CLONAL VARIABILITY STUDIES IN ALPHONSO MANGO (*Mangifera indica* L.) BY PHENOTYPIC CHARACTERS AND MOLECULAR MARKERS

Thesis submitted to the
University of Agricultural Sciences, Dharwad
in partial fulfilment of the requirements for the
Degree of

Master of Science (Agriculture)

In

HORTICULTURE

By

MAKARAND DHONDU MANCHEKAR

DEPARTMENT OF HORTICULTURE
COLLEGE OF AGRICULTURE, DHARWAD
UNIVERSITY OF AGRICULTURAL SCIENCES,
DHARWAD - 580 005

SEPTEMBER, 2008

Advisory Committee

DHARWAD **(A. N. MOKASHI)**
SEPTEMBER, 2008 **MAJOR ADVISOR**

Approved by :

Chairman : ____________________________
(A. N. MOKASHI)

Members : **1.** ____________________________
(R. V. HEGDE)

2. ____________________________
(C. K. VENUGOPAL)

3. ____________________________
(A. S. BYADGI)

CONTENTS

Contd…..

LIST OF ABBREVIATIONS

Code	Abbreviations
bp	Base pair
cDNA	Complementary DNA
CTAB	Hexadecyl trimethyl ammonium borate
cpDNA	Chloroplast DNA
DNA	Deoxy ribonucleic acid
EDTA	Ethyl diamine tetra acetic acid
IARI	Indian Institute of Agricultural Research
IIHR	Indian Institute of Horticultural Research
kbp	Kilo base pairs
MT	Metric tonnes
NaCl	Sodium choride
ng	Nanogram
PCR	Polymerase chain reaction
p moles	Picomoles
PVPP	Polyvinyl poly pyrrolidine
QTL	Qualitative trait loci
RFRS	Regional Fruit Research Station
RNA	Ribonucleic acid
STR	Short tadom repeates
TNAU	Tamilnadu Agricultural University
µg	Microgram
mbp	million base pairs
pg	picogram
mg	Milli gram
RAPDNTys	Random amplified polymorphic DNA Numerical Taxonomical System analysis
UBC	University of British Columbia
UV	Ultra Violet
UPGMA	Unweighted pair-group method with arithmetic average

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

1. INTRODUCTION

Mango (*Mangifera indica* L.) is the most popular fruit crop in the orient particularly in India, where it is considered as the best choice among all indigenous fruits. It occupies relatively the same position as that enjoyed by apple in temperate America or Europe. It ranks first among all the fruits of India in area and production.

Global production of mango is concentrated mainly in Asia and more precisely in India. Mango is grown in 85 countries, among which 63 countries produce more than 1000 metric tonnes in a year. In these countries, mango serves as an integral part in human life since it is not only a rich source of nutrients but also a common good shared in culture, life style and religion. In India, mango is distributed throughout the length and breadth of country, except in hilly regions at 915 meters above mean sea level. India is the largest producer and consumer of mango in the world. The country reportedly produces about 50 varieties of mango. In 2005, the country had an area of 1.962 million ha and production of 11.61 million metric tonnes. The major mango growing states in India are Maharashtra, Andhra Pradesh, Uttar Pradesh, Bihar, Karnataka and Gujarat. Uttar Pradesh is known for growing a host of mango varieties which are not found else were. The other important mango growing states in the country comprises of Goa, Haryana, Madhya Pradesh, Orissa, Punjab, Tamil Nadu and West Bengal. The region wise popular varieties grown in different parts of the country comprise of 'Alphonso' and 'Kesar' in western India, 'Bangenpalli', 'Totapuri' and 'Neelum' in southern states, 'Fazli' in eastern states and 'Langra' and 'Chausa' in northern states (Anon., 2006). In Maharashtra, mango is cultivated in an area of 0.43 million ha and produced at 0.63 million metric tonnes, while in Karnataka, the area and production are 0.12 million ha and 1.11 million metric tonnes respectively (Anon., 2006).

The cultivation of 'Alphonso' is spread across Ratnagiri, Sindhudurg, Raigad and Thane districts of Maharashtra, as well as Dharwad and Belgaum districts of Karnataka. The demand for 'Alphonso' mangoes from specific pockets in South Konkan region of Maharashtra is high. This demand is plausible explanation for genetic purity and relativity among 'Alphonso' mangoes from different locations in Maharashtra and Karnataka states.

It was observed that significant variation exists, among trees of the same clone in an orchard with respect to fruit shape, size, colour and quality, which is ascribed to bud mutation. Asexual propagation enables us to preserve the accumulated mutations which would normally be sieved-out by sexual propagation. Thus, during the course of evolution, number of mutations accumulated in different clones, might have created polymorphism among the cultivated 'Alphonso' mangoes from different pockets. Therefore, it becomes necessary to establish the phyllogenetic relationship and investigate any possible differences at molecular level in the 'Alphonso' mangoes from different pockets. Simultaneously, it may also help to assess the extent of variability for morphological traits according to geographical variability.

Indian mango varieties like 'Alphonso' and 'Dashehari' are in great demand abroad. A number of countries are attempting to compete with India in the production of high quality mangoes. Majority of the varieties grown in Florida and other places of the world are of Indian origin. Hence, there is an urgent need to characterize the Indian cultivars using the most reliable techniques (Welsh and Mc-clelland, 1990, Williams *et al*., 1990). Characterization through morphological characters, requires extensive observation of plant at different growth stages especially at flowering and fruiting. Biochemical markers offer greater diversity as compared to the morphological markers, irrespective of the stage of the crop. But, they also suffer from some limitations like insufficient polymorphism among closely related cultivars and dependence on development stage of tissue for expression.

On the contrary, a more sophisticated and reliable biotechnological tool *viz.,* RAPD (Random Amplified Polymorphic DNA) detects nucleotide sequence polymorphism in a DNA amplification-based assay using only a single primer of arbitrary nucleotide sequence. In this reaction, a single species of primer binds to genomic DNA at two different sites on opposite strands of DNA templates. If these primary sites are within amplifiable range, the presence of each amplification product identifies complete or partial nucleotide sequence homology, between the genomic DNA and the oligonucleotide primer at each end of amplification cycle. On an average each primer will direct the amplification of several discrete loci in the genome, making the assay an efficient way to screen for nucleotide sequence polymorphism between individuals. The major advantage of this assay is that there is no requirement for DNA sequence information. The protocol is also relatively quick, easy to perform and uses fluorescence in lieu of radio activity (Williams *et al.* 1992). Since the RAPD technique is amplification based assay, only nanogram quantities of DNA are required, and automation is feasible.

In the light of above, the present investigation was undertaken with following objectives:

1. To study the morphological and qualitative traits of 'Alphonso' mango clones from different geographical locations
2. To establish location specific RAPD pattern in 'Alphonso' mangoes from different geographical locations, and use the same to know the variability, if any

2. REVIEW OF LITERATURE

A brief review of literature related to the present study entitled ‘Clonal variability studies in ‘Alphonso’ mango (*Mangifera indica* L.) by phenotypic characters and molecular markers’ is discussed under different headings.

2.1 CLONAL VARIATIONS

Naik (1948) observed significant variation among the trees of same clones in an orchard with respect to fruit shape, colour and quality which was ascribed to bud mutations. Oppenheimer (1956) reported a wide variability in the performance of the trees belonging to same variety in same orchard, after surveying many orchards in India. Mukherjee *et al.* (1985) conducted a survey of mangoes in eastern India and identified some superior clones. Singh and Chadha (1981) in a study of mango orchards of ‘Dashehari’ located four clones which were superior in performance. Singh *et al.* (1985) isolated two high yielding clones from the orchards of ‘Langra’ with improved resistance to bacterial black spot. Whiley *et al.* (1993) identified certain strains within ‘Kensington’ clone, having improved resistance to bacterial black spot disease.

2.2 SOMATIC MUTATIONS

Mangoes are classified into two groups, monoembryonic and polyembryonic, based on their ability to reproduce from seeds. Most of the monoembryonic cultivars are propagated vegetatively by grafting or budding onto monoembryonic or polyembryonic seedling rootstocks. Polyembryonic cultivars are propagated either from seed or by grafting onto polyembryonic seedling rootstocks. There appears to be a considerable variability reported within certain cultivars grafted onto different rootstocks, which attributed either to somatic mutations (Young and Ledin, 1954) or to the influence of non-uniform monoembryonic rootstocks. The ‘Davis-Haden’ originated as a sport from ‘Haden’. ‘Alphonso’ and ‘Puthi’ were thought to be chimeras (Singh, 1960). Gan *et al.* (1981) observed considerable variations within the vegetatively propagated mango cultivars based on polymorphism of four enzymes isolated from leaf tissues.

Lakshminarayana (1980) reported that in mango, same clones were called by different names. ‘Philippine’ was believed to be the same clone as ‘Manila', and 'Cecil' and 'Carabao' were to be the same cultivar.

2.3 MARKER ASSISTED CHARACTERISATION IN MANGO IMPROVEMENT

Studies involving *in vitro* culture, selection, micropropagation, embryo rescue, genetic transformation, marker-assisted characterization and DNA fingerprinting, etc. are underway on mango at different centres worldwide. *In vitro* culture and somatic embryogenesis of several genotypes were achieved. The nucellus excised from immature fruitlets was found to be the appropriate explant for induction of embryogenic cultures. High frequency somatic embryogenesis was achieved in some genotypes. However, some abnormalities can occur during the germination of somatic embryos. DNA fingerprinting and studies on genetic diversity of mango cultivars and *Mangifera* species are also being conducted at several research stations (Hare Krishna and Singh, 2007).

2.3.1 Morphological markers

The oldest and most widely used markers were the morphological traits, which may still be optimal for certain cases, where the cultivars were identified based on leaf, panicle, fruit and other physical characteristics. These characters, however, may change with environmental conditions. Furthermore, actual identity of some cultivars is still in question, because similar cultivars grown in different areas often have various names (Lakshminarayana, 1980). Their prime advantages are simplicity and rapid, inexpensive assays, even from herbarium specimens and other dead tissues. However, the morphological markers have many disadvantages such as lack of decisiveness.

2.3.2 Biochemical markers

Karibasappa (1995) reported those three enzyme systems *viz.,* peroxidase, catalase and aspartate amino transferase were studied for their electrophoretic patterns in 67 accessions of mango. Among these peroxidase and aspartate amino tranferase were resolved into discrete bands at various positions on starch gel. In all the three enzyme systems, the buffer systems and running conditions were more or less similar. It was possible to identify zygotic seedlings in the seedling progeny of polyembryonic cultivars by peroxidase, catalase and aspartate amino tranferase isozymes. Peroxidase system was more convenient to use as a tool in identification of nursery scion plants. Thus his investigation clearly explained the diversity for various quantitative characters in 67 mango accessions at University of Agricultural Sciences, Dharwad. The diversity among the quantitative characters including the variation in isozymes was further quantified using relevant techniques and estimations.

Degani *et al.* (1993) and Schnell and Knight (1992) demonstrated the feasibility of using isozymes as biochemical markers in mango. Degani *et al.* (1992) analysed leaf phosphoglucoisomerase (PGI) isozyme from 139 mango cultivars and seedlings of Mango. Six distinct banding patterns of PGI-2 consisting of single and triple banded phenotypes were detected. The genetic control of PGI-2 isozyme was inferred from segregating progenies of self pollinated parent cultivars having triple banded phenotypes. Comparison of the banding patterns of PGI-2 isozymes, extracted from pollen and leaf of the same heterozygous cultivars, demonstrated the allelism of PGI-2 locus.

Schnell and Knight (1992) used five isozyme systems to detect zygotic seedlings from five polyembryonic cultivars of mango. The enzyme systems studied were isocitrate dehydrogenase, luceinamino peptidase, phosphogluco isomerase. They found that there were significant differences for the frequency of occurrence of nucellar and zygotic seedlings.

Degani *et al.* (1993) distinguished the zygotic seedlings and nucellar seedlings of two polyembryonic mango cultivars viz. Tarpentine and '13-1'. Leaf extracts of the seedlings were analysed for isozymes of isocitrate dehydrogenase, phosphoglucose isomerase, phosphoglucomutase and triosephosphate isomerase. The distinction made was based on the fact that nucellar seedlings should have the same isozyme allele as the maternal parent. A variation at a locus coding for an enzyme indicated that the plant was originated by sexual reproduction. Zygotic seedlings arising from self pollination were distinguished from nucellars by being homozygous at one or more loci at which the female parent was heretozygous. Zygotic seedlings resulting from cross pollination were distinguished from those resulting from self-pollinated if they expressed an allele not carried by the female parent.

Eiadthong *et al.* (1999) analysed leaf extracts of 58 mango cultivars for variation of isocitrate dehydrogenase, phosphogluco isomerase and alcohol dehydrogenase. Polymorphism was found in all enzyme systems examined and intracultivar variations of the samples collected at different locations were confirmed in banding patterns. No correlation between the polyembryonic and monoembryonic groups was found. Banding patterns of the three enzyme systems allowed classification of Thai cultivars into 14 groups.

Results of the studies made by Jintanawongse and Changtragoon (2000) in the commercial Thai mango cultivars ‘Kniew Sawoey’, ‘Nam Dok Mai’, ‘Nan Klang Wan’, ‘Red’, ‘Tong Dum’ and ‘Kaew’ showed that resolution was more in case of nine enzyme systems out of 11 systems studied. The nine systems were of the enzymes glutamate dehydrogenase, glutamate oxaloacetate, transaminase, isocitrate dehydrogenase, diaphorase, 6-phosphogluconate dehydrogenase, shikimate dehydrogenase, phosphoglucomutase, malate dehydrogenase and glucose-6 phosphate dehydrogenase.

2.3.3 Molecular markers

The discovery of molecular markers in recent years greatly enhanced the scope for detailed genetic analysis and approaches for improvement of crop plants. The recently developed DNA-based markers like restriction fragment length polymorphism (RFLP) and RAPD provided excellent tools to study the genetic diversity for eliminating duplicates in germplasm, study genetic relationships, gene tagging and genome mapping etc. These markers measure diversity at DNA level and they are seldom influenced by environmental conditions as in case of morphological markers.

The most exciting feature of DNA based markers is the extent of detectable polymorphism. The DNA content in higher plants is highly variable. Armuganathan and Earle (1991) estimated the DNA content in over 100 important crop species. DNA content varied from 0.30 pg per 2C nuclei or 145 mbp in *Arabidopsis* spp. to over 50 pg or 24,255 mbp in Leek. However, the DNA content of most of the intensively mapped diploid species (tomato, rice, *Arabidopsis* spp.) was in the range of 0.30 to 1.0 pg. The DNA content of mango in 2C nuclei was 0.90 pg or 439 mbp.

Most of the higher plants have a considerable portion of DNA as repetitive non-coding DNA that is not transcribed. Species with larger genomes normally have more repeated DNA and a higher proportion of repeated DNA to single copy DNA (Tanksley and Pichersky, 1988, Saiprasad *et al.* 2004). Thus, only a small fraction of the total genetic variation at a DNA nucleotide sequence level reveals itself as a distinct trait, showing Mendelian inheritance. The degeneracy of the genetic code ensures that about one in three nucleotide changes will not affect the amino acid sequence of the protein produced. Thus, the great bulk of genetic variation at the nucleotide level may not have any detectable expression at phenotypic level. It is this genetic variation that is expressed as DNA-based polymorphism.

RFLP and RAPD are the two types of DNA based markers which were widely used and the literature on these techniques is presented below. Since RAPD was adopted in the present study much of the literature reviewed pertains to it.

2.3.3.1 RFLP

Adato *et al.* (1995) analysed mango DNA fingerprint patterns obtained by mini satellite multilocus probes. The genomic DNA of 25 mango cultivars and 14 mango rootstocks probed with 33.6, R28.1, 22.3 and simple repeat oligonucleotide probes revealed resolvable and complex band patterns. The DNA fingerprints were obtained for each cultivar by the probe 33.6. The probability of obtaining a similar pattern for two different cultivars was 9.4×10^{-6}.

Genetic analysis was carried out on offspring of a cross between ‘Tommy Atkins’ and ‘Keit’ using hind III / 33.6 enzyme probe combination. The average transmission frequency detected in this analysis was 65% for material specific bands. It did not appear in any of the 12 progenies. No pair of bands was found to be completely linked and no polymorphic band seemed to be homozygous.

Application of DNA fingerprint (DFP) markers shed light on some horticultural dilemmas. It was widely accepted that ‘Edward’ resulted from a cross between ‘Haden’ and ‘Carabao’. However DFP pattern of these two cultivars showed that ‘Edward’ could not be their progeny.

2.3.3.2 RAPD

Schnell and Knight (1993) examined nine *Mangifera* spp. using RAPD molecular markers to determine phylogenetic relationships. Ten selected primers produced 109 bands. Data were scored as presence or absence of bands. Analysis was conducted using average taxonomic distance, UPGMA and principal coordinate analysis. RAPD generated clusters did not always agree with the taxonomic classification based on phenotypic traits. When the two subsections of the genus were analysed separately, the classification more closely agreed with the traditional taxonomic analysis.

