2001) also had reported inverse correlation of number of nuts panicle^{-1} with apple weight. Therefore, both these parameters may not be considered simultaneously to improve yield in cashew. This is in corroboration with the findings of Manoj *et al.* (1994) and Sena *et al.* (1994).

Apple weight and nut weight had significant positive correlation with each other. This was also reported by Reddy *et al.* (1996) and Lenka *et al.* (1999).

The high residual effect revealed in the path studies indicate the influence of other factors like environment on yield. Variability due to other yield contributing characters not included in the study may also account for high residual effect. Rao (2002) had also reported significant influence of climate on cashew.

The improvement of these characters can be made simultaneously.

5.1.3 Genetic Divergence based on morphological characters

The study of genetic divergence based on the morphological characters showed the genotype H-1593 to be the most unique or divergent from the rest of the genotypes. The genotypes were grouped into 4 clusters. Members of the Cluster I (Sulabha, Priyanka and P-3-2) and the Cluster II (Mdk-1, Akm-1 and K-22-1), the two

farthest clusters can be used for hybridisation programmes in all possible combinations to get maximum heterosis. Cluster analysis showed Dharasree, H-1600 and Kanaka were more closer. Rao (1999) also had grouped Mdk-1 and AKM-1, the small nut varieties in one cluster and Kanaka and Dhana into another cluster based on flowering and fruiting habit. The present study shows that Cluster I with bold nut genotypes and Cluster II with small and medium nut weight were the farthest emphasising the role of nut characters in assessing the genetic divergence.

5.2 BIOCHEMICAL STUDIES

Based on the phenolic content estimated in the mature leaves, the genotypes could be grouped into those with high phenolic content and those with low phenolic content. There is no report of relationship between phenols and tannins in leaves and the consumable parts (Cashew apple and kernel). However, high phenol content in the apple had been reported which hinders the raw consumption of cashew. The results of leaf phenol and tannin estimations points out that these can be exploited for variability studies. Daggade (1999) reported the total phenol content in pepper genotypes and found that the genotype Kalluvally had higher phenolic content (3.802 mg/g) compared to Panniyur I (2.483 mg/g). Giridharan (1993) observed that the total phenol content varied from 2.9 mg/g to 5.03 mg/g among the grape cultivars.

The seed storage protein electrophoresis showed three major zones of protein seperation. Genotypes did not show any variation with regard to major polypeptides. However, it showed variation with respect to minor polypeptides. The same was reported by Samanta *et al.* (1993). Eventhough certain bands (band numbers 1, 2, 5, 6, 7, 8, 12, 13, 14, 15, 17, 18 and 19) could distinguish genotypes, only one unique band (band number 12) was noted in the study. Samanta *et al.* (1993) have reported that eventhough it is possible to identify cashew varieties through SDS-PAGE study of seed proteins, further resolutions by combining IEF or Urea-PAGE with SDS-PAGE to give two-dimensional separation (Shewry *et al.*, 1977, 1978a) can reveal polymorphisms in the minor polypeptides better. Most of the polypeptides were within the range 66 KDa and 20 KDa. Samanta *et al.* (1993) also reported in their study that maximum number of bands were obtained within 25KDa and 76 KDa.

Isozyme studies revealed the specificity of enzymes in cashew. Electrophoretic separation and activity studies confirmed the above statement. Streak formation and steady reaction rate after the initial reaction were indicating the same. Detailed studies on the standardisation of analytical methods may give a true picture of the nature and properties of these enzymes as a tool for characterisation as well as pest and disease infestation and effect of temperature on cashew crop. This may be a landmark for developing eco-friendly control measures. Enzyme studies show the importance of the stage in which it is to be performed. Due to this reason and other factors, the attempts for standardizing the protocol for isoenzyme analysis were not successful. In case of axillary buds, phenolic interference was not noticed and still, no clear banding pattern was seen. The possible reasons maybe degradation of the enzyme before loading. Dominance of some proteolytic inhibitors present in the crop may have resulted in the degradation and thus, be one possibility for no resolution. Enzymatic activity was studied for the enzymes peroxidase and polyphenol oxidase using the procedure given by Mallik and Singh (1980) (Table 13a and 13b; Fig.11 and 12). The activity was seen to be lost within seconds after the reaction had commenced indicating the fast reaction at the initial stage.