Lycett *et al.* (1997) reported that a gamma-ZAP cDNA library was constructed from mango (*Mangifera indica*) cv. Tommy Atkins fruits which had been induced to ripen with ethylene. The library was screened with a tomato ACC oxidase 1 (ACO) cDNA. A cDNA clone (pNY601) was isolated that appeared to encode the full amino acid sequence of ACO. Northern analysis showed that the corresponding message was expressed in untreated green fruits and in fruits after 24 hours of ethylene treatment, although it decreased in the later stages of ripening. A differential screen was then conducted to find ripening-specific clones. Two abundant clones (pNY608 and pNY642) and four less common clones (pNY602, pNY627, pNY633 and pNY634) were identified. None of these six clones was expressed in green fruits, although all of them increased after ethylene treatment. When the cDNA inserts were sequenced and translated, pNY608, pNY627, pNY633 and pNY642 showed no significant homology to protein sequences in the SWISSPROT database. However, pNY634 showed strong homology to a plastid chaperonin, and pNY633 showed strong homology to the Ypt3/Rab11 class of small GTPases. These substances were thought to have possible roles in extracellular and intracellular pathways. None of the clones was related to known fruit ripening specific genes of tomatoes or other species.

Fifteen mango cultivars collected from Mexico, Phillipines, Florida and Reunion, were examined using RAPD markers with decamer primers of arbitrary sequence. Thirteen of the 40 primers screened were informative and 109 amplified DNA bands were selected as RAPD markers. Specific RAPD markers for some mango cultivars were identified. Cluster analysis based on the RAPD markers produced a dendrogram of the genetic relatedness of the 15 mango cultivars. 'Manila' and 'Carabao' were the most similar, which is in good agreement with their putative pedigrees. The four major bifurcations in the dendrogram clearly separated the genotypes into four groups according to their geographic origin. Bulk segregant analysis of polyembryonic and monoembryonic cultivars detected a specific RAPD marker for polyembryony. These markers may facilitate the management of mango germplasm for breeding purposes Lopez *et al.* (1997).

Lavi *et al.* (1998) estimated phenotypic and genetic variance components for mango traits. The analyzed population composed of 745 progenies derived from both controlled crosses and open pollination. The average percentage of selfing was estimated from the statistical model to be 50 per cent. Both the additive and dominance (non-additive) genetic variance components were calculated. In all the 13 traits analyzed, the additive genetic variance components were not statistically significant, while in eight of the traits, the dominance genetic variance was significant. In three pairs of traits, significant phenotypic correlation was detected and in one pair of traits, significant genetic correlation was found. Conclusions for mango breeders were discussed with the main recommendation being to widen the genetic background of the breeding population.

Jayasankar *et al.* (1998) isolated genomic DNA from embryogenic cultures of mango cultivars 'Hindi' and 'Carabao' that had been selected for resistance to the culture filtrate of *Colletotricum gleosporioides* [*Glomerella cingulata*]. The genomic DNA was analyzed using RAPD. *In vitro* selection caused changes in RAPD markers in the selected embryogenic cultures with respect to the unchallenged control cultures and the stock plants. The differences involved both the absence and the presence of additional RAPD markers in the resistant lines, although the former was most commonly observed. The absence of differences between the unchallenged control of either of the cultivars and DNA from the leaves of parent trees confirmed that the changes were not due to prolonged maintenance in liquid cultures.

Deng-Jiu-Sheng *et al.* (1999) isolated DNA from mango cultivars Hongmang and Kuixiangmang and a selection Hongpingmang. The DNA was used for RAPD analysis. The primers S273, S281 and S286 were found to be the most suitable for RAPD amplification of the genomic DNA. The differences among the genotypes with respect to DNA sequence were documented.

Samanta *et al.* (1999a) investigated the genetic variability of sexual fruit characters among 25 genotypes of mango. Differences between the PCV and GCV were found to be less for fruit volume, fruit weight and pulp weight only indicating a small influence of environment. High heritability along with a high genetic advance was found for the same characters, indicating the presence of additive gene actions where selection would be effective. However, for other characters a low genetic advance was observed, where hybridization was suggested for an improvement of those traits.

Samanta *et al.* (1999b) studied genetic variability and heritability of several morphological characters among 25 genotypes of mango grown in West Bengal. The genotypes showed significant differences for all the characters studied. A broad range of variation was observed for leaf length, average number of leaves per twig, number of tertiary branches, and chlorophyll 'a' and chlorophyll 'b' contents. A great degree of difference was observed between phenotypic and genotypic coefficients of variation for number of tertiary branches and number of scaffold branches, indicating the influence of environment for these traits. High heritability along with high genetic advance was recorded for average number of leaves per twig, indicating the importance of additive gene action. Leaf length, leaf breadth, petiole length, internodal length, thickness of one-year-old shoots, and chlorophyll 'a' and 'b' contents showed high heritability and low genetic advance, indicating the presence of non-additive gene action. Low heritability along with low genetic advance was recorded for number of scaffold branches and number of tertiary branches, indicating the large influence of the environment.

At IIHR, Banglore, Ravishankar *et al.* (2000) reported that RAPD markers were used extensively to study genetic relationships in a number of fruit crops. They concluded that a wide genetic diversity existed among the mango cultivars in India. Present day commercial cultivars of mango were originated mainly from this subcontinent. In their study, 18 commercial mango cultivars, traditionally grown in western, southern, northern and eastern parts of India, were selected to assess genetic relatedness. Total genomic DNA was extracted and subjected to RAPD analysis using 30 arbitrary 10-mer primers, of these, 27 primers amplified mango genomic DNA. None of these primers produced unique band pattern for each cultivar. RAPD data were used to calculate a squared Euclidean distance matrix, and based on this, cluster analysis was done using a minimum variance algorithm. Cluster analysis clearly showed two groups - the first consisting of western, northern and eastern mango cultivars and the second group consisting of southern cultivars. From the analysis of results, it was felt that the majority of mango cultivars originated from a local mango gene pool and was domesticated later.

Hemantkumar *et al.* (2001) working at UAS, Banglore, screened fifty mango cultivars, chosen from a number of South Indian institutes to represent a wide range of geographical sources. They were screened using RAPD markers with decamer primers of arbitrary sequence. Out of the 80 primers screened, ten were selected which gave 139 clear and bright fragments. A dendrogram based on Jaccard's co-efficient of similarity implied a moderate degree of genetic diversity among the cultivars used for experimentation. The hybrids which had one parent in common were placed together. In the cluster, alternate bearers and regular bearers formed separate groups, and the members in each group were very closely linked. Another analysis based on Pearson's co-efficient of similarity revealed a high degree of genetic diversity. In both the analyses, 'Mulgoa' was found to be very distinct. This study showed clearly that cultivars from South India possess a high degree of genetic diversity and indicated the potential of RAPD markers for the identification and management of mango germplasm for breeding purposes.

A study was carriedout at IIHR, Bangalore by Dinesh (2003) to determine the presence of reciprocal effects and to assess the parents that are best suited for the transfer of desirable characters among commercial cultivars of mango. The cultivars 'Alphonso', 'Banganapalli', 'Neelum', 'Kalapadi' and 'Janardhan Pasand' were utilized for crossing, including reciprocals. The F_1 intervarietal progenies of the combinations Alphonso x Banganapalli, Alphonso x Neelum, Alphonso x Kalapadi and Alphonso x Janardhan Pasand were evaluated for different characters, i.e., fruit weight, fruit volume, TSS, skin weight, stone weight and pulp percentage. The study showed that non-additive variance controlled the characters. Heritability was low and the chances of hybrid vigour manifesting for these characters in the F_1 generation were high. It was suggested that selection of progenies could be made based on fruit size i.e., medium-sized fruits having high TSS and vice verse. For the characters studied, PCV was higher than GCV.

Karihaloo *et al.* (2003) carriedout RAPD analysis in 29 Indian mango cultivars comprising of popular land races and some advanced cultivars. PCR amplification with 24 primers generated 314 bands, 91.4 per cent of which were polymorphic. Jaccard's coefficient of similarity between pairs of cultivars ranged between 0.318 and 0.75 with a mean of 0.565. A UPGMA dendrogram showed the majority of the cultivars from northern and eastern regions of India clustering together and were different from southern and western cultivars. Analysis of molecular variance revealed that 94.7 per cent of the genetic diversity in mango existed within regions. However, differences among regions were significant; northern and eastern regions formed one zone for western and southern regions formed another zone of mango diversity in India.

Souza *et al.* (2004) examined forty mango genotypes of the Embrapa Meio-Norte mango collection centre RAPD markers with 32 random primers. Thirteen, of these 32 initially screened primers that indicated more polymorphic DNA amplification patterns, were selected for RAPD reactions. Each genotype was characterized by its banding pattern, using 1 kb DNA ladder as standard DNA. A genetic similarity matrix based on Jaccard's coefficient, and a phenogram using UPGMA clustering was constructed. Initially, the genotypes were grouped into two distinct groups (J = 0.36), one formed by 'Mallika' and 'Amrapali' and another comprised of the other genotypes, which in turn was sub divided into two groups (J = 0.41), one formed by 'Manzanilo', 'Van Dyke', 'Palmer' and 'Keitt', and the other including the remaining genotypes. This latter group was further divided into two other groups (J = 0.54), one including 'Edward', 'Winter', 'Alfa Emprapa 142', 'Kensington' and the advanced breeding selection CPAC 98/86 ('Beta') and the other, including 'Santa Alexandrina', 'Sensation', 'Glenn', 'Irwin' and all 25 Rosa's genotypes. In Rosa's group, the similarity coefficient ranged from 0.55 to 0.98, indicating a reasonable amount of genetic variation within this cultivar. Bootstrap consistency test, however, indicated that only three groups ('Mallika' and 'Amrapali', 'Van Dyke', 'Palmer' and 'Keitt', and 'Irwin' and 'Glenn') were really diverged.

Ravishankar *et al.* (2004) observed that the genetic relationship among 10 polyembryonic and monoembryonic cultivars each traditionally grown in the west coast of southern India were determined using RAPD markers. Eight mango cultivars from each of these groups were used for chloroplast DNA RFLP analysis. The primers viz., ORF 106-rbcL and GIF-GI460 were used. All the products were restriction digested with enzymes. Data from RAPD markers and chloroplast DNA RFLP markers were used for cluster analysis and principal component analysis (PCA), separately. In both genomic DNA and chloroplast DNA RFLP analyses, the grouping of cultivars based on their embryo types indicated that monoembryonic and polyembryonic types of Indian mango cultivars had a different genetic base. These results suggested that the polyembryonic types might have been introduced from other parts of Southeast Asia and are unlikely to have originated from India.

Srivastava *et al*. (2004) observed that major efforts in mango (*Mangifera indica* L.) breeding resulted in the release of several new promising mango hybrids such as 'Amrapali' (Dashehari x Neelum), 'Mallika' (Neelum x Dashehari), 'Ratna' (Neelum x Alphonso) and 'Sindhu' (Ratna x Alphonso). In his work, the application of molecular markers for parentage analysis of commercial mango hybrids was studied i.e., primarily, three different Single Primer Amplification Reaction (SPAR) methods, RAPD, Inter-Simple Sequence Repeat (ISSR) and Directed Amplification of Minisatellite DNA (DAMD), were used for establishing parent-hybrid relationship, in case of three commercially important mango hybrids, that were developed using Neelum as one of the parents. They showed that hybrid 'Ratna' (Neelum x Alphonso) was genetically closer to its male parent Alphonso, while reciprocal hybrids 'Amrapali' (Dashehari x Neelum) and 'Mallika' (Neelum x Dashehari) were closer to 'Neelum'. Further, one RAPD and two DAMD primers revealed that the Neelum-specific bands were present in all the three hybrids and 'Neelum' exclusively. Such bands were suggested to be useful in breeding programs by tagging genes as well as by enabling a more efficient early selection of progeny with desirable qualities.

Cordeiro *et al*. (2006) conducted a study in Brazil during 2003 to estimate the male genitor of mango hybrids through a parentage test using RAPD markers. Five mango cultivars (female, 'Mallika' and males, 'Roxa', 'Alfa', 'Keitt' and 'Tommy Atkins') were densely planted in a Latice Square Design (LSD). RAPD markers efficiently showed the genetic similarity among cultivars and produced specific DNA fragments for the parentage test of hybrids selected in open crosses. Open crosses did not occur due to the closest spacing of cultivars, since the actual stage of development did not permit full canopy contact.

Iqbal *et al*. (2006) collected a total of 20 Fusarium isolates of mango malformation affected plants from 14 locations in Pakistan and assessed genetic diversity using the RAPD technique. The RAPD proved a reproducible and traceable means of differentiating Fusarium isolates. These findings also suggested that some infections originate not from adjacent plants within an orchard but from geographically distant areas; indicating that most probably infection occurs in nurseries prior to plants being transported around the country for subsequent cultivation, and that improved plant hygiene could significantly curb mango malformation infection and spread.

2.3.3.3 RAPD studies related to other fruit crops

Rout *et al*. (2002) developed an efficient protocol for DNA extraction and purification from fresh leaves of different cashewnut varieties for RAPD analysis at Orissa University of Agriculture and Technology, which could be useful for other woody species. In the same laboratory, similar studies by Samal *et al*. (2002) on genetic relatedness in 20 cashew germplasm collections determined by RAPD, showed the highest similarity index of 87 per cent between 'Ullal-3' and 'H-1608'. it was observed that 'Vengurla-2' and 'Vengurla-3', were not grouped into a single cluster, but 'Vengurla-4' had 82 per cent similarity with 'Vengurla-3', which, in turn, had a close similarity of 85 per cent with 'VRI-3', a variety from altogether a different geographical location. Samal *et al*. (2003) reported that the analysis of genetic relationship in cashewnut using morphological traits and RAPD banding data can be useful for plant improvement, description of new varieties and also for assessment of varietal purity in plant certification programmes.

Simon *et al*. (2007) studied that RAPD markers used to estimate genetic diversity in 12 high-yielding jackfruit (*Artocarpus heterophyllus* L.) accessions obtained from different locations in southern India. Marker data were compared with morphological data obtained over three successive seasons. PCR-amplifiable DNA was isolated using CTAB method and 171 amplified fragments were obtained using 23 random primers. The genetic dissimilarity matrix was calculated based on Squared Euclidian Distances, which revealed a maximum genetic distance of 7.9 per cent between a clone of 'Mottavarica'(M_0), and 'Chandrahalasu' from distant locations, while the minimum genetic distance (5%) was found between the genotypes M_0 and 'Kerala' indicating their similar geographical origin. Ward's method of cluster analysis grouped all individuals on the Dendrogram into two major clusters according to their geographical locations. He showed low to moderate genetic diversity among the 12 jackfruit accessions, which will assist in the identification and management of jackfruit germplasm for breeding purposes.

Damasco *et al*. (1996) detected dwarf off-types in micropropagated Cavendish (*Musa* spp. AAA) bananas using RAPD makers. New Guinea Cavendish and Williams were identified following an analysis of 57 normal and 59 dwarf plants generated from several micro propagation events of 66 random decamer primers used in the initial screen, 28.8 per cent revealed polymorphisms between normal and dwarf plants. Primer OPJ-04 (5'-CCGAACACGG-3') amplified a 1.5 kb band which was consistently present in normal but absent in all dwarf plants of both cultivars. Reliable detection of dwarf plants was achieved using this marker, providing a suitable means of *in vitro* detection. Other micro propagation induced RAPD polymorphisms were not associated with the dwarf trait. Use of this marker could facilitate early detection and elimination of dwarfs from batches of micropropagated bananas, and may help to determine the factors responsible for dwarfing.

Abkenar and Isshiki (2003) used RAPD markers to evaluate genetic similarity and relationship among 31 acid citrus species and cultivars, including sour oranges (six accession),'Yuzu' (four accession) and its relatives (21 accession). Out of the 60 decamer primers screened, 27 were selected which produced 108 markers, 76 of which were polymorphic. Species or cultivars with specific RAPD markers were also found. A dendrogram based on genetic distance implied that sour oranges were very distinct from 'Yuzu' and its relatives 'Yuzu' accessions were very closely linked to each other, however, for the other specimens genetic polymorphism could easily be detached by RAPD and the genetic variation between accessions was quite high and revealed that different origin. In this study some RAPDs allowed the destinction of very close cultivars, for instance 'Kabousu' from 'Aka kabosu'.

2.4 GENETIC DIVERGENCE

Singh *et al.* (2004a) conducted an experiment to assess the genetic divergence among 43 genotypes of mango collected from different research centres in India, using Mahalanobis D^2 statistics. Data were recorded for fruit yield per plant, cumulative yield per plant, fruit weight, TSS and acidity. Analysis of variance revealed highly significant differences among genotypes for all characters. The genotypes were grouped into seven clusters. Cluster VI, which consisted of the genotypes 'Kala Pahar' and 'Amrapali', had the highest TSS (21.45%) and lowest acidity (0.22%) with a desirable fruit weight (206.50 g). Cluster IV, comprising the genotypes 'Dudhia Maldah', 'Bangalora', 'Maldah Surajgarha' and 'Dholikothi Maldah', recorded the highest fruit yield per plant (84.78 kg), cumulative yield per plant (771.07 kg) and fruit weight (189 g), with desirable values for TSS (19.58%) and acidity (0.23%). No relationship between geographical origin and genetic diversity was established. Selecting genotypes from divergent clusters and utilizing them in hybridization programme was thought to produce desirable segregates in subsequent generations that might lead to desirable improvement in mango for yield and quality traits.

Singh *et al.* (2004b) reported that the genetic variability for five traits (yield per plant, cumulative yield per plant, fruit weight, TSS and acidity) was assessed in 43 genotypes of mango, planted during 1980-81 in Bihar. A wide range of variability was observed for all the characters, except for TSS. 'Bangalora' recorded the highest cumulative yield (1012.28 kg) and yield per plant (95.28 kg), but produced fruits of poor quality. 'Dudhia Maldah' had a cumulative yield of 780.10 kg and yield of 88.54 kg per plant, but had high TSS (20.74%) and low acidity (0.21%). The magnitude of PCV was higher than that of GCV. High heritability coupled with high genetic advance was observed for cumulative yield and yield per plant.

Singh *et al.* (2004c) conducted field and laboratory experiments in Bihar, to determine correlations among some yield and quality traits in mango. Yield per plant was positively and significantly associated with cumulative yield per plant and fruit weight. Fruit weight was positively and significantly associated with cumulative yield per plant. Per cent acidity was negatively but non-significantly associated with all the traits. Path analysis for fruit weight showed a high direct effect on yield per plant and very high indirect effect on cumulative yield per plant via fruit yield per plant. Fruit weight and yield per plant appeared to be important traits for bringing the desired improvement in mango.

Pradeepkumar *et al*. (2006) evaluated physico-chemical properties of the fruits of 31 mango genotypes from north Kerala. Based on the physical and chemical characteristics, these genotypes were divided into four homogenous clusters. Large table fruited varieties like 'Mulgoa', 'Phirangiladua', and 'Banganapalli' formed a single group, while 'Bangalora', 'Gudad', and 'Chotta Jehangir' deviated from that considerably. The highest significant correlation was noted between fruit length and pulp weight. Clustering pattern obtained in this study was suggested for use in future genetic improvement of these cultivars.

3. MATERIAL AND METHODS

The present investigation was carried out during the year 2007-2008 in Department of Horticulture, University of Agricultural Sciences, Dharwad. The details of the experiments and the material and methods employed are presented here.

3.1 DETAILS OF EXPERIMENTS

The investigation was carried out in two experiments, one based on phenotypic and chemical characters of ripe fruits and the other on RAPD. Both the experiments had the same sampling locations.

3.1.1 Locations

In Maharashtra: Devgad, Ratnagiri, Vengurla and Dapoli

In Karnataka: Dharwad (three clones) and Belgaum (two clones)

The ‘Alphonso’ clones collected from the above locations were abbreviated as follow:

T_1 : DVG-I

T_2 : RTN-I

T_3 : VEN-I

T_4 : DPL-I

T_5 : DWR-I

T_6 : DWR-II

T_7 : DWR-III

T_8 : BGM-I

T_9 : BGM-II

3.2 EXPERIMENT NO. 1: STUDY OF CLONAL VARIABILITY AMONG THE ‘ALPHONSO’ CLONES, BASED ON PHENOTYPIC PARAMETERS OF FRUITS

3.2.1 Collection of samples

The ‘Alphonso’ mango fruits were collected from Ratnagiri and Sindhudurg districts of Maharashtra, as well as Belgaum and Dharwad districts of Karnataka. The exact addresses were presented in Table 1. The fruits were collected at maturity stage and transported through corrugated fibre boxes with paddy straw and paper and brought to laboratory. The fruits were kept in ripening chamber for observing their shelf life and some fruits were used for taking observations on phenotypic characteristics as well as quality parameters such as total soluble solids, titrable acidity, and ascorbic acid content.

3.2.2 Design and layout

There were nine locations, from where samples were collected in five replications from each. The nine sites represented nine treatments and the data was analysed in Completely Randomised Design.

3.2.3 Observations recorded

3.2.3.1 Physical parameters

Various physical parameters of fruits were recorded as described below,

a. Weight of fruit: Weight of mature fruit was determined with the help of a ‘top loading electronic balance’ and expressed in grams.

b. Length and diameter of fruit: The length of the fruit was recorded by measuring the linear distance from the point of attachment of stalk to the stylar end and the diameter was measured as the maximum linear distance between the two shoulders of the fruits with the help of Vernier callipers and expressed in centimetres.

c. Weight of peel, pulp and stone: At ripe stage the skin was peeled-out by hand and the pulp adhering to it was scooped-out with a spoon. Weight of peel, pulp and stone were recorded for five fruits and was averaged.

d. Stone thickness: Maximum thickness of stone was measured as the maximum vertical distance between the two sides of stone with the help of Vernier callipers and expressed in centimetres.

e. Shelf life: The fruits of each clone of ‘Alphonso’ were stored under ambient temperature (27-30°C) and were thoroughly examined for any visible symptom of infection during storage. Shelf life was expressed as number of days taken to reach the end point (15% and more spoilage).

3.2.3.2 Chemical parameters of fruits

Samples of pulp weighing five grams from each fruit were taken and analyzed for the following parameters.

a. Total soluble solids (TSS)

TSS was found by using Refractometer (0-30 °Brix range). The values were corrected at 20°C and expressed as °Brix (Anon., 1970).

b. Total titratable acidity

The acidity was determined by diluting a known volume of clear juice, filtered through muslin cloth, with distilled water and titrating the same against standard 0.1N sodium hydroxide solution, using phenolphthalein indicator. The appearance of light pink colour was marked as the end point. The result was expressed in terms of maleic acid as per cent total titratable acidity of the fruit juice (Anon., 1970).

c. Ascorbic acid

Ascorbic acid content of the juice was determined by diluting a known volume of clear juice filtered through muslin cloth to appropriate volume. A ten millilitres of aliquot was titrated against 2, 6-dichlorophenol indophenol solution till light pink colour appeared. The result was expressed as mg of ascorbic acid per 100 g of fruit (Anon., 1970).

d. Sugars

The alcohol free aqueous extract was clarified by precipitation with saturated lead acetate and excess lead acetate was removed by precipitation with saturated solution of sodium dihydrogen phosphate. The clarified filtrate was made up to 25 ml. Sample from this aliquot was used for determination of sugars.

Table 1. Details of farmers from whose orchards samples were collected

Sl. No.	Clones	Original source of the planting material	Farmer's name and addresses	Year of planting
1	DPL-I	Dapoli Dt.: Ratnagiri	Mr. Yogesh Vilas Nevse, Krishidhan Nursary, At-Po: Tetavali, Tq:Dapoli, Dt.- Ratnagiri	1998-99
2	RTN-I	Ratnagiri	Mr.Mukund Yadav, At-Po: Neve budruk, Tq:Devrukh, Dt: Ratnagiri	1990-91
3	DVG-I	Lanja Dt: Ratnagiri	Mr.Rajan Vaman Gurav, At-Po: Manche, Tq:Devgad, Dt- Sindhudurg	1994-95
4	VEN-I	RFRS Vengurla Center, Dt:Sindhudurg	Mrs. Sulbha Arolkar, Arolkar Nursery, At-Po-Tq: Vengurla, Dt: Sindhudurg	1993-94
5	DWR-I	Ratnagiri	Dr.Mamaledesai, Dharwad	1993-94
6	DWR-II	Ratnagiri	Mr. Mahesh Matti, Saudatti, Dharwad	1992-93
7	DWR-III	Ratnagiri	Dr. B.C Patil, At: Jogellapur, Tq,-Dt:Dharwad	1988-89
8	BGM-I	Ratnagiri	Mr. Prasad Manerikar, Amarkung Nursery, At-Po:Asoga Tq:Khanapur, Dt: Belgaum	1960-61
9	BGM-II	Belgaum (Local Nursery)	Department of Horticulture, At-Po-Tq: Khanapur, Dt: Belgaum	1972-73

The content of reducing sugars was estimated by Nelson-Somogyi's method (Nelson, 1944). Working standard with concentration ranging from 10 to 100µg ml^{-1} was prepared. A reagent blank with 1 ml distilled water was also maintained one ml of alkaline copper reagent was added to all the tubes and placed in boiling water bath for 20 minutes, allowed to cool one ml of Arsenomolybdate reagent was added to all tubes and volume was made up to 20 ml with distilled water. The absorbance readings of the standards and samples against reagent blank, at 540 nm were recorded using a spectrophotometer.

The total sugar in the sample was estimated by the same method as that of reducing sugar after inversion (Anon., 1970). One millilitre of clarified extract was hydrolyzed with equal volume of 1N HCl on water bath at 49^oC for 45 minutes. This hydrolyzed mixture was neutralized with in 1N NaOH using methyl red as indicator and was made up to a volume 5 ml. The total sugar in the hydrolysate was estimated by Nelson Somogyi's method (Nelson, 1944).

3.3 STATISTICAL ANALYSIS

3.3.1 Physical and chemical characters

Fisher's method of analysis of variance as given by Panse and Sukhatme (1989) was applied for analysis and interpretation of data in Completely Randomised Design. The level of significance used for 'F' and 't' test was at P: 0.05 for field experiment and 0.01 for laboratory experiment. Critical difference (CD) values were worked out wherever 'F' test was significant.

3.3.2 Mahalanobis D^2 analysis

Mahalanobis (1936) D^2 statistics was used for assessing the genotypic divergence between populations belonging to the location specific clones. The generalized distance between any two populations is given by formula.

$$D^2 = \Sigma\Sigma\ \lambda_{ij}\ \sigma_{ai}\sigma_{aj}$$

Where D^2 = Square of generalized distance

λ_{ij} = Reciprocal of the common dispersal matrix

$\sigma_{ai} = (\mu_{i1} - \mu_{i2})$

$\sigma_{aj} = (\mu_{j1} - \mu_{j2})$

μ = General mean

Since, the formula for computation requires inversion of higher order determinant transformation of the original correlated unstandardized character mean (Xs) to standardized uncorrelated variable (Ys) was done to simplify the computational procedure. The D^2 value were obtained as the sum of squares of the differences between pairs of corresponding uncorrelated (γs) values of any two uncorrelated genotype of D^2 value (Rao, 1952).

3.3.2.1 Cluster of D^2 values

All n (n-1)/2 D^2 value were clustered using Tocher's method described by Rao (1952).

3.3.2.2 Intra cluster distance

The intra cluster distances were calculated by the formula given by Singh and Choudhary (1977).

$$\text{Square of the intra cluster distance} = \frac{\Sigma D^2 i}{n}$$

Where,

ΣD^2i is the sum of distance between all possible combinations of the entries included in a cluster.

n = Number of all possible combinations.

3.3.2.3 Inter cluster distance

The inter cluster distances were calculated by the formula described by Singh and Choudhary (1997).

$$\text{Square of the inter cluster distance} = \frac{\Sigma D^2 i}{n_i n_j}$$

Where,

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

n_i = Number of entries in cluster i

n_j = Number of entries in cluster j

3.4 EXPERIMENT NO.2 STUDY OF CLONAL VARIABILITY AMONG THE ‘ALPHONSO’ CLONES, BASED ON RAPD

3.4.1 Collection of samples

The ‘Alphonso’ mango scion sticks were collected from Ratnagiri and Sindhudurg districts of Maharashtra, as well as Belgaum and Dharwad districts of Karnataka. The exact addresses were presented in Table 1. They were transported through corrugated boxes with wet sphagnum moss and brought to the ‘Silver Jubilee’ Orchard for grafting purpose, to carry out further analysis. The leaves of these well maintained grafted plants were used for RAPD. DNA was isolated from these young leaves by following the method given by Doyle and Doyle (1990) *i.e.* CTAB method. After the extraction, the DNA was stored in deep freezer at - 20 °C in $T_{10}E_1$ buffer.

3.4.2 RAPD procedure

Molecular characterization was carried out using PCR based RAPD technique. The investigation was carried out in collaboration with Plant Molecular laboratory, Department of Pathology, College of Agriculture, Dharwad. Details of primers given in Table 2.

3.4.2.1 Plant material used

Young flush of leaves from the selected grafts were used for analysis. They were collected in the evening time and kept in plastic bags containing moist cotton and were carried immediately to the laboratory, where the samples were frozen immediately in liquid nitrogen and stored in -70˚C until further use.

3.4.2.2 Plant DNA extraction

DNA was isolated following the protocol of Doyle and Doyle (1990) with slight modification in buffer composition and concentration.

Table 2. Details of random primers

Sl. No.	Operon/ UBC Code	Sequence (5' 3')
1	UBC 83	GGGCTCGTGG
2	OPC 08	TGGACCGGTG
3	OPD 13	GGGGTGACGA
4	OPD 20	ACCCGGTCAC
5	OPA 11	CAATCGCCGT
6	OPA 13	TCGGCGATAG
7	OPA 18	AGGTGACCGT

Note : 0.4 μM of each RAPD primer was used in each reaction.

The steps followed were as given below:

1. A tissue sample of 3 g was grind to a fine powder in liquid nitrogen using mortar and pestle.
2. The powder along with some liquid nitrogen was transferred to a 50 ml of pre warmed (65℃) DNA extraction buffer when liquid nitrogen was completely evaporated.

Concentration of extraction Buffer

CTAB	4%
NaCl	1.4 M
EDTA (pH 8.00)	20 mM
Tris-HCl (pH 8.00)	100 mM
Mercaptoethanol	2%
PVPP	2%

3. The suspension was incubated at 65℃ for one hour with swirling after every 10 minutes.
4. The mixture was then allowed to cool at room temperature and emulsified with an equal volume of chloroform: isoamyle alcohol (24:1) for 5 minutes by inversion.
5. The contents were spun at 10,000 rpm for 15 minutes at room temperature.
6. The aqueous phase was transferred to a fresh tube and re-extracted with chloroform: isoamyl alcohol.

7. The aqueous phase was mixed with equal volume of chilled isopropanol and mixed gently.
8. The precipitated DNA was spooled out using a disposable pipette tip and washed twice with 70 per cent ethanol.
9. The spooled DNA was dried under vacuum in centrovap (Centrovap concentrator, LABCONCO)

3.4.2.3 DNA Purification

Purification of DNA was done to remove RNA, proteins and polysaccharides which were the major contaminants. RNA was removed by RNAase treatment. The RNAase was added to the DNA sample @100 µg ml^{-1} and incubated at 37˚C for 1 hour.

3.4.2.4 DNA Quantification

Concentration of DNA in the sample was determined after electrophoresis with standard DNA i.e., uncut lambda on 0.8 per cent agarose and by comparison of the intensity of staining with ethidium bromide.

The final concentration of DNA was adjusted to 250 ng μl^{-1} with sterile water.

3.4.2.5 Standardization of DNA concentration

Various dilutions of DNA were tried for standardization of DNA concentration to be used for the PCR reaction. The different dilutions tried were 1:1, 1:5, 1:10, 1:20, 1:50 and 1:100, 1: 150. The one which gave optimal amplification was chosen for actual work.

3.5 PCR- RAPD REQUIREMENTS

3.5.1 Template DNA

The dilutions corresponding to 2.5 ng μl^{-1} were used in PCR reaction.

3.5.2 DNA amplification

For the purpose of RAPD amplification arbitrary decamer primers obtained from Operon Technologies Inc. (Alameda, Ca, USA) and University of Columbia (Canada) were used. Initially six Operon Primers and two UBC primers were screened against the nine clones of Alphonso mango to study their suitability for the present study.

3.5.3 Taq DNA polymerase

Taq DNA polymerase and 10x Taq dilution buffer were obtained from M/S Bangalore Genei Pvt. Ltd., Bangalore.

3.5.4 dNTPs

Individual dNTPs viz. dATP, dGTP, dCTP and dTTP were obtained from M/S Bangalore Genei Pvt. Ltd., Bangalore.

3.5.5 Thermocycler

Eppendorff, Master cycler gradient supplied by Eppendorff gradient, 2231, Hamburg Germany was used for cyclic amplification of DNA.

3.5.6 Reaction mixture

One primer at a time was used to study the polymorphism among the nine clones by PCR-RAPD assay, with genomic DNA extracts from all clones as template DNA with one control (without template DNA). Master mix required was prepared a fresh together to avoid handling errors. The master mix was distributed to nine tubes (24µl per tube) and 1 µl of template DNA from the respective location was added to make the total reaction volume to 25 µl.

3.5.7 PCR-amplification

The PCR-amplification was carried out according to the following programme.

Stage	Step	Temperature (℃)	Duration (minutes/ seconds)	No. of cycles
I.	1. Initial Denaturation	94℃	3 minutes	1
II.	1. Denaturation	94℃	45 seconds	35
	2. Annealing	36℃	30 seconds	-
	3. Extension	72℃	2 minutes	-
III.	1. Complete Primer Extension	72℃	7 minutes	1
	2. Soak	4℃	-	-

After the completion of required cycles of amplification the samples were stored at 4℃ in a refrigerator and the contents were loaded on to agarose gels for electrophoresis.

3.5.8 Separation of amplified products by agarose gel electrophoresis

About 15 µl of the amplified products from each tube along with 2 µl of loading dye were loaded on 1.2 per cent agarose, using 1x TAE buffer of pH 8.0 along with λ DNA-EcoRI/ Hind III double digest as DNA molecular weight marker at100 v for 3 h and photographed using gel documentation system (UV1 Tech. Cambridge, England).