Based on the several trials for standardisation of the protocol for isozyme analysis, the following suggestions are made:

To modify the extraction procedure considering the high phenol content.

Very young leaf buds are suggested as explants.

The possibility of presence of proteases or any other secondary metabolite which interfered with the enzyme activity has to be explored.

5.3 MOLECULAR STUDIES

Molecular markers have been proved to be a fundamental and reliable tool for fingerprinting varieties, establishing the fidelity of progenies etc. The advent of automated PCR technology made a new set of markers available to scientists interested in comparing organisms at molecular level. Williams *et al.* (1990) were the

Table 13a. Peroxidase activity at different sample concentrations in two cashew genotypes

Time in seconds after the start of the reaction	P-3-2 60µl	P-3-2 0.1ml	Akm-1 60µl	Akm-1 0.1ml
30	0.033	0.09	0.104	0.154
60	0.031	0.088	0.101	0.152
90	0.031	0.087	0.101	0.15
120	0.029	0.087	0.099	0.148
150	0.029	0.086	0.099	0.148
180	0.028	0.084	0.098	0.148
210	0.028	0.084	0.098	0.148

Table 13b. Polyphenol oxidase activity in six cashew genotypes

Time in seconds after the start of the reaction	Sulabha	Mdk-2	K-22-1	Kanaka	Dhana	P-3-2
30	0.166	0.162	0.143	0.315	0.143	0.173
60	0.146	0.151	0.132	0.305	0.127	0.138
90	0.139	0.146	0.127	0.297	0.119	0.125
120	0.133	0.143	0.124	0.293	0.113	0.117
150	0.130	0.141	0.121	0.295	0.108	0.115
180	0.127	0.140	0.119	0.295	0.105	0.112
210	0.125	0.139	0.117	0.297	0.104	0.110
240	0.121	0.141	0.116	0.295	0.101	0.109
270	0.118	0.136	0.116	0.291	0.100	0.109
300	0.117	0.134	0.115	0.295	0.099	0.108

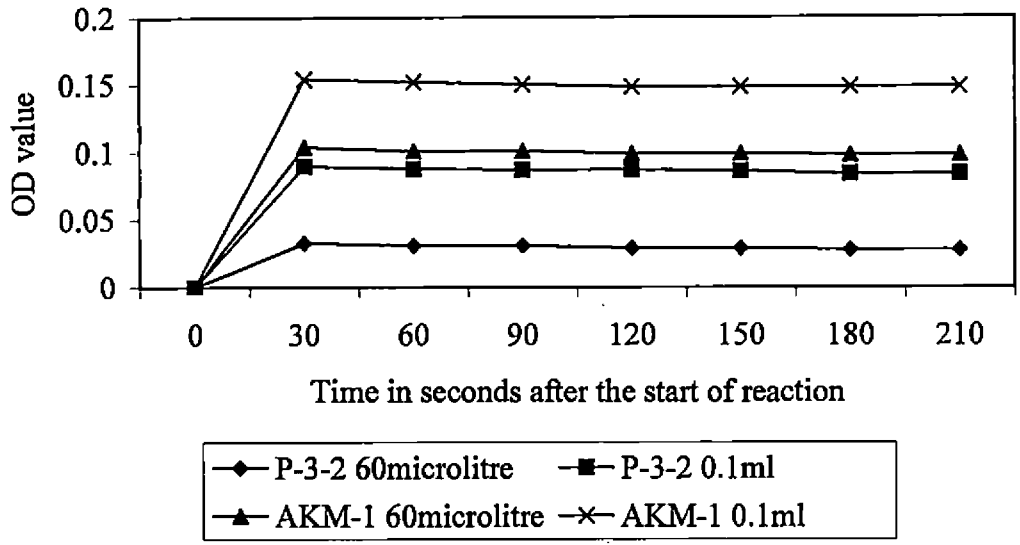


Fig. 7a. Peroxidase activity at different sample concentrations in two cashew genotypes

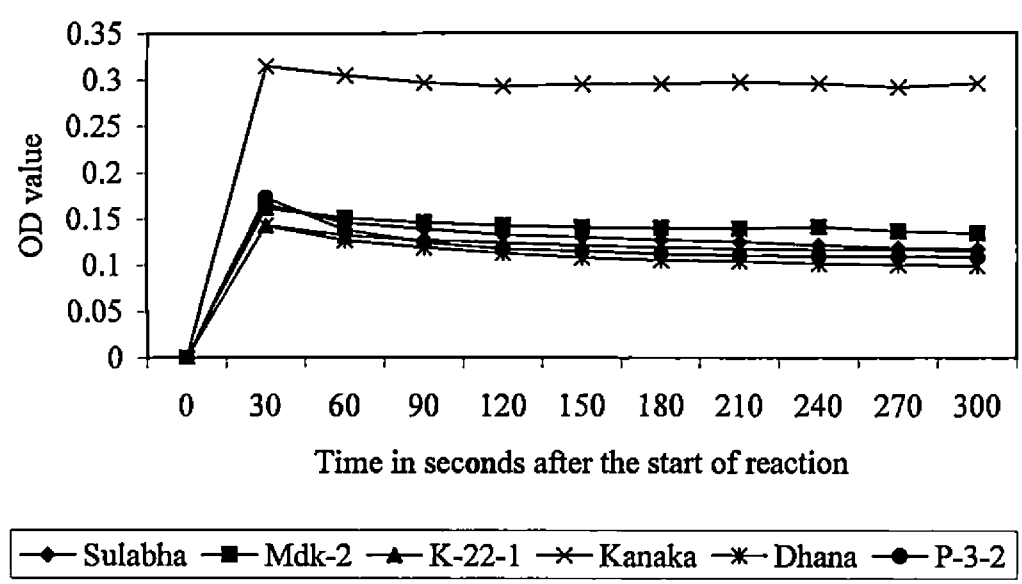


Fig. 7b. Polyphenol oxidase activity in six cashew genotypes

first to use random amplified polymorphic DNA markers obtained by PCR amplification of DNA segments with single arbitrary primers.

The random amplified polymorphic DNA (RAPD) reaction performed on genomic DNA with an arbitrary oligonucleotide results in the amplification of several discrete DNA products. These are usually separated on agarose gel and visualised by ethidium bromide staining. The polymorphism between individuals result from sequence difference in one or both of the primer binding sites and are visible as presence or absence of a particular band. Such polymorphism, in general, behave as dominant genetic markers. The banding pattern differences existing between 2 species or varieties can be used for species or varietal identification. Also, it can be used to study the pattern of introgression in hybrids.

The RAPD amplification generated can be classified into two types: constant (monomorphic) and variable (polymorphic). These differences can be used to examine and establish systematic relationship (Hadrys *et al.*, 1992).

In this study, 4 random primers were used to amplify the genomic DNA of 12 cashew accessions. These primers could produce a total of 44 amplification products of which 30 were polymorphic (68.18%). This shows that RAPD markers can be effectively used for assessing genetic diversity in cashew. Dhanraj *et al.* (2002) had also stressed the use of RAPD markers in estimating diversity and identifying a core collection in cashew. Mneney *et al.* (1998) also have determined genetic diversity within and between populations of cashew using RAPD markers. In this study, primers of OPP series have been found to be more effective in screening the cashew germplasm.

The primers chosen for the study reveal the advantage of GC-rich primers in bringing about amplification. Williams *et al.* (1990) tested a set of primers with GC-content ranging from 0-100 per cent in the amplification of soyabean genomic DNA to find that GC content of 40 per cent or more generated detectable levels of amplification products. Fukoka *et al.* (1992) found that in rice, increasing GC content

in the range between 40 per cent-60 per cent tended to increase the number of amplification products. In this study, all the primers which gave good amplification had GC content of more than 70 per cent. Primer OPP-5 gave maximum number of amplification products which shows that primers gave maximum number of amplification products with an increase in GC content.

Among the different primers studied, OPP-5 could produce maximum number of polymorphic products. This primer could distinguish var. Mdk-2 ,with a specific amplicon 22 with less than 564 bp (Plate 5b). Amplification product number 19 was present in all the varieties except Sulabha. Primer OPP-10 also produced 66.66 per cent polymorphism. This primer could distinguish variety Mdk-1 with amplicon 25 having 1365 bp approximately(Plate 6b).