3.5.9 Agarose gel electrophoresis

The amplified products in RAPD reaction were separated by electrophoresis in 2 per cent agarose gel (SRL, India), containing Ethidium Bromide in 0.5x Tae Buffer (pH 8.00) and separation was carried-out by applying constant voltage of 60 volts for 5 hours.

The standard molecular marker used was Øx 174/Hae III.

PCR and gel electrophoresis were carried-out for three times and only reproducible patterns were used for data analysis.

3.5.10 Photography and Gel documentation

The gels were photographed under UV light using Pentax K 100 camera. The images of gels were also taken by the documentation systems (Pharmacia Biotech, USA) and they were saved in Liscap capture function for further analysis.

3.6 ANALYSIS OF PROFILES

3.6.1 Fingerprinting of genotypes

The gels were carefully studied and amplicons which occurred only once for a particular clone were marked which constituted the fingerprint for that particular clone. Additionally, the band fragments present in two clones were also marked, which in combination with other bands generated with other primers would constitute the fingerprints.

3.6.2 Statistical analysis

RAPD markers across the seven accessions were scored for their presence ‘1’ or absence ‘0’ of bands for each primer. By comparing the banding patterns of genotypes for a specific primer, genotype-specific bands were identified and faint or unclear bands were not considered. The binary data so generated were used to estimate levels of polymorphism by dividing the number of polymorphic bands by the total number of scored bands.

Pair-wise similarity matrices were generated by Jaccard’s coefficient of similarity by using the SIMQUAL format of NTSys-pc. A dendrogram was constructed by using the unweighted pair group method with arithmetic average (UPGMA) with the SAHN module of NTSys-pc to show a phenetic representation of genetic relationships as revealed by the similarity coefficient. The binary data was also subjected to principal component analysis (PCA) using the EIGEN and PROJ modules of NTSys Pc (Ganesh *et al*., 2007).

4. EXPERIMENTAL RESULTS

The results of the investigation are presented in this chapter under following headings.

Experiment No. 1: 4.1 Clonal variability among 'Alphonso' clones based on phenotypic parameters of ripe fruits

Experiment No. 2: 4.2 Clonal variability among 'Alphonso' clones based on RAPD

4.1 CLONAL VARIABILITY AMONG 'ALPHONSO' CLONES BASED ON PHENOTYPIC PARAMETERS OF RIPE FRUITS

The results pertaining to the phenotypic characters of fruits are presented in the Tables 3 and 4 (Fig. 1 and 2).

4.1.1. Physical parameters of fruits

4.1.1.1 Total fruit weight (g)

The total fruit weight at ripen stage exhibited significant differences among the clones. The Alphonso mango clone of BGM-I recorded significantly higher total fruit weight (260.80 g), which was on par with DVG-I (254.46 g), DPL-I (249.40 g), BGM-II (243.17 g) and VEN-I (226.98 g). The lowest fruit weight was recorded in DWR-II (193.70 g).

4.1.1.2 Length of fruit (cm)

The length of fruit ranged from 8.81 cm to 14.16 cm. RTN-I clone of alphonso mango recorded significantly higher fruit length (14.16 cm). However, it was on par with BGM-II (14.03 cm), DWR-II (13.85 cm), VEN-I (13.52 cm) and DWR-III (12.92). The lowest fruit length was observed in DWR-I (8.81 cm).

4.1.1.3 Diameter of fruit (cm)

The diameter of fruit was significantly higher in DVG-I clone of alphonso (7.78 cm) and it was on par with DPL-I (7.40 cm), BGM-II (7.32 cm) and VEN-I (7.28 cm). The least diameter of fruit was recorded in DWR-II (6.50 cm).

4.1.1.4 Weight of peel (g)

The ripe fruits of BGM-I clone recorded significantly higher weight of peel (40.79 g) followed by the other clones viz., DPL-I (37.10 g), BGM-II (36.12 g), DVG-I (35.90 g), DWR-III (30.85 g), VEN-I (30.32 g), DWR-I (28.43 g) and DWR-II (25.76 g). RTN-I clone was characterised by the lowest weight of peel (25.45 g).

4.1.1.5 Stone weight (g)

The stone weight of BGM-I was significantly higher (41.88 g). However, it was on par with that of DVG-I (40.55 g) and BGM-II (38.92 g) followed by DPL-I (35.44 g), VEN-I (34.85 g), DWR-I (34.67 g), DWR-II (30.95 g) and DWR-III (29.68 g), respectively. The RTN-I clone recorded significantly lower stone weight (27.14 g).

4.1.1.6 Stone thickness (cm)

The DVG-I alphonso fruit showed the highest thickness of stone (2.4 cm) which was on par with VEN-I (2.32 cm), BGM-II (2.3 cm), RTN-I (2.28 cm), DWR-II (2.28 cm) and DPL-I (2.22 cm), whereas, DWR-III recorded the lowest thickness of stone (2.08 cm).

Table 3. Physical parameters of fruits

Sl. No.	Alphonso clone	Total fruit weight (g)	Length of fruit (cm)	Diameter of fruit (cm)	Weight of peel (g)	Weight of stone (g)	Stone thickness (cm)
1	DPL-I	249.40	12.31	7.40	37.10	35.44	2.22
2	RTN-I	208.38	14.16	7.20	25.45	27.14	2.28
3	DVG-I	254.46	12.24	7.78	35.90	40.55	2.40
4	VEN-I	226.98	13.52	7.28	30.32	34.85	2.32
5	DWR-I	217.73	8.81	7.03	28.43	34.67	2.12
6	DWR-II	193.70	13.85	6.50	25.76	29.68	2.28
7	DWR-III	213.20	12.92	6.94	30.85	30.95	2.08
8	BGM- I	260.80	12.48	7.22	40.79	41.88	2.21
9	BGM- II	243.17	14.03	7.32	36.12	38.92	2.30
	Mean	229.76	32.30	7.18	32.30	34.90	2.24
	SEm±	13.131	0.43	0.191	1.02	1.78	0.07
	CD at 5%	37.825	1.25	0.549	2.93	5.14	0.19

Table 4. Pulp characters of fruits

Sl. No.	Alphonso clone	Weight of pulp (g)	Percentage of fruit pulp (%)	Pulp to stone ratio	Pulp to peel ratio	Shelf life of fruits (days)
1	DPL-I	176.65	71.16	4.99	4.77	23.00
2	RTN-I	155.72	74.89	5.75	6.12	22.40
3	DVG-I	177.83	70.28	4.48	4.97	22.60
4	VEN-I	161.33	72.11	4.64	5.33	23.20
5	DWR-I	154.61	71.96	4.50	5.45	17.40
6	DWR-II	138.00	71.28	4.52	5.35	17.60
7	DWR-III	151.42	71.32	4.81	5.03	16.00
8	BGM- I	178.46	69.84	4.34	4.38	17.20
9	BGM- II	167.98	69.71	4.37	4.67	16.40
	Mean	162.44	71.39	4.71	5.12	19.56
	SEm±	5.88	3.69	0.26	0.24	0.53
	CD at 5%	16.93	10.62	0.75	0.69	1.52

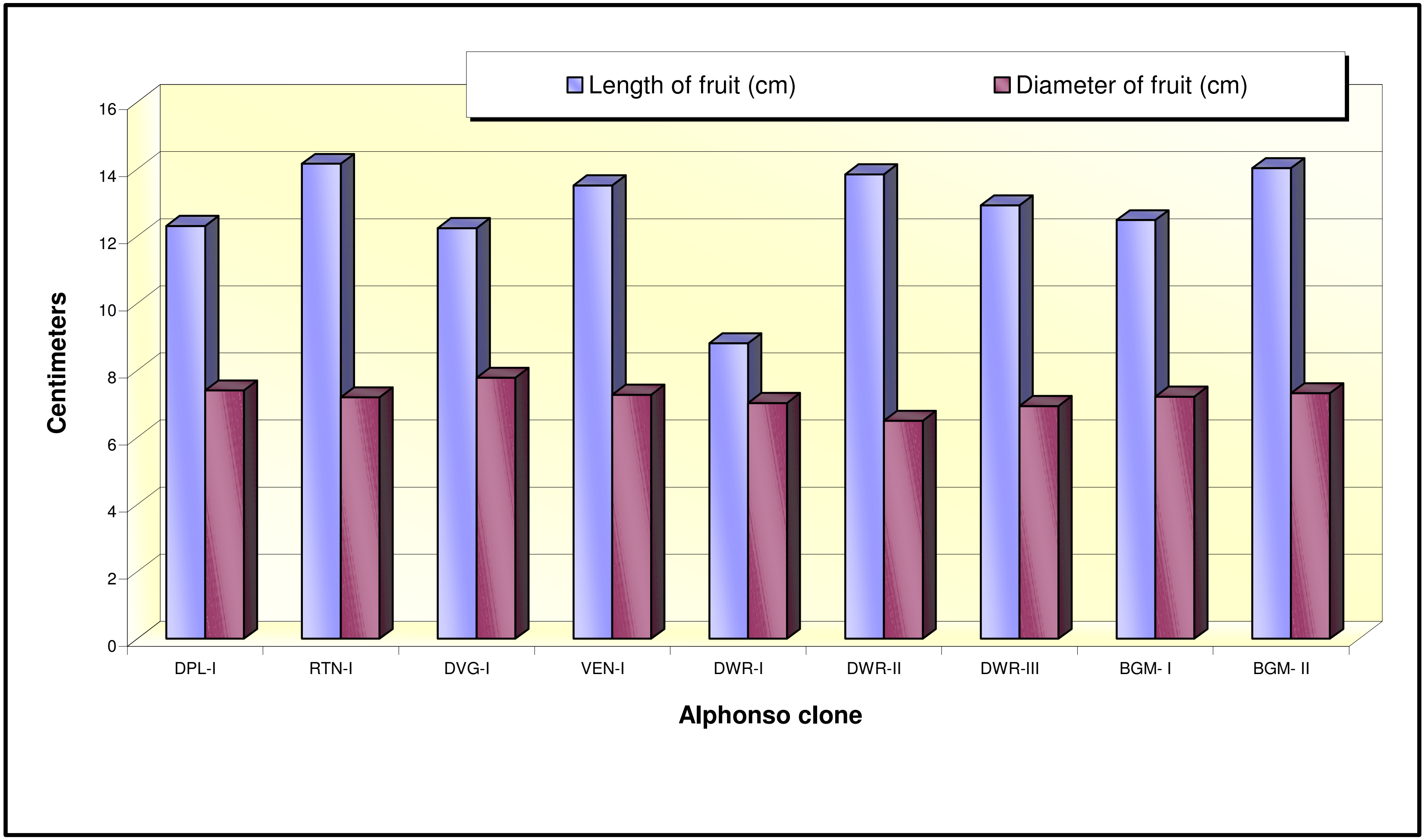


Fig. 1. Length and diameter of fruit

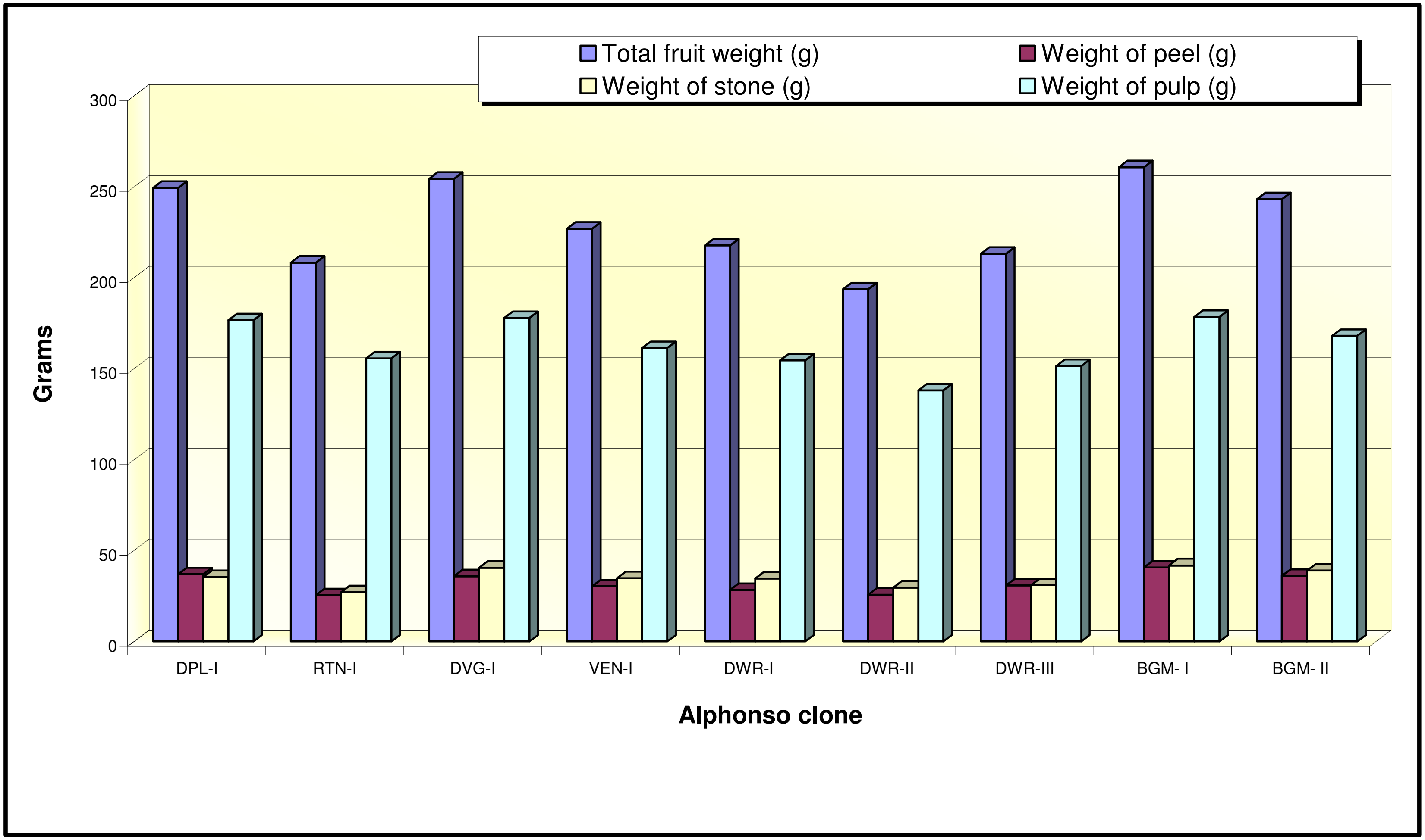


Fig. 2. Total fruit weight, weight of stone, peel and pulp (g) of fruit

4.1.1.7 Weight of pulp (g)

The clone, BGM-I recorded significantly higher weight of pulp (178.46 g), which was on par with DVG-I (177.83 g), DPL-I (176.65 g) and BGM-II (167.98 g). The minimum pulp weight was recorded by the fruits of DWR-II (138 g) at ripen stage.

4.1.1.8 Percentage of fruit pulp (%)

The percentage of fruit pulp did not differ significantly among the clones of the Alphonso mango. The highest percentage of fruit pulp was recorded in RTN-I (74.89%) and the lowest percentage of fruit pulp (69.71%) was recorded in BGM-I.

4.1.1.9 Pulp to stone ratio

The values of pulp to stone ratio of fruits of differed significantly among the location specific clones. The highest pulp to stone ratio was recorded by RTN-I (5.75:1), while the lowest pulp to stone ratio was recorded by BGM-I (4.34:1).

4.1.1.10 Pulp to peel ratio

Among the different Alphonso clones, the pulp to peel ratio of fruits was significantly higher in RTN-I (6.12:1), which was on par with DWR-I (5.45:1), whereas, the lowest pulp to peel ratio was observed in BGM-I (4.38:1).

4.1.1.11 Shelf life of fruit (days)

The highest shelf life of fruit was observed in VEN-I (23.20 days), which was on par with DPL-I (23.00), DVG-I (22.60) and RTN-I (22.40), whereas, the lowest shelf life was observed in the clone of DWR-III (16.0 days).

4.1.2 Chemical parameters

The results pertaining to chemical parameters such as total soluble solid (°Brix), titrable acidity (%), reducing sugars (%), total sugars and ascorbic acid content (%) of the Alphonso fruit at ripening stage are presented in the Table 5 and Fig. 3.

4.1.2.1 Total soluble solids (TSS) (°Brix)

The TSS of fruit at ripen stage recorded significant differences among the different location specific clones of alphonso mango. It was found that the DVG-I clone recorded a significantly higher TSS (18.28°B), which was on par with DPL-I (18.11°B) and the lower TSS was recorded in BGM-II clone (15.27°B).

4.1.2.2 Titrable acidity (%)

The titrable acidity differed significantly among the nine clones of Alphonso mango. Significantly higher titrable acidity was recorded in BGM-I (0.56%), which was on par with BGM-II (0.53%), DWR-II (0.51%), DWR-I (0.5%) and DWR-III (0.49%). The significantly lower titrable acidity was recorded in RTN-I clone (0.38%).

4.1.2.5 Ascorbic acid (mg/100 g fruit)

The ascorbic acid content of fruit differed significantly among the nine clones. VEN-I fruit exhibited significantly higher ascorbic acid content (62.66 mg/100 g fruit) and it was on par with the DVG-I (62.22 mg/100 g fruit) and DPL-I (60.62mg/100 g fruit) clones. Significantly lower Ascorbic acid content was recorded by the fruits of BGM-I clone (42.68 mg/100 g fruit).