OPF-3 and OPP-15 could not produce unique products for any of the varieties. However, OPF-3 could distinguish H-1593 with product number 34 which was present in all the varieties except H-1593. OPP-15 could distinguish K-22-1 from the other varieties by the absence of two products 37 and 38.

This study points out that varieties under study are divergent with respect to RAPD markers. Rao (1999), while studying the use of molecular markers in assessing the genetic diversity in cashew cultivars had reported that the hybrids and varieties released from Kerala were much more diverse than those of other regions.

Similarity indices among the varieties show that H-1600 (a hybrid developed at Cashew Research Station, Madakkathara) and P-3-2 (an exotic collection from Panama) were found to be the closest followed by Kanaka and Dharasree. While studying the use of RAPD markers to reveal the genetic divergence within and between the population of cashew, Mneney *et al.* (1998) have reported that accessions from India, Mozambique and Tanzania showed closer relationships with accessions from Brazil.

Dendrogram constructed based on similarity indices grouped Mdk-1 and Mdk-2 together. Dhanraj *et al.* (2002) had also found similar results in a study involving 90 accessions at NRCC, Puttur.

Williams *et al.* (1990) attributed polymorphism between genotypes to nucleotide changes that prevent amplification by introducing a mismatch at one primary site, deletion of a priming site, insertions that render priming sites too distant to support amplification and insertions or deletions that change the size of the amplified product. Therefore, these differences between the cashew genotypes would lead to the polymorphism in the RAPD analysis. Such polymorphism make RAPD well suited for studies on genetic diversity and genetic relationships. Many workers could use RAPDs in estimation of genetic variability in crop plants. Applications include fingerprinting of a genotype, identification of duplicate accessions and analysis of genetic diversity in a collection. This technique would, therefore, be of high value for germplasm characterisation and genetic resource maintenance in cashew. RAPD markers will be helpful to clarify the confusion of experienced cashew breeders in classifying certain cultivars or types.

5.4 MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR MARKERS IN ASSESSING GENETIC DIVERGENCE

On comparing the analysis of genetic divergence based on the three markers (Figs. 8,9 &10), it was seen that even though certain similarities are present between the dendrograms, complete similarity was not seen between any of the markers.

Based on the morphological characters, H-1593 was the most divergent. This genotype also differed significantly from the others based on phenol content. But, this variety showed more than 70 per cent similarity with H-1600 (Damodar) and P-3-2 at the molecular level. H-1593 and H-1600 are hybrids both having AKM-1 (BLA-139-1) as the common female parent.

H-1593 and H-1591 (Priyanka), both having common pedigree, were closer in both morphological and molecular studies.

H-1600 (Damodar) was tied with Dharasree (H-3-17) in biochemical studies which has a Brazilian accession as male parent; and also with P-3-2 in molecular studies. In morphological studies also, H-1600 was placed close to P-3-2. This shows close proximity of Indian accessions with those of South America as has been reported by Mneney (1997, 2001).

Eventhough AKM-1 was the female parent for the hybrids Kanaka (H-1598), Damodar(H-1600), Priyanka (H-1591) and H-1593, it was placed far from these hybrids in all the three studies. AKM-1 and Dhana were placed close together in all the three studies but both were found to be diverse by pedigree. Similarly, Kanaka and Dharasree were tied together both in the morphological and molecular studies though both the genotypes were diverse by pedigree.

AKM-1 and Mdk-1 are Bapatla accessions and both are early flowering varieties. They were closer in both morphological and molecular studies.

Cashew being a highly heterozygous crop, pedigree is not completely answerable to variability.

The morphological and molecular markers were found to have a more similar trend in assessing the genetic divergence rather than the biochemical markers which require more resolution to assess the genetic divergence. However, the molecular studies using RAPD markers by screening with more number of primers can definitely give precise information on the genetic divergence.

Summary

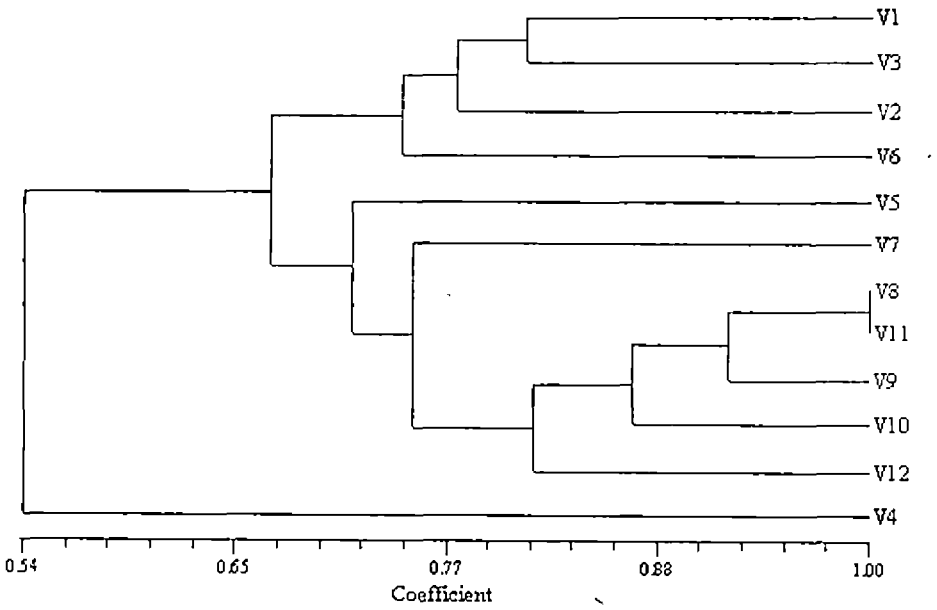
6. SUMMARY

The present study " Morphological, biochemical and molecular markers for the genetic analysis of Cashew (*Anacardium occidentale* L.)" was conducted in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during the year 2000-2002. Morphological observations were recorded at Cashew Research Station, Madakkathara. Molecular studies were done at Centre for Plant Biotechnology & Molecular Biology. College of Horticulture, Vellanikkara and the biochemical studies were done at the Biochemistry Laboratory, College of Horticulture, Vellanikkara and at Cashew Research Station, Madakkathara.

The study intended to characterize 12 cashew genotypes based on morphological, biochemical and molecular markers and to analyse the genetic divergence based on these markers.

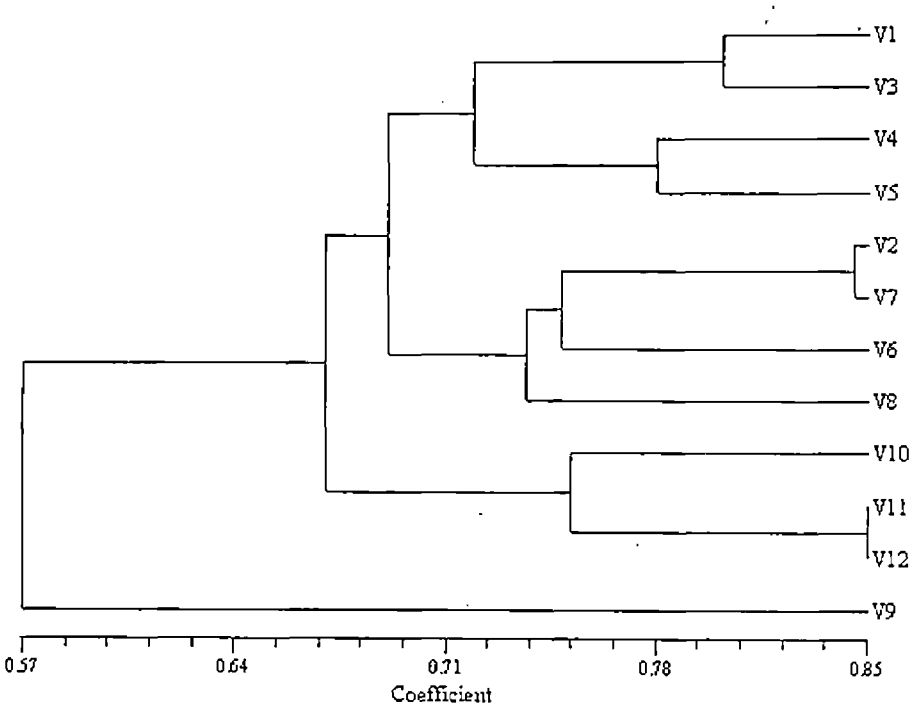
As a basic step, the genetic variability existing was assessed in the different cashew genotypes. Morphological data on the 14 characters for the twelve genotypes were analysed statistically. The analysis revealed that all the genotypes differed significantly for all characters except number of primary branches. The characters number of perfect flowers per m^2, nut weight, number of nuts per m^2, apple weight and kernel weight provide a clear separation of the genotypes. Number of perfect flowers per m^2 showed high heritability, genotypic coefficient of variation, phenotypic coefficient of variation and genetic advance. Similarly, number of nuts per m^2 showed a high heritability, genotypic coefficient of variation, phenotypic coefficient of variation and a moderate genetic advance.