4.1.2.3 Reducing sugars (%)

The VEN-I clone showed significantly higher content of reducing sugar content (4.49%) which was on par with DVG-I (4.16%) and DPL-I (4.03%), whereas, significantly lower content of reducing sugars (3.08 %) was observed in DWR-III clone of Alphonso (3.08%).

Table 5. Chemical parameters of fruits

Sl. No.	Alphonso clone	TSS (°Brix)	Titrable acidity (%)	Ascorbic acid (mg/100 g of fruit)	Reducing sugars (%)	Total sugars (%)
1	DPL-I	18.11	0.40	60.62	4.03	14.10
2	RTN-I	16.65	0.38	53.71	3.30	13.22
3	DVG-I	18.28	0.43	62.22	4.16	14.49
4	VEN-I	17.30	0.39	62.66	4.49	14.04
5	DWR-I	16.16	0.50	49.69	3.35	13.14
6	DWR-II	16.20	0.51	51.09	3.10	13.06
7	DWR-III	15.78	0.49	52.67	3.08	13.12
8	BGM-I	15.63	0.56	42.68	3.14	13.22
9	BGM-II	15.27	0.53	46.77	3.38	12.96
	Mean	16.58	0.468	54.73	3.72	13.59
	SEm±	0.29	0.04	1.93	0.22	0.20
	CD at 5%	0.84	0.11	5.55	0.64	0.57

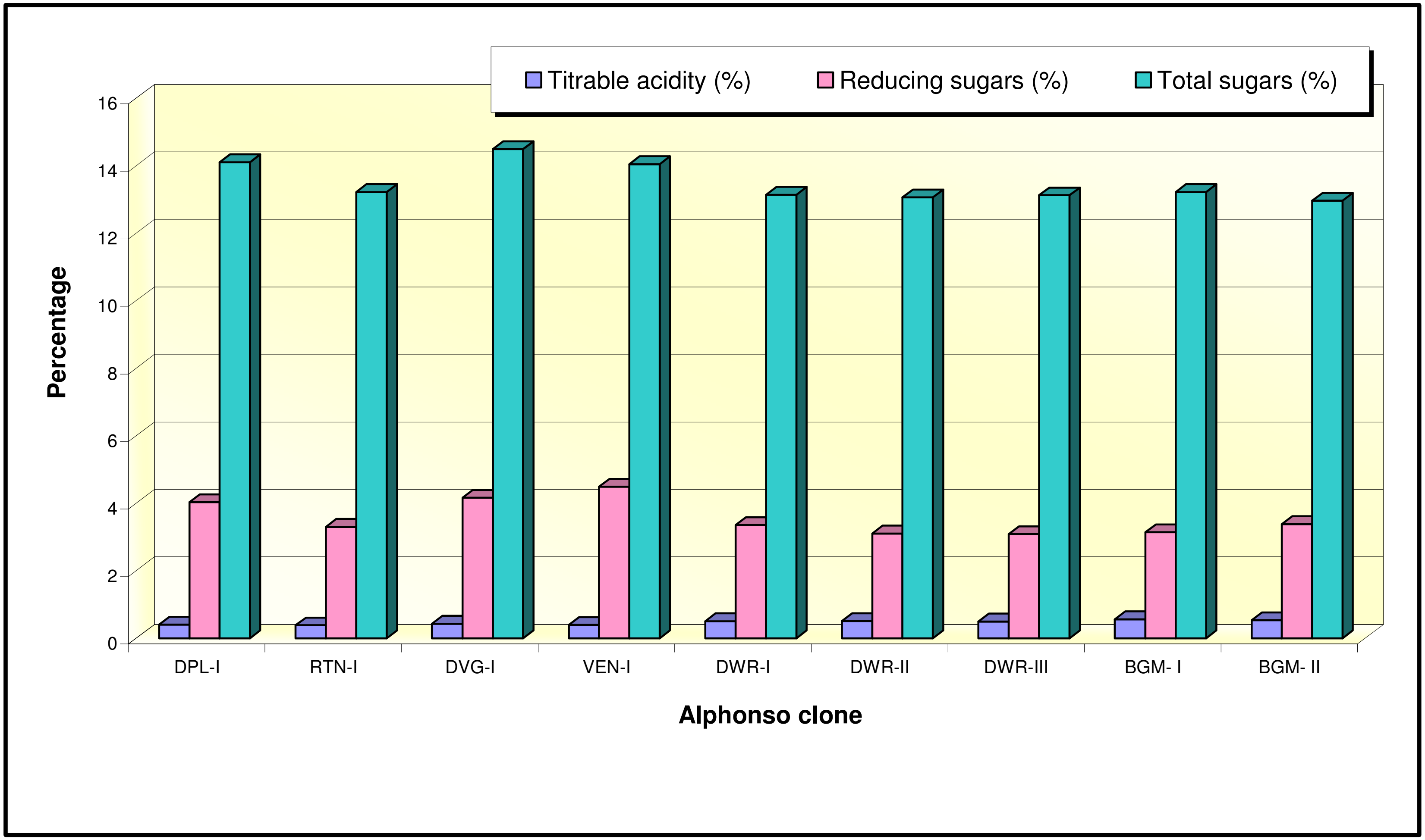


Fig. 3. Chemical parameters of fruits

4.1.2.4 Total sugars (%)

The total sugar content showed significant differences among the fruits of different location specific alphonso clones of mango at ripening stage. DVG-I fruit recorded significantly higher total sugar content (14.49%). However, it was on par with that of DPL-I (14.10%) and VEN-I (14.04%). The significantly lower total sugar content was recorded in DWR-II fruit (13.06%).

4.1.3 Genetic divergence (D^2) among the nine location specific clones of Alphonso

The data obtained from evaluation of nine location specific clones of Alphonso were used for testing divergence among the clones by employing Mahalanobis D^2 statistics.

4.1.3.1 Group constellations

A method suggested by Toucher (Rao, 1952) was used to group the genotype into different clusters based on D^2 values.

In the present study, nine location specific clones of Alphonso mango were grouped into two clusters using 16 characters. Among the two clusters, Cluster-I comprised of eight clones of alphonso mango. It was followed by Cluster-II, comprising of only one clone. It was presented in Table 6 and Fig. 4.

4.1.3.2 Per cent contribution of characters towards divergence

Per cent contribution of each character towards divergence was presented in Table 7 and Fig. 5. It was observed that weight of pulp contributed as much as 22.22% towards the divergence followed by total soluble solids (19.44%), total fruit weight (16.67%), stone thickness (13.89%) and shelf life of fruit (11.11%)

The remaining characters *viz.,* length of fruit, diameter of fruit, weight of peel, weight of stone, percentage of fruit pulp, pulp to stone ratio, pulp to peel ratio and chemical parameters *viz*., titrable acidity, ascorbic acid, reducing sugars, total sugars did not contribute significantly to the divergence of nine clones of Alphonso mango.

4.1.3.3 Average intra and inter relationship between clusters

For the nine clones of Alphonso, divergence (D^2) values of intra and inter cluster distance are presented in Table 8. The highest difference of the clones within the same cluster (Intracluster) was shown by cluster-II to cluster-I from 0.000 to 2.739 and Intercluster distance shown in cluster-I is 5.362

4.1.3.4 Cluster means

The results on cluster means in respect of 16 characters and overall character wise score across the two clusters are presented in Table 9.

In case of cluster-I, among the physical characteristics of the fruits, highest mean value was contributed towards the divergence by length of fruit (14.37 cm), thickness of stone (2.24 cm), percentage of fruit pulp (71.58), pulp to stone ratio (4.75), pulp to peel ratio (5.21), and shelf life of fruit (19.85 days). Among the quality parameters total soluble solids recorded a value of 16.69 followed by ascorbic acid (54.92 mg/100 g) and total sugars (13.62 mg/100 g).

In case of cluster-II, among the physical characteristics of fruit, the highest mean value was observed in total fruit weight (260.80 g) followed by diameter of fruit (7.22 cm), weight of peel (40.79 g), weight of stone (41.88 g), weight of pulp (178.46 g). The quality parameters were significantly higher in titrable acidity (0.56%), and reducing sugars (3.81 mg/100 g).

Table 6. Distribution of nine Alphonso clones in two clusters

Cluster	No. of Alphonso clones	Name of Alphonso clones
1	8	DPL-I, RTN-I, DVG-I, VEN-I , DWR-I, DWR-II, DWR-III, BGM-I
2	1	BGM-II

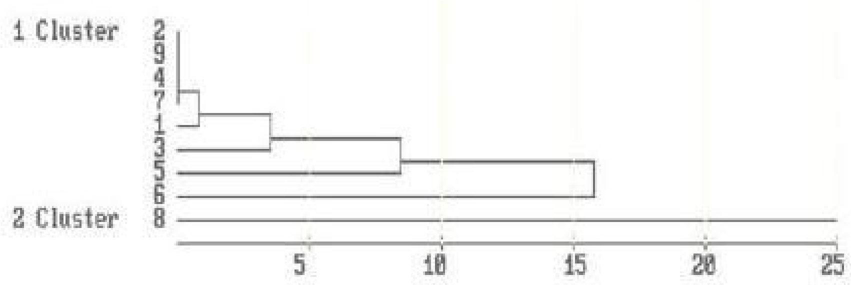


Fig.4. Genetic distance (D^2) analysis of the nine clones of alphonso mango

Table 7. The per cent contribution of characters towards divergence

Sl. No.	Divergence	
	Characters	Contribution (%)
1	Total fruit weight	16.67
2	Stone thickness	13.89
3	Weight of pulp	22.22
4	Shelf life of fruit	11.11
5	Total soluble solid	19.44

Table 8. Average intra and inter cluster D^2 value of nine Alphonso mango clones

Cluster	I	II
I	2.739	5.362
II	5.632	0.000

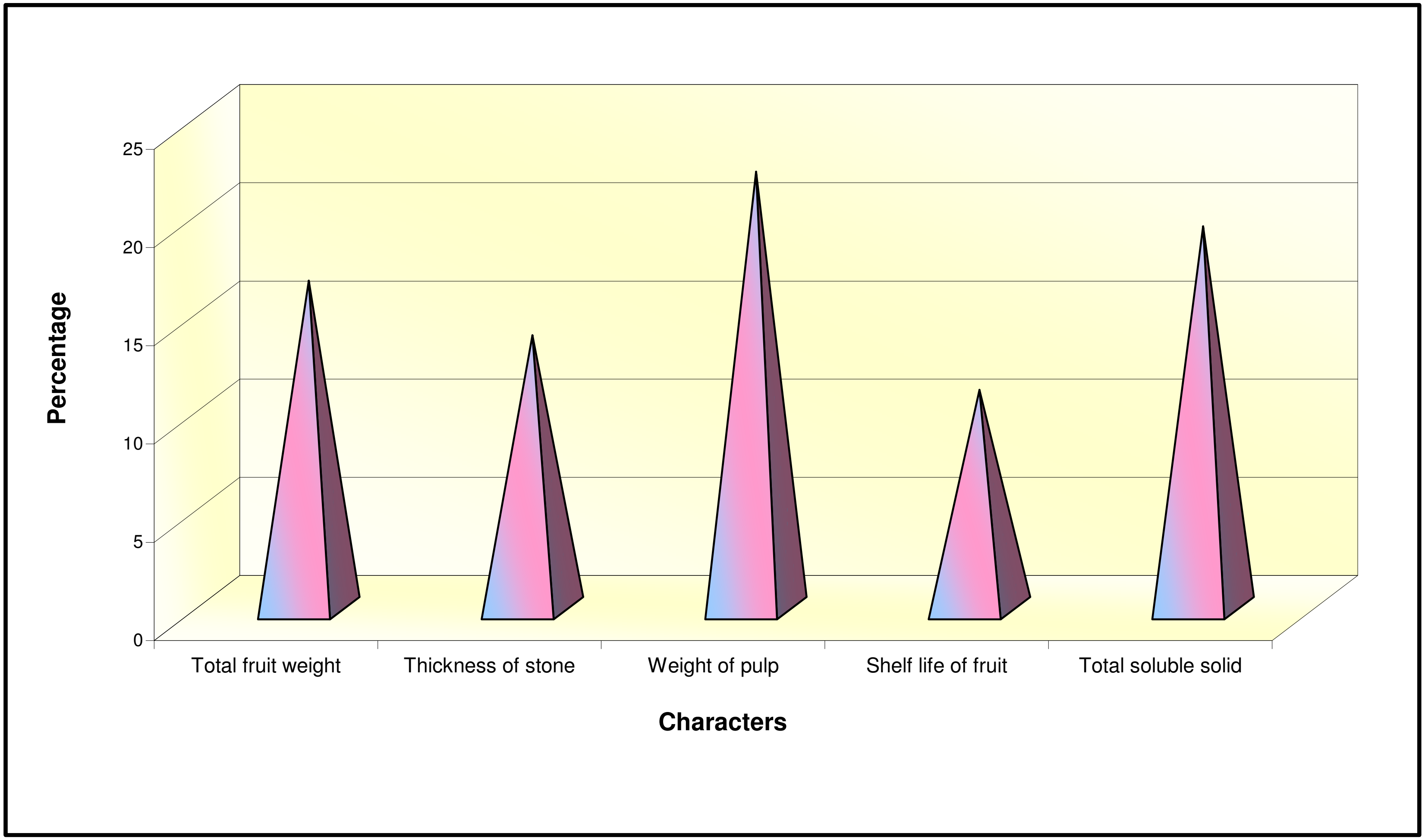


Fig. 5. Per cent contribution of characters towards divergence

Table 9. Mean values of 16 characters in two clusters of nine Alphonso clones

Sl. No.	Characters	Cluster-I	Cluster-II
1	Total fruit weight (g)	225.87 (2)	260.8 (1)
2	Length of fruit (cm)	14.37 (1)	12.48 (2)
3	Diameter of fruit (cm)	7.18 (2)	7.22 (1)
4	Weight of peel (g)	31.24 (2)	40.79 (1)
5	Weight of stone (g)	34.02 (2)	41.88 (1)
6	Thickness of stone (cm)	2.24 (1)	2.20 (2)
7	Weight of pulp (g)	160.44 (2)	178.44 (1)
8	Per cent of pulp of fruit	71.58 (1)	69.84 (2)
9	Pulp to Stone Ratio	4.75(1)	4.34 (2)
10	Pulp to peel ratio	5.21(1)	4.38 (2)
11	Shelf life (days)	19.85 (1)	17.20 (2)
12	Total soluble solids	16.69 (1)	15.63 (2)
13	Titrable acidity (%)	0.45 (2)	0.56 (1)
14	Ascorbic acid (mg/100gm)	54.92 (1)	42.68 (2)
15	Reducing sugars (m/100gm)	3.70 (2)	3.81 (1)
16	Total sugars (mg/100gm)	13.62 (1)	13.22 (2)
	Total	Mean-41.63 score 24 (1)	Mean-44.71 score 25 (2)

4.2 CLONAL VARIABILITY AMONG 'ALPHONSO' CLONES BASED ON RAPD

4.4.1 Primer screening

In all, eight random primers comprising of six Operon primers and two UBC primers, were screened in the present study.

All the six Operon primers screened were amplified, of which three were polymorphic and the other three were monomorphic. Whereas, only one out of the two amplified UBC primers showed polymorphism.

Thus, out of eight random primers screened seven primers gave scorable bands, out of which three were monomorphic and the rest were polymorphic, across the nine location specific 'Alphonso' clones.

4.4.2 Per cent polymorphism

The analysis of binary data showed that seven random primers produced 36 bands in the range of 0.53 to 1.61 Kb (Table 10). Out of these 36 bands, twelve showed polymorphic patterns. The number of bands per primer varied from two (UBC 83, OPA 11) to eight (OPD 20), across the seven random primers (Plate 1 and Plate 2). The mean polymorphism observed was only 36.38 per cent across the seven primers. Primers with the highest per cent polymorphism were UBC 83, OPA 12, OPC 08 and OPA 13. The primers OPD 20, OPA 18 and OPA 11 did not reveal any polymorphism.

4.4.3 Genetic distance

Jaccard's similarity coefficient was generated based on PCR it is presented in Table 11. From the similarity matrix of nine Alphonso clones, it was evident that there is 100 per cent similarity among the clones namely DVG-I, RTN-I, VEN-I, DPL-I, DWR-I and DWR-II clones with a similarity coefficient of 1.00. That means to say that there is no clonal variation among these six clones of Alphonso mango. Whereas, the clone DWR-III with similarity coefficient was 0.964, slightly differed from the above six clones. However two clones namely BGM-I and BGM-II showed the least similarity coefficient of 0.885 and 0.827 respectively and differed from the rest of clones.