Correlation and path studies revealed that tree height and number of nuts per m^2 had significant positive correlation and direct effect on yield. Number of primary branches showed positive direct effect but negative correlation with yield due to the negative indirect effects of tree girth, number of secondary branches, nut weight and kernel weight. Tree girth showed positive correlation with yield while canopy spread showed a weak positive correlation with the same. Number of perfect flowers per m^2



V1 : P-3-2
V2 : Dhana
V3 : Kanaka
V4 : Priyanka
V5 : K-22-1
V6 : AKM-1
V7 : Mdk-2
V8 : H-1600
V9 : Mdk-1
V10 : Sulabha
V11 : Dharasree
V12 : H-1593

Fig 9. Dendrogram constructed on the basis of the similarity indices of the cashew genotypes for SDS-PAGE of the kernel protein



V1: Anakkayam -1
V2: Kanaka
V3: Dhana
V4: Mdk-1
V5: Mdk-2
V6: Priyanka
V7: Dharasree
V8: Sulabha
V9: K-22-1
V10: H-1593
V11: Damodar
V12: P-3-2

Fig. 10. Dendrogram constructed on basis of similarity indices for DNA amplification products of different cashew genotypes

showed a significant negative direct effect and a weak positive correlation with yield. Apple weight showed significant negative correlation with yield and significant negative direct effect on yield. Significant positive direct effects and a weak positive correlation with yield is reported for nut weight and kernel weight. Among the intercorrelations, number of nuts per m^2 showed significant negative correlation with apple weight while apple weight showed significant positive correlation with nut weight.

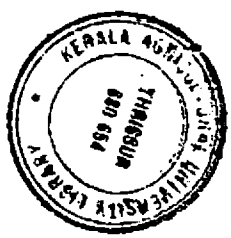
Genetic divergence studied using Mahalanobis D^2 analysis revealed H-1593 to be the most divergent of all the genotypes. Cluster analysis revealed that the members of Cluster I (Sulabha, Priyanka and P-3-2) and Cluster II (Mdk-1, AKM-1 and K-22-1), showing highest genetic distance, were found to be best suited for hybridisation. The genotypes Dharasree, H-1600 (Damodar) and Kanaka were clustered on the basis of morphological characters. Mdk-1 and AKM-1 were, similarly, closer in the present study and also on basis of flowering and fruiting habit.

Biochemical studies on phenol and tannin content of mature leaves could group the twelve genotypes into those with high phenolic content and those with low phenolic content. Seed storage protein studies showed three major zones of protein separation. A unique band (number 12) could distinguish the genotype K-22-1 from all the others. The protocol for isoenzyme analysis could not be standardised due to degradation possibly due to some proteolytic inhibitors or due to loss of enzymatic activity within seconds.

Molecular studies using RAPD produced 44 amplification products of which 30 were polymorphic. GC-rich primers were found to give more number of polymorphic products. Among the four primers, OPP-5 could distinguish Mdk-2 with specific product Band-22 and OPP-10 could distinguish Mdk-1 with specific product Band-25. The varieties under study were found to be divergent with respect to RAPD markers. Similarity indices indicated H-1600 (Damodar) and P-3-2 to be closest among the genotypes. Kanaka and Dharasree; Mdk-1 and Mdk-2 were similarly grouped together.

On comparative study, genotype H-1593 was found to be genetically divergent based on morphological attributes and also had the lowest phenol content. At the molecular level, it showed similarity with H-1600 and P-3-2. H-1593 and H-1591 (Priyanka), both with common pedigree, were closer in molecular and morphological studies. H-1600 (Damodar) was tied to Dharasree in biochemical studies and with P-3-2 in molecular studies. In morphological studies also, it was placed close to P-3-2 indicating the proximity of Indian accessions with those of South America. Kanaka and Dharasree were tied together both in morphological and molecular studies but both were diverse by pedigree. Eventhough AKM-1 was the female parent for the hybrids Kanaka (H-1598), Damodar (H-1600), Priyanka (H-1591) and H-1593, it was placed far from these hybrids in all the three studies. AKM-1 and Dhana were placed close together in the three studies both of which were diverse by pedigree.AKM-1 and Mdk-1 are Bapatla accessions and both are early flowering varieties. They were closer in both morphological and molecular studies. To conclude, pedigree is not completely answerable to variability.

The study had revealed a similar trend for morphological and molecular markers than biochemical markers with molecular markers being capable of giving a more precise information for the characterisation of the genotypes.



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* Originals not seen

MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR MARKERS FOR THE GENETIC ANALYSIS OF CASHEW *(Anacardium occidentale* L.*)*

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ABSTRACT OF THE THESIS

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ABSTRACT

The research project ' Morphological, biochemical and molecular markers for the genetic analysis of cashew' was carried out in the College of Horticulture, Vellanikkara, Thrissur during the period 2000-2002. The major objectives of the study were to fingerprint cashew genotypes based on genetic analysis carried out and the genetic relationship deduced between the morphological, biochemical and molecular parameters studied and also to identify genetically diverse genotypes among those selected for the study to be used in breeding programmes.

The study revealed that among the fourteen characters selected i.e., tree height, tree girth, number of primary branches, number of secondary branches, canopy spread, leaf area, number of panicles m^{-2}, number of nuts m^{-2}, number of perfect flowers m^{-2}, apple weight, nut weight, kernel weight, shelling percentage and nut yield, all showed significant variation except number of primary branches. Number of perfect flowers m^{-2}, number of nuts m^{-2}, apple weight, nut weight and kernel weight provide a clear seperation of the genotypes.

Correlation and path studies revealed tree height and number of nuts m^{-2} had significant positive correlation and direct effect on yield. Tree girth showed positive correlation but significant negative direct effect on yield. Number of primary branches showed significant positive direct effect but a significant negative correlation with yield. Apple weight showed significant negative correlation and significant negative direct effect with yield.

Genetic divergence studied using Mahalanobis D^2 analysis revealed H-1593 to be the most divergent genotype. Cluster analysis could group them into four clusters. The members of Cluster I (Sulabha, Priyanka and P-3-2) and Cluster II (Mdk-1, AKM-1 and K-22-1) were found to be best suited for hybrdisation being the farthest.

Biochemical studies on phenol and tannin content could group the twelve genotypes into those with high and low contents. The genotype H-1593 had the lowest

phenol content. Seed storage protein studies could distinguish K-22-1 from all others by a single unique band. Isozyme analysis in cashew showed only high initial rate of reaction. Further studies to standardise the protocol for isozyme studies needs to be done.

Molecular studies involved RAPD analysis using four primers which gave 44 amplification products out of which 30 (68.19 per cent) were found to be polymorphic. Two primers OPP-5 and OPP-10 could distinguish varieties Mdk-2 and Mdk-1 with amplicons 22 and 25 respectively. Dendrogram constructed based on the study grouped together Kanaka and Dharasree; Mdk-1 and Mdk-2 and H-1600 and P-3-2 with the latter two being the closest of all.

On comparative study, H-1600 (Damodar) was tied to Dharasree in biochemical studies and with P-3-2 in molecular studies. In morphological studies also, it was placed close to P-3-2 indicating the proximity of Indian accessions with those of South America. Kanaka and Dharasree were tied together both in morphological and molecular studies but both were diverse by pedigree. Similarly, AKM-1 and Dhana were placed close together in the three studies both of which were diverse by pedigree. H-1593 and H-1591 were found to be close in molecular and morphological studies. AKM-1 and Mdk-1,Bapatla accessions and early flowering varieties, were closer in both morphological and molecular studies. It can be said that pedigree is not completely answerable to variability.

The study had revealed a similar trend for morphological and molecular markers in deducing the genetic divergence. Biochemical markers need more refinement so as to get as precise information as has been obtained for the characterisation of the genotypes through molecular studies.