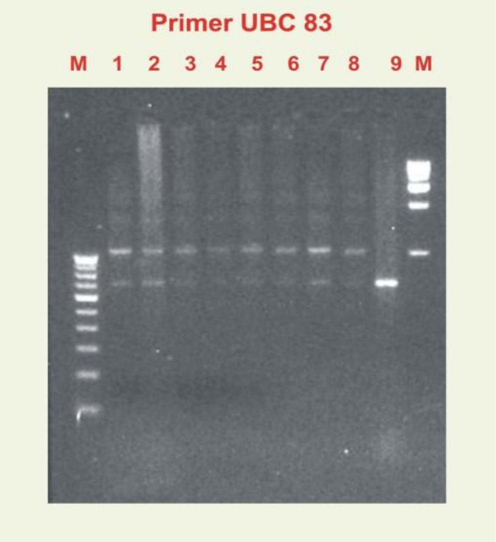
The dendrogram based on genetic relatedness grouped all the nine Alphonso clones into two major clusters. The first major cluster in turn comprised of two sub cluster one having six clones (DVG-I, RTN-I, VEN-I, DPL-I, DWR-I and DWR-II clones) and the other having one (DWR-III) clone. On the other hand, the second major cluster had two clones namely BGM-I and BGM-II (Table 12 and Fig. 6).

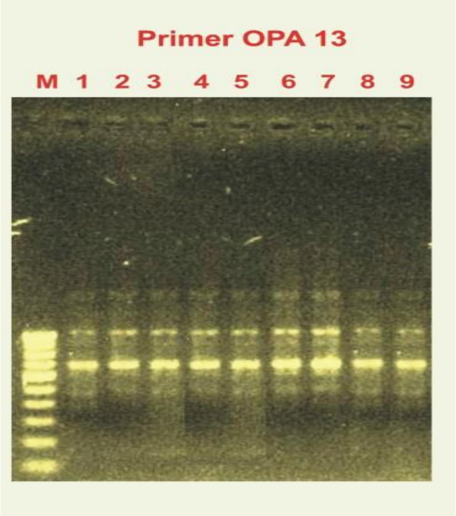
Table 10. Summary of statistics about the random primers and per cent polymorphism with respect to nine clones of Alphonso mango

Primer	Total number of bands generated	Number of polymorphic bands	Range or size of bands	Percentage of polymorphism
UBC 83	2	2	0.7 to1.1 kb	100
OPA 13	6	1	0.5 to 1.6 kb	16.66
OPD 20	8	0	0.18 to 1.9 kb	0
OPC 08	6	4	0.05 to 2.0 kb	66.66
OPA 12	7	5	0.3 to 3.0 kb	71.4
OPA 18	3	0	0.4 to 1.0 kb	0
OPA 11	2	0	0.5 to 0.7 kb	0
Total	36	12	0.53 to 1.61 kb	36.38

Table 11. Similarity matrix of nine Alphonso clones using Jaccard's coefficient of similarity

Alphonso clones	DVG-I	RTN-I	VEN-I	DPL-I	DWR-I	DWR-II	DWR-III	BGM-I	BGM-II
DVG-I	1.000								
RTN-I	1.000	1.000							
VEN-I	1.000	1.000	1.000						
DPL-I	1.000	1.000	1.000	1.000					
DWR-I	1.000	1.000	1.000	1.000	1.000				
DWR-II	1.000	1.000	1.000	1.000	1.000	1.000			
DWR-III	0.964	0.964	0.964	0.964	0.964	0.964	1.000		
BGM-I	0.885	0.885	0.885	0.885	0.885	0.885	0.847	1.000	
BGM-II	0.827	0.827	0.827	0.827	0.827	0.827	0.857	0.918	1.000





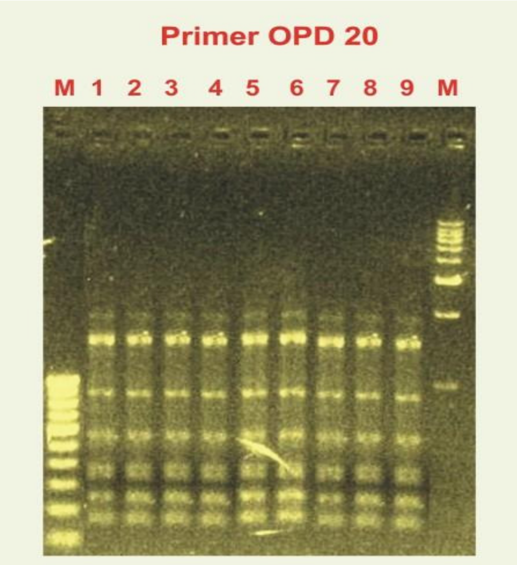


Plate 1. RAPD gel profiles of nine Alphonso clones using UBC 83, OPA 13 and OPD 20

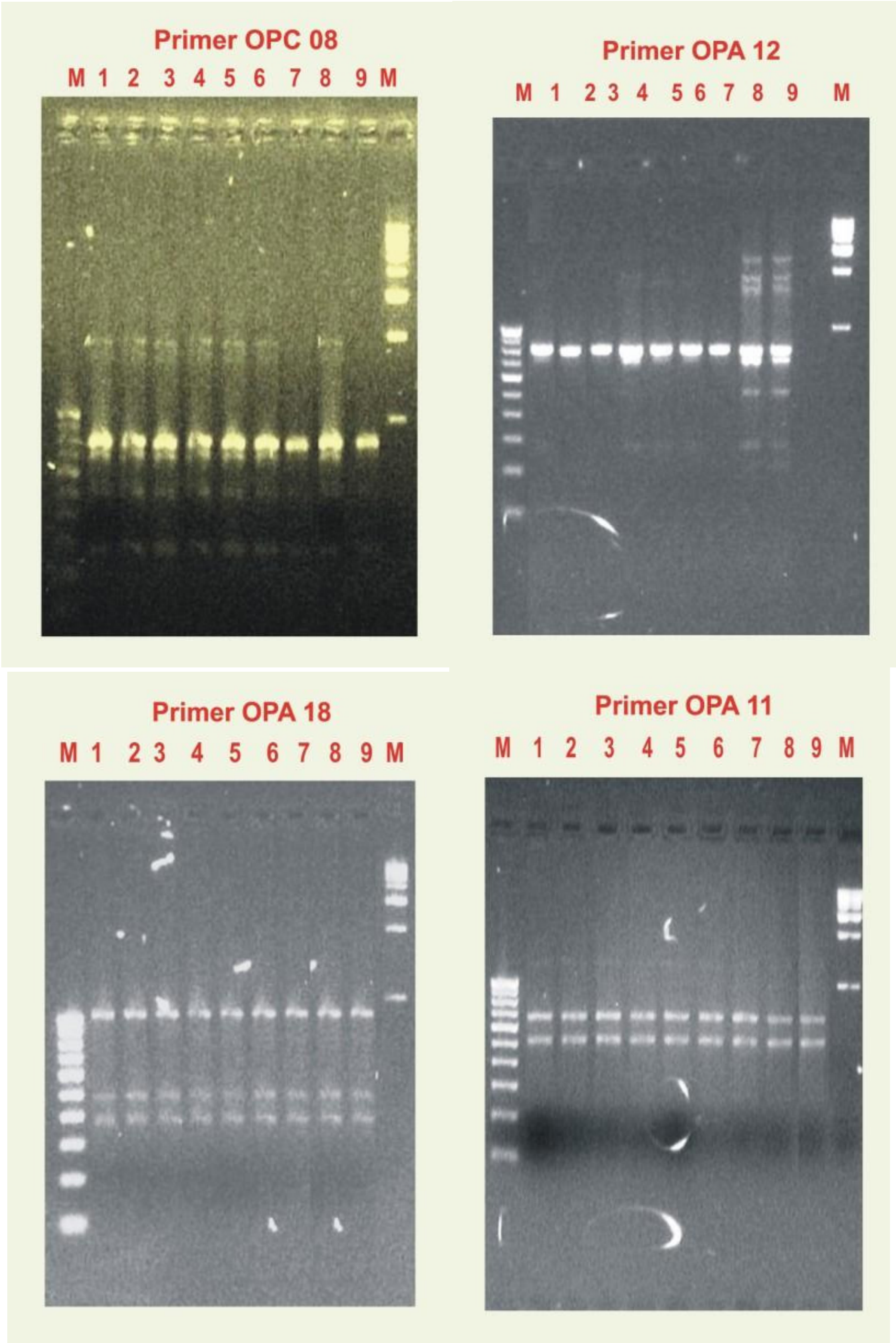


Plate 2. RAPD gel profiles of nine Alphonso clones using OPC 08, OPA 12, OPA 18 and OPA 11

Table 12. Distribution of Alphonso clones into two clones in clusters

Cluster- A		Cluster- B	
DVG-I		BGM-I	
RTN-I			B
VEN-I		BGM-II	
DPL-I	A1		
DWR-I			
DWR-II			
DWR-III	A2		

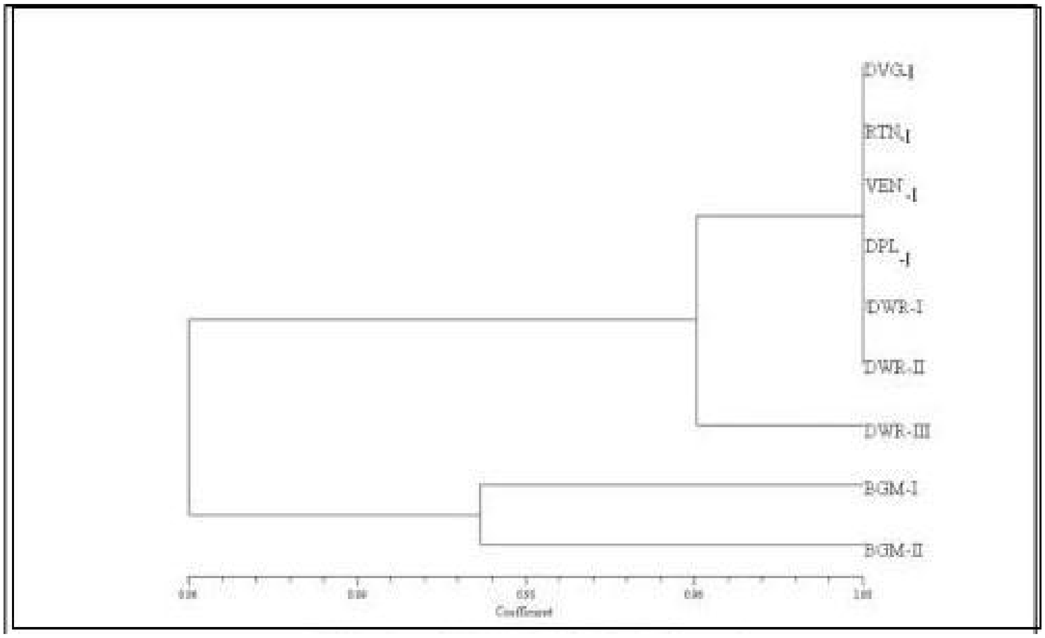


Fig. 6. Jaccard similarity coefficient of nine alphonso clones

5. DISCUSSION

Mango (*Mangifera indica* L.) is rightly known as 'King of fruits' and 'The pride fruit of India', on account of its extensive cultivation and the most appealing taste, among different tropical fruits, widely accepted by consumers. Although the crop is characterised by having enormous cultivars, only a group of three to four cultivars occupies more than 60 per cent of mango area in our country 'Alphonso' is one such cultivar, preferred by domestic as well as foreign fruit markets. Besides its export potential, this cultivar has a lion share in the domestic fruit market. The cultivar is highly popular in Maharashtra and Karnataka regions, where a large number of public and private nurseries are involved in the supply of plant material. This ensured the spread of 'Alphonso' variety on commercial scale. Even though the planting material has been multiplied vegetatively (softwood grafting or epicotyl grafting), often clonal variation is routinely observed under field conditions (Rosetti *et al.*, 2004, Gurudatta *et al.,* 2004, Islam *et al*., 2004, Patil *et al.,* 2006, Prasanth *et al.,* 2007). Clonal variations are manifested many times in fruit characteristics besides other vegetative characteristics and yield attributes. Such clonal variations in Alphonso have led to the selection of new cultivars within Alphonso blood. In view of the above observations it was felt necessary to understand the extent of clonal variations, if any, among the clones of Alphonso variety, planted in different areas of Maharashtra and Karnataka states.

Since the crop is vegetative propagated, the trees of Alphonso cultivar were subjected to sub-cloning repeatedly over a period of time, across several geographical locations. This would have resulted in the production of bud sports or somatic mutants. Intra-cultivars study of genome from different locations can confirm whether there are any genetic differences among the location specific clones or not.

Molecular techniques have become very handy in solving such biological problems (Karp *et al*., 1997, Neto *et al*., 1995, Lee 1995). A number of DNA markers namely RFLP, RAPD, AFLP, SSR (Simple sequence repeat marker) *etc*. are being used for solving various types of biological problems and in molecular breeding (Staub and Serquen, 1996) RAPD markers have been used most widely for their simplicity in technique and easy handling nature in instance, Schnell and Knight (1993) studied the genetic relationship among *Mangifera* species by employing RAPD markers.

In the present investigation, an attempt was made to cluster the eco-types of Alphonso, based on Random Amplified DNA Polymorphic pattern as well as morphological markers. The results obtained are discussed here under the following major sub-sections,

5.1 Clonal variability among clones for physical parameters of fruits

5.2 Clonal variability among clones for chemical parameters of fruits

5.3 Genetic divergence (D^2) analysis

5.4 Clonal variability of alphonso mango based on molecular study

5.1 CLONAL VARIABILITY AMONG CLONES FOR PHYSICAL PARAMETERS

The total fruit weight differed significantly among various clones of Alphonso mango. BGM-I recorded significantly highest total fruit weight, which was on par with DVG-I, DPL-I, BGM-II and VEN-I while the minimum fruit weight was recorded by DWR-II. The differences in total fruit weight of clones of alphonso under study appeared to be due to the basic clonal difference amongst them. These differences could be also due to the differences in the size of fruit which were revealed by the differences in the values of fruit length and fruit diameter. The length of the fruit differed significantly among the different clones of Alphonso mango. Ratnagiri clone recorded significantly maximum length, which was on par with BGM-II, DWR-II, VEN-I and DWR-III, while DWR-I recorded the minimum fruit length. Similarly, the diameter of fruit was maximum in DVG-I fruit followed by DPL-I and BGM-II, while it was minimum in DWR-I. An examination of data these parameters revealed that, most of the clones that produced heavier fruits, also had large sized fruits. This was more clearly revealed when the trend in product of length and breadth of fruits was examined, rather than that in either length or breadth of fruit alone. Thus, most of the fruit dimensions were similar among RTN-I, VEN-I, DWR-I, DWR-II and BGM-I clones. These results are also confirmed by the clustering pattern as observed in the genetic divergence analysis. Similar results in these parameters among different cultivars of mango, were also reported by Patil (1990), Doreyappagowda and Ramanjaneya (1994), Gunjate *et al*. (1995) and Kumar and Jaiswal (2006), in different clones of Alphonso mango.

The weight of peel as well as that of stone showed significant differences among the different clones. It is interesting to note that both peel and stone weights were maximum in the fruits of BGM-I, while both of them were minimum in the fruits of RTN-I clone. This indicated that the fruits of RTN-I clone had minimum photosynthetic substances diverted towards peel and stone, as compared to BGM-I. It is also evident from the results of pulp percentage, pulp to peel as well as pulp to stone ratio values. All the three characters had the maximum values in Ratnagiri fruits and minimum in BGM-I fruits. The maximum pulp per unit of peel and stone was recorded by the fruits of Ratnagiri clone, while the accumulation of pulp per unit of peel and stone was minimum in the fruits of the clone BGM-I of Alphonso mango. This clearly suggests that there were differences in the proportion of photosynthetic substances diverted towards the pulp component of fruit, which might be probably due to genetic differences or environmental differences. The farthest genetic location of RTN-I clone is evident from the RAPD pattern, which is discussed under forthcoming sub section. With regard to environmental differences, Ratnagiri is characterised by relatively more even rainfall during the months of June to September and more clear weather during the months of March to May, as compared to Belgaum conditions (Appendix II and III). This might have enabled the fruits of Ratnagiri clone, to attract a greater proportion of photosynthetic substances, some of which would have diverted to vegetative growth, in case of moist or rainy weather during fruit development, as under Belgaum conditions. However, the clone BGM-I had significantly higher weight of fruit, peel, stone and pulp as compared to other clones, which might be due to lower number of fruits per tree under Belgaum conditions, as compared to Ratnagiri conditions. Further, it is not the highest individual fruit weight, that actually is important, but the highest proportion of pulp to total fruit weight, which enables a fruit highly preferable by consumer. However, there is less magnitude of divergence based on these characters as evident from the values of per cent contribution towards divergence from the D^2 analysis. The maximum value of contribution towards divergence was only 22.22 per cent recorded by the character weight of pulp. Therefore, it can be inferred that there is less clonal variability among the nine locations, which is confirmed by the clustering pattern since most of the clones were grouped under the same cluster.

The shelf life of fruits differed significantly among various clones of ‘Alphonso’ mango. The fruits of VEN-I clone recorded significantly maximum shelf life, which was on par with DPL-I, RTN-I and DVG-I, whereas, the minimum shelf life was observed in DWR-III. The differences in shelf life among the clones is also in conformity with those obtained in case of pulp to peel ratio, pulp to stone ratio, pulp percentage. The results on shelf life are also in favour of the clones from RTN-I and surrounding geographical locations, recording the maximum values. These results suggest that these clones are benefited by favourable environmental parameters under Ratnagiri conditions, which, on the other hand, relatively unfavourable in case of Dharwad and Belgaum regions, as inferred from the lower values recorded by the clones DWR-III, BGM-I and BGM-II. The studies on genetic diversity as well as RAPD also confirmed the same.

Kulkarni and Rameshwar (1981), Reddy and Singh (1991), Yadav *et al.* (1984) and Kumar and Jaiswal (2006), Doreyappagowda and Ramanjaneya (1994), Ramsingh *et al.* (2003), Sanjay Singh (2003), Wasker and Dhemre (2004) and Abu-Goukh and Mohammed (2004), also observed significant differences in these parameters among different mango varieties. However, it is inferred that even though there were differences among the nine location specific clones, the magnitude of differences were less and in overall, most of the locations represented one and the same clone of ‘Alphonso’ mango.

5.2 CLONAL VARIABILITY AMONG CLONES FOR CHEMICAL PARAMETERS

The total soluble solids of fruit pulp registered a significantly maximum value in DVG-I alphonso clone, while BGM-II was found to have the lowest value among the nine clones. The highest titrable acidity was recorded in BGM-I followed by BGM-II and DWR-II, while the lowest titrable acidity was observed in RTN-I clone. Reducing sugar content was significantly maximum in VEN-I at ripe stage followed by DVG-I and DPL-I, whereas, the minimum reducing sugar content was recorded by DWR-III. The total sugar content of fruit pulp registered a significantly maximum value in DVG-I alphonso clone, while BGM-II was found to have the lowest value among the nine clones of Alphonso mango. The content of ascorbic acid was maximum in the fruits of VEN-I clone and minimum in case of BGM-I.

The results on total soluble solids, titrable acidity, reducing and total sugar content as well as ascorbic acid content indicated that there were significant differences among the nine location specific alphonso clones under study. Either DVG-I, VEN-I or RTN-I clones recorded significantly maximum values in case of total soluble solids, reducing sugar content, total sugar content as well as ascorbic acid content, while the minimum values were recorded by either BGM and DWR clones. On the contrary, titrable acidity was found to be maximum in BGM-II and minimum in RTN-I. The trend exhibited in these results also confirms that there was a favourable influence of environmental parameters that prevailed along the Konkan coast, on the quality parameters. Partitioning of a higher proportion of carbohydrates to the pulp in the fruits belonging to Maharashtra clones, might have led to greater values of total soluble solids and sugar content in them as compared to BGM and DWR clones. On the contrary, accumulation of a lesser proportion of carbohydrates with respect to organic acids, in the pulp might have resulted in greater values of titrable acidity among the fruits belonging to BGM and DWR clones. RTN-I, DVG-I and VEN-I clones are on par with each other in case of all the chemical characters of ripe fruits. The fruits of these clones were found to have significant superiority as compared to those of the rest of clones, as seen from the results of chemical parameters, especially total soluble solids, reducing and total sugar contents. However, there is less magnitude of divergence based on physical as well as chemical characters ripe fruits as evident from the values of per cent contribution towards divergence from the D^2 analysis. The per cent contribution towards divergence was only 19.44 recorded by total soluble solids. Therefore, it can be inferred that there is less clonal variability among the nine locations, which is confirmed by the clustering pattern since most of the clones were grouped under the same cluster.

Roy *et al.* (1980), Kalra and Tandon (1983) and Yadav *et al.* (1984), Lakshminarayana *et al.* (1980), Shyamalamma (1993), Singh (1991), Kaushik and Ranjit Kumar (1992), Sahani and Khurdiya (1994), Deng-Yi-Cai *et al.* (1995), Doreyappagowda and Huddar (2001), Gautam *et al.* (2003), Gohil (2006) and Shivshankar *et al.* (2007) also observed significant differences in these parameters among different mango varieties.

5.3 GENETIC DIVERGENCE (D^2) ANALYSIS

Before aiming for improvement in crop, it is essential to have knowledge of variability present in the population. The existence of genetic variability among the clones for the characters to be improved is the most important basic factor for successful selection. Evaluation of large number of genotypes is the basic step for successful breeding programme. The genotypes and environmental variance and their interaction (G×E) can be determined by employing useful biometrical tools. Mahalanobis D^2-statistic is a very sensitive tool for measuring genetic divergence based on phenotypic and qualitative traits and is widely used by many geneticists and breeders for selecting divergent parents for hybridization among the genetic improvement in Alphonso mango. However, the utility of generalised distance concept in classifying different clones would depend upon the consistency of the results from location to location. The present investigation was planned to conduct among the nine different location specific clones with an objective of identifying the genetically diverse alphonso clones for their use in mango improvement programme.

The nine clones of Alphonso studied formed two clusters with different levels of coefficients. In the first cluster, eight clones were grouped together, where as in second cluster only one clone was involved at a certain level of divergence. Most of the clones are on par with each other when fruit dimensions, pulp characters and chemical parameters are considered. No single character recorded more than 25 per cent of contribution towards genetic divergence. Therefore, the population of 'Alphonso' clones among the selected locations mostly represented the same clone, even though there were differences, they are proved to be of lesser magnitude.

5.3.1 Genetic divergence in two clusters

The nine alphonso clones were grouped into two clusters using Toucher's method (Rao, 1952) employed on the Mahalanobis generated distance (D^2) values. The cluster-I accounted for eight clones of Alphonso followed by cluster-II accounting for one clone. Formation of solitary cluster is due to geographical barriers preventing gene flow, intensive natural clonal selection or artificial clonal selection for diverse adoptive gene complexes. However, grouping pattern indicated that geographic diversity according to location specific clones seems to be hundred per cent direct association with genetic diversity. This is as evidenced by the clones of Alphonso originating in the same geographical region and occupying the same cluster, while different genotypes with same origin occupied different cluster. Similar findings were earlier reported by Wahab and Gopalkrishnan (1993) and Varalakshmi *et al*. (1994) in ridge gourd.

The maximum contribution to the total divergence was from weight of pulp at 22.22 per cent followed by total soluble solids (19.44%), total weight of fruit (16.67%), stone thickness (13.89%) and shelf life (11.11%). This contribution is important for the purpose of further selection and choice of parents. For hybridization, however, Parhi *et al.* (1993) reported that hundred seed weight contributed maximum towards divergence, followed by number of seeds per fruit and yield per plant. These differences in the contributing factors for genetic divergence could be attributed to differences among the clones under study, which in term might be due to environmental conditions of locations associated and interacted with the clones of Alphonso.

The main objective of D^2 analysis is to identify diverse clusters and to select genotypes from them for hybridization. In the present study, cluster-I showed the lowest distance (D^2=2.739), whereas, the Intercluster distance was maximum in cluster-II (D^2=5.362). Similar views were expressed by Wahab and Gopalkrishnan (1993), Parhi *et al.* (1993), Prasad and Singh (1997) and Ram (2001) in pointed gourd.

The means obtained for various characters from varying number of clones in each cluster gives an idea of divergence among the clusters compared. It also helps to group the cluster according to their average performance.

5.4 CLONAL VARIABILITY OF ALPHONSO MANGO BASED ON MOLECULAR STUDY

The Department of Horticulture under the jurisdiction of University of Agricultural Sciences, Dharwad is a major centre for germplasm collection and maintenance of mango. Therefore, the present work of characterization of genetic diversity among the selected Alphonso clones from different locations of both the Maharashtra and Karnataka states using molecular markers was initiated. In this work, an initial step was to explore the use of RAPD markers to assess the diversity in a representative set of clones available at selected locations in both the states of Maharashtra and Karnataka. As a pre-requisite, the PCR protocol was optimized and stringently followed without any variation throughout the analysis.

A preliminary screening of random decamer primers from two different sources *viz.,* Operon (O-series) and University of British Columbia (UBC series) was carried out to select primers that gave reliable polymorphism. The primers were carefully selected for further consideration in the analysis and only those bands which appeared reproducibility in three PCR runs were scored. Thus, by taking all these precautions, it was possible to use RAPD markers effectively for diversity assessment of mango and they proved to be promising method to detect genetic diversity and study the genetic relationships among the nine alphonso clones of Maharashtra and Karnataka state. About 36.38 per cent of mean polymorphism was detected among in the clones under consideration.

5.4.1 Comparison of UBC and Operon Random Primers

In the present study, Operon primers revealed a little higher per cent polymorphism than UBC primers and therefore, can be considered good for mango diversity analysis. Among the primers studied, three Operons and one UBC primers namely, OPA-13, OPC-08, OPA-12 and UBC-83 were observed to be good for revealing maximum polymorphism. In fact, some of the primers showed 100 per cent polymorphism. As far as Alphonso clones are concerned, there is no record of UBC primers in RAPD analysis. So far, only Operon primers have been used for mango diversity analysis according to Schnell and Knight (1993), Schnell and Knight (1995), Lopez *et al*. (1997) and Ravishankar *et al.* (2000). Most of the Operon primers, screened in the present study showed polymorphism, as compared to only one UBC primer, showing polymorphism.

5.4.2 DNA fingerprint pattern for Alphonso mango clones

DNA finger print can be considered as DNA band profile which is unique to variety or line generated by a highly polymorphic marker. With RAPDs, there are no reports of obtaining a fingerprint for any mango variety with a single primer so far. The present study also accorded with the previous findings. In general, it is observed that to obtain a fingerprint, many polymorphic markers need to be used, since, number of polymorphic bands generated is less using a single primer as compared to other marker systems like AFLP and hybridization based microsatellites or minisatellites.

Schnell *et al.* (1995) analyzed RAPD data from each primer, used in pairs and obtained a unique pattern for 25 mango individuals with such primer combinations. It was suggested that similar primer combinations can be used to develop fingerprints for Indian Alphonso mango varieties. It is also possible to obtain unique patterns for cultivar identification purpose if a large number of random primers are used.

Although finger print could not be developed for individual Alphonso mango clones using single RAPD primers, in this study, a few variety specific bands were observed. Details of these bands are given in the Table 10. These clone specific bands have a potential use in germplasm maintenance. RAPD markers have the ability to target the regions which are unique to genome and can be used to rectify the cases of misidentified clones. However for this application the specificity of the markers needs to be established using a large number of accessions of the same clones. Apart from clones specific bands no group specific bands or pattern was obtained using any of the primers singly.

Plant genetic resources in horticulture include the sexually or vegetative propagated material of cultivars in current use and newly developed varieties, absolute cultivars, farmers traditional cultivars and land races, wild relative of cultivated species, special genetic stock including elite and current breeder lines etc. Mango has an enormous varietal wealth indigenous to India.

The results on RAPD based diversity among the nine clones of Alphonso mango are discussed here. The results revealed that out of seven random primers four primers showed polymorphism across nine alphonso clones based on combined banding patterns of all the nine mango clones. Pair wise similarity coefficient for nine clones indicated that first six clones (DVG-I, RTN-I, VEN-I, DPL-I, DWR-I and DWR-II) were observed to have 100 per cent similarity without any clonal variations, which were grouped together in sub-cluster-I of cluster-I. Similarly one clone, *i.e.* DWR-III was monoclinic grouped under sub-cluster-II of cluster-I with a similarity coefficient of 0.964.

The first four clones grouped in cluster-I, *viz.*, DVG-I, RTN-I, VEN-I and DPL-I are from more or less the same geographic locations. Obviously these clones showed similarity to the highest degree and indicated their clonal purity. The environmental conditions of the locations from where the first four clones collected appeared to be the same because these areas are the part of the same coastal area of Konkan region of Maharashtra. The source of collection of clones DWR-I and DWR-II, was the same as that of RTN-I clones. Therefore, similar clones were also grouped together in cluster-I.

In Konkan region of Maharashtra where alphonso is largely cultivated, half-sib stones will be invariably used for raising the rootstocks for grafting alphonso variety. The rootstock effect on scion appeared to be the same, which was evident in the absence of clonal variations in scion material, as in the case of the first four clones of the Konkan region of Maharashtra. Same might be the reason for the DWR-I and DWR-II, which were originally brought from Konkan region of Maharashtra. The clone DWR-III, though grouped under cluster-I, differed to some extent from the others in the same cluster. It appears that the original source of DWR-III clones might be half-sib seedling progeny manifesting most of the Alphonso fruits characters.

The clones of BGM-I and BGM-II showed the similarity coefficients of 0.847 and 0.918 compared to other clones. These clonal variations are attributed to the seedling progeny of the original source, which appears to be evident due to its very old age of more than 50 years. Similar clonal variations in Alphonso were reported by Karihaloo *et al.* (2003) and were attributed to the seed propagation instead of grafting. In another study in Mexico, based on 109 markers, mango cultivars 'Manila' and 'Carabao' were observed to be the most similar through cluster analysis (Lopez *et al.,* 1997). Further, RAPD analysis of Indian mango cultivars identified two groups on the basis of cluster analysis, representing the geographical location (Ravishankar *et al*., 2000).

A high degree of similarity among the first six clones of Alphonso mango was observed through in RAPD analysis. This similarity was also observed in physico-chemical characteristics of mango fruit among different clones with slight variations. These variations between molecular analysis and phenotype based morphometric analysis, can be attributed to the non-descript rootstock used for grafting, Hartmann and Kester (2002) reported such a variation due to rootstock and scion interaction in perennial tree species. Besides these, such clonal variations could be also due to other environmental factors. In fact, such clonal variations are well known in a number of widely grown mango cultivars like 'Langra' and 'Banarasi' (Singh, 1985).

5.4.3 Clone wise RAPD pattern to assess genetic diversity among the selected Alphonso clones of mango

As discussed previously, reproducible RAPD pattern were established for the selected nine location specific clones of mango. Various statistical parameters used to analyse the data for diversity observed among the selected clones of mango indicated that it is still possible to use RAPD markers effectively for diversity estimation, given a representative sample of Alphonso clones and a large number of polymorphic primers being used.

5.4.4 Assessment of clonal differences

In mango, there is a possibility of preservation of accumulated mutations which may lead to significant variations among trees of even the same clone. In this study, nine different location specific clones were selected with varied performance with respect to morpho-physiological characters and biochemical contents. These clones formed separate clusters of 'A' and 'B', within 'A' cluster, there are sub-clusters A_1 comprising of DVG-I, DPL-I, VEN-I, Ratnagiri, DWR-I and DWR-II and subcluster A_2 comprising of DWR-III. On the other side, cluster-B had subcluster B_1 comprising of BGM-I and subcluster B_2, comprising BGM-II. The clones originated in Konkan region were found to be very close to each other, while the clones of BGM-I and BGM-II were distant from them. Also these clones had a mean polymorphism 36.38 per cent among them. Thus, in the present study, very less genetic distance was found among the selected Alphonso clones using RAPD markers. One reason for this could be that the phenotypic variations associated with these clones might be due to environmental influence or otherwise, more sensitive techniques like AFLP giving more number of bands or primers need to be used to investigate, in detail, whether these clones are really distant or not.

5.5 PRACTICAL UTILITY

1. The results of the study indicated that seven out of nine clones of Maharashtra and Karnataka are genetically similar. The phenotypic variations observed may be due to agro-climatic situations (environment). Hence emphasising specific locations of origin of planting materials is not necessary.

2. While collection and maintenance of germplasm of mango, one should not take into account the phenotypic variations only.

5.6 FUTURE LINE OF WORK

1. Variability studies may be conducted by using more number of primers and collecting samples from some more locations

2. Relationship between quality parameters and DNA fingerprint patterns may be studied in detail, so that fingerprint specific clones can be preferred for crop improvement

3. Data on morpho-phenological characters may also be taken into consideration to study the clonal variability pattern among the location specific clones of a cultivar

6. SUMMARY AND CONCLUSIONS

The present investigation *viz.*, ‘Clonal variability studies in ‘Alphonso’ mango (*Mangifera indica* L.) by phenotypic characters and molecular markers’ was carried-out in the Department of Horticulture, College of Agriculture, UAS, Dharwad, Karnataka during the year, 2007-2008.

Totally nine Alphonso clones were selected from different locations of both Maharashtra such as Devgad, Dapoli, Ratnagiri and Vengurla and Karnataka such as Belgaum-Khanapur and Dharwad. Data on physical and chemical characters of ripe fruits were collected and analysed for genetic divergence. Similarly, the samples of DNA were extracted and the purified DNA samples were used for amplification with random primers. Amplification products were then separated by agarose gel electrophoresis. These gels were photographed under UV light and were scored as (1) for the presence and (0) for the absence of band. This binary matrix of (1)-(0) was fed to RAPD NTSys distance software to generate similarity matrix. Further analysis was performed using UPGMA.

The nine alphonso clones were found differed to a small extent according to physico-chemical characters of ripe fruits and with regard to Mahalanobis D^2 values and thus, it was inferred that there is genotypic similarity among most of the location specific clones. The nine clones of Alphonso studied formed two clusters with different levels of coefficients. In the first cluster, eight clones were grouped together, whereas in the second cluster only one clone was involved.

Out of seven (six Operon and one UBC) primers screened, it gave scorable bands. These seven primers generated a total of 36 bands, out of which twelve were polymorphic revealing 36.38 per cent mean polymorphism.

In UPGMA cluster analysis, the selected nine location specific ‘Alphonso’ clones of both Maharashtra and Karnataka state formed two major clusters. In the first cluster there were two sub-clusters. Subcluster-I comprised of six clones *viz.*, DVG-I, DPL-I, RTN-I, VEN-I, DWR-I and DWR-II., whereas subcluster-II comprised of one clone *viz.*, DWR-III. Similarly second cluster formed two sub-clusters, each of which comprised of only one clone *viz.*, BGM-I and BGM-II respectively.

In this study clone wise RAPD patterns were established for all the nine clones of alphonso, Thus in present study, it was proved that RAPD is a promising method to detect genetic diversity and to study the genetic relationship among the nine clones of alphonso mango. However, still a number of polymorphic random primers should be used to get actual picture of levels of diversity and heterozygosity in alphonso mango.

REFERENCES

Abkenar, A. A. and Isshiki, S., 2003, Molecular characterization and genetic diversity among Japanese acid citrus (*citrus* spp.) based on RAPD marker. *J. Hort. Sci. and Biotech.,* **78** (1): 108-112.

*Abu Goukh, A. B. A and Mohammed, H. I., 2004, Effect of harvesting method on quality and shelf-life of mango fruits. *Tropical Sci*., **44** (2): 73-76.

Adato, A., Sharon, D. and Lavi, U., 1995, Application of DNA fingerprinting for identification and genetics analysis of mango (*Mangifera indica* L.) genotypes. *J. Amer. Soc. Hort. Sci*., **120** (2): 259-264.

Anonymous, 1970, *Official Methods of Analysis*. Association of official agricultural chemists, Washington.

Anonymous, 2006, Area and production of horticultural crops. Bulletin of National Horticulture Board, India.

*Armuganathan, K. and Earle, E. D., 1991, Nuclear DNA content of some important plant species. *Plant Mol. Bio. Rep.,* **9** (3): 208-218.

*Cordeiro, M. C. R., Pinto, A. C. De. Q., Ramos, V. H. V., Faleiro, F. G. and Fraga, L. M. S., 2006, RAPD markers utilization and other parameters in the determination of mango hybrids genitors. *Revista Brasileira de Fruticultura*, **28** (2): 164-167.

*Damasco, O. P., Graham, G. C., Henry, R. J., Adkins, S. W., Smith, M. K. and Godwin, I. D., 1996, Randomly amplified polymorphic DNA (RAPD) detection of dwarf off types in micro propagated Cavendish (*Musa spp*-AAA) Banana. *Plant Cell Reports,* **16** (1-2): 118-123.

Degani, C., Cohen, M. and Ei-Batsri, R., 1992, PGI isozyme diversity and its genetic control in mango. *Hort. Sci.,* **27** (3): 252-254.

Degani, M., Cohen, O. Reuveni, R. F. and Basstri., 1993, Frequency and characteristics of zygotic seedling from polyembryonic mango cultivars determined using isozyme as genetic markers. *Acta Hort.,* **341**: 256-259.

*Deng. Jiu. Sheng., Lai. Zi. Chang. and Bai. Zhi. Ying., 1999, RAPD analysis of mango cultivars. *J. Fruit Sci.,* **16** (2): 156.

*Deng. Yi. Cai., Zhu. Here., Yin-Miaoyan., Li. Anni. Deng, V.C., Zhu-H-Y., Xu, My and Li, A. N., 1995, Study on proper harvesting of mango. *Guangdong Agric*. *Sci.,* **4**: 21-23.

Dinesh, M. R., 2003, Genetic studies in mango (*Mangifera indica* L.). *J. Applied Hort.,* **5**(1): 27-28.

Doreyappagowda, I. N. and Huddar, A. G., 2001, Studies on ripening changes in mango (*Mangifera indica* L.) fruits. *J. Food Sci. Tech.,* **389**(2): 135-137.

Doreyappagowda, I. N. and Ramanjaneya., 1994, Studies on physico-chemical characteristics of some commercial cultivars of mango*. Indian Food Pac.,* **48**(2): 45-50.

Doyle, J. J. and. Doyle, J. L., 1990, Isolation of plant DNA from fresh tissue. *Focus,* **12**: 13-15.

Eiadthong, W., Yonemori, K., Sugiura, A., Utsunomiya, N. and Subhadrabandhu, S., 1999, Analysis of phylogenetic relationship in *Mangifera* by restriction site analysis of an amplified region of cpDNA. *Sci. Hort.,* **80**: 145-155.

Gan, Y. Y., Zaini, S. and Idris, A., 1981, Genetically induced off-season flowering in mango. *J. Hort. Sci.,* **63**: 361-367.

Ganesh, R.S, Parthiban, K.T., Senthil Kumar, R., Thiruvengadam, V. and Paramathma, M., 2007, Genetic diversity among Jatropha species as revealed by RAPD markers. *Genetic Res. Crop Evolution*, DOI 10.1007/S 10722-007-9285-7.

Gautam, B., Sarkar, S. K and Reddy, T. N., 2003, Effect of post harvest treatments on shelf life and quality of Banganpalli mango. *Indian J. Hort.,* **60** (2): 135-139.

Gohil, S. N., Garad, B. V., Shisath, H. K. and Warade S. D., 2006, Study on physico-chemical constituents of mango cultivars under sub-arid regions of Maharashtra. *Sci. Hort.,* **10**: 91-97.

Gunjate, R. T., and Burondkar, M. M., 1993, Parthenocarpic mango developed through hybridization. *Acta Hort.,* **341**: 107-109.

Gurudutta, P. S., Vijay Jain and Singh, P. N., 2004, Response of mango cultivars to epicotyl grafting. *Indian J. Hort.,* **61**(3): 267.

Hare-Krishna and Singh, S. K., 2007, Biotechnological advances in mango (*Mangifera indica* L.) and their future implication in crop improvement. *Biotech. Advances,* **25** (3): 223-243.

Hartman, T. H., Kester, D.E, Davis, F.T. and Geneve, R.I., 2002, Topic:Genetic control in propagation, Sexual Versus Asexual. *In*: *Plant propagation,* 6th edition, pp. 10-16.

Hemant Kumar, N. V., Narayanswamy, P., Theertha Prasad D., Mukund, G. K. and Sondur, S., 2001, Estimation of genetic diversity of commercial mango (*Mangifera indica* L) cultivars using RAPD markers. *J. Hort. Sci. and Biotech.,* **76** (5): 529-533.

*Iqbal, Z., Mehboob, U. R., Dasti, A. A., Ahmad S. and Zafar, Y., 2006, RAPD analysis of Fusarium isolates causing "Mango malformation" disease in Pakistan. *World J. Microbiology and Biotech.,* **22** (11): 1161-116.

Islam, M. N., Rahim, M. A. and Farooque, A. M., 2004, Standardization of time and grafting techniques in mango under Bangladesh condition. *Asian J. Plant Sci.*, **3**(3): 378-386.

Jayasankar, S., Litz, R E., Schnell, R J. and Hernandez, A. C., 1998, Embryogenic mango cultures selected for resistance to *Colletotrichum gleosporioides* culture filtrate using RAPD markers*. In Vitro Cellular and Developmental Biology Plant.*, **34** (2): 112-116.

*Jintanawongse, S. and Changtragoon S., 2000, Identification of cultivars and certification of hybrids in mango (*Mangifera indica* L.) by isozymes gene markers. *Acta Hort.,* **509**: 177-184.

Kalra, S. K. and Tandon, D. K., 1983, Ripening behaviour of Dashehari mango in relation to harvest period. *Sci. Hort.,* **19**: 267-229.

Karibasappa, G. S. 1995, Morpho-phenological, fruit physico-chemical and isozymes characterization of (*Mangifera indica* L) germplasm, using principal component analysis. *M.Sc. (Agri.),* Univ. Agri. Sci., Dharwad, Karnataka, India.

Karihaloo, J. L., Dwivedi, Y. K., Sunil Archak and Gaikwad, A. B. 2003, Analysis of genetic diversity of Indian mango cultivars using RAPD markers. *J. Hort. Sci. and Biotech.,* **78**(3): 285-289.

*Karp A., Kresovich, S., Bhat K. V., Ayad, W. G. and Hodgkin, T., 1997, Molecular tools in plant genetic resources conservation: a guide to the technologies. *IPGRI Technical bulletin,* No. 2: 46.

Kaushik, R. A. and Ranjit Kumar., 1992, Physico-chemical changes in different mango cultivars during storage. *Haryana J. Hort. Sci.,* **21** (3-4) 166-173.

Kulkarni, V. and Rameshwar, 1981, Biochemical and physical composition of fruits of some important Indian mango cultivars. *Prog. Hort.,* **13** (3/4): 5-8

Kumar, N. and Jaiswal, U. S., 2006, Bearing behavior of some west and south Indian mangoes fruit morphology and its storage life. *Sci. Hort.,* **10**: 85-90.

Lakshminarayana, S., Subhadra, N. V. and Subrhmanyam, H., 1980, Some aspects of developmental physiology of the mango fruit. *J. of Hort. Sci.,* **45**: 133-142.

Lavi, U., Tomer, E., Gazit, S. and Hillel, J., 1998, Components of the genetic variance and genetic correlations between traits in mango. *Sci. Hort.,* **75** (1/2): 11-25

Lee, M., 1995, DNA markers and plant breeding programmes. *Advances in Agronomy,* **55**: 265-344.

Lopez, V. J A., Martinez, O. and Lopez, O. P., 1997, Geographic differentiation and embryo type identification in *Mangifera indica* L. cultivars using RAPD markers*. Hort. Sci*., **32**(6): 1105-1108.

Lycett, G. W., Zainal, Z. B., Los, M., Findlay, C. L. and Tucker, G. A., 1997, Novel ripening-specific cDNA clones from mango fruit. *Acta Hort.,* **455:** 277-286.

Mahalanobis, P.C., 1936, The generalized distance in statistics. *Proc. Nation. Acad. Sci. (India)*, **2**: 79-85.

*Mukherjee, S. K., 1985, Systematic and ecogeographic studies of crop genepools (*Mangifera indica* L.). Bulletin by *International Board for Plant Genetic Res.,* Rome*,* **92:** 235-240.

Naik K.C., 1948, Improvement of the mango (*Mangifera indica* L.) by selection and hybridization. *Ind. J. Agri. Sci.,* **18:** 35-41.

Nelson, N., 1944, A photometric adaptation of the Somogyi's method for determination of glucose*. J. Biology and chemistry*, **153:** 375-380.

Neto, S. P., Maruta, I, Takaiwa, F., Oono, K. and Matsumoto, K., 1995, Identification of cashew (*Anacardium occidentale* L.) seedlings with RAPD markers. *Acta Hort.,* **370**: 21-25.

*Oppenheimer, C., 1956, Study tour report on subtropical fruit growing and research in India and Ceylon. *State of Israel Ministry of Agriculture. Report, October.* Bulletin, **3.**

Panse, V.G. and Sukhatme, P.V., 1989, *Statistical methods for Agricultural workers.* (Revised by: Sukhatme, P.V. and Amble, V. N.) *ICAR Publications,* New Delhi, pp 100-109.

Parhi, G., Mishra, H. N. and Tripathy, P., 1993, Genetic divergnce in bittergourd (*Momordica charantia* L). *South Ind. Hort.,* **41**(6): 344-349.

Patil, P. V., Patil, V. K. and Navale, P. A., 2006, Grafting in mango. *Sci. Hort*., **10:** 45-66.

Patil, S. A., 1990, Evaluation of mango (*Mangifera indica* L.) cv. Alphonso, Ratna and Kesar fruits for physico-chemical composition and processing. *M.Sc.(Agri) Thesis,* Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India.

*Pradeepkumar, T., Philip, J., and Johnkutty, I., 2006, Variability in physico-chemical characteristics of mango genotypes in northern Kerala. *J. Trop. Agric.,* **44**(1/2): 57-60.

Prasad, V. S. R. K and Singh, D. P., 1997, Genetic divergence in parval (*Trichosanthes dioca* Roxb). *Indian J. Plant Genetic Res*, **10** (1): 91-96.

Prasanth, J. M., Reddy, P. N., Patil, S. R. and Pampanagouda, B., 2007, Effect of cultivars and time of softwood grafting on graft success and survival in mango. *Agricultural Sci. Digest,* **27** (1): 18-21.

Ram, D., 2001, Non-hierarchical Eucliden cluster analysis in pointed gourd (*Trichosanthes dioca* Roxb). *Ind. J. Hort.*, **58** (3): 264-268.

Ramsingh, Sharma, R K., Kumar J. and Goyal, R. K., 2003, Effect of oil emulsions and precooling on the shelf life of mango cv. Amrapali. *Haryana J. Hort. Sci.,* **32**(1/2): 54-55.

Rao, C. R., 1952, Genetic divergence: Methods and analysis of agriculture crop. *In: Advanced Statistical Methods in Biometrical Research.* John Wiley and sons, New York, pp. 357-362.

Ravishankar, K. V., Chandrashekara, P., Sreedhara, S. A., Dinesh, M. R., Lalitha-Anand and Saiprasad, G.V.S., 2004, Diverse genetic bases of Indian polyembryonic and monoembryonic mango (*Mangifera indica* L.) cultivars. *Current Science*, **87** (7): 870-871.

Ravishankar, K. V., Lalitha Anand and Dinesh, M. R., 2000, Assessment of genetic relatedness among mango cultivars of India using RAPD markers. *J. Hort. Sci. Biotech.,* **75** (2): 198-201.

Reddy, Y. T. N. and Singh. G., 1991, Further studies on the relationship between leaf number and area and fruit development in mango. *J. Hort. Sci.,* **66** (4) 471-478.

Rossetti, A. G., Cavalcante, A. T., Barros, L. and De. M., 2004, Variability of mango seedlings as a function of container type, age of rootstock and grafting method - a case of evaluation of experimental designs. *Acta Hort.*, **645**: 691-695.

Rout, A.V.V. D., Samal, S., Nayak, S. Rashmi, M. N., Lenka, P.C. and Das, P., 2002 DNA extraction protocol. www.*gartenwissenschaft*, **67** (3): 1-4.

Roy, B. N., Saha, S. K., Pramanick, S. and Biswas, S., 1980, Effect of wax emulsion and maleic hydrazide on the storage behaviour of mango (*Mangifera indica* L.) cv. Langra and Himsagar, *Progr. Hort.,* **11** (4): 35-38.

Sahani, C. K. and Khurdiya, D. S., 1994, Physico-chemical changes during in Dashehari, Chousa, Neelum and Amrapali mango. *Ind. Food Pac.*, **43** (1): 36-41.

Saiprasad, G. V. S., Lalitha-Anand., Ravishankar, K. V., Mythili, J. B., Nagesh, M. and Joshi, R., 2004, Isolation and characterization of mRNAs differentially expressed during ripening of mango fruits. *Indian J. Biotech*, **3** (4): 533-537.

Samanta, A. K., Chattopadhyay, T. K. and Roy, S., 1999a, Genetic variability, heritability and genetic advance relating to some plant characters in mango (*Mangifera indica* L.). *Hort. Journal,* **12** (2): 41-45.

Samanta, A. K., Chattopadhyay, T. K. and Roy, S Z., 1999b, Genetic variability of some fruit characters in mango (*Mangifera indica* L.). *Environment and Ecology*, **17** (2): 488-490.

Samal, S. Lenka, P. C. Nanda, R. M., Nayak, S., Rout, G. R. and Das, P., 2002, Genetic relatedness in cashew (*Anacardiun occidentale* L) germplasm collection as determined by RAPD. *Genetic Res. and Crop Evolution,* **48** (1): 25-30.

Samal, S. Rout, G. R. and Lenka, P. C., 2003, Analysis of genetic relationship between population of cashew (*Anacardiun occidentale* L) by using morphological characterisation and RAPD markers. *Plant Soil Environment,* **49** (4): 176-182.

Sanjay Singh, 2003, Studies on storage behaviour of South and East Indian mango cultivars. *Ann. Agric. Res.,* **24** (3): 498-501.

Schnell, R. J. and Knight, R. J., 1992, Frequency of zygotic seedling from five polyembryonic mango rootstocks. *Hort. Sci.,* **27** (2): 174 -176.

Schnell, R. J. and Knight, R. J., 1993, Genetic relationship among *Mangifera* spp. based on RAPD markers. *Acta Hort.,* **341:** 168-170.

Schnell, R. J., Ronning, C, M. and Knight, R. J., 1995, Identification of cultivars and validation of genetic relationship in (*Mangifera indica* L.) using RAPD markers. *Theor. Appl. Gent*., **90** (2): 269-274.

Shivashankar, S., Ravindra, V. and Louis, L., 2007, Biochemical changes in seed and mesocarp of mango (*Mangifera indica* L.) cv. 'Alphonso' and their significance during the development of spongy tissue. *J. Amer. Soc. Hort. Sci.,* **82** (1): 35-40.

Shyamalamma, S., 1993, Standardization of maturity indices for harvesting mango fruit of cultivars Mallika (*Mangifera indica* L.). *M. Sc. (Agri.) Thesis,* Univ. Agric. Sci., Bangalore*,* Karnataka, India.

Simon, L., Shaymalamma, S. and Narayanswamy, P., 2007, Morphological and molecular analysis of genetic diversity in jackfruit. *J. Hort. Sci. Biotech.,* **82**(5): 764-768.

Singh, D. B., 1991, Post-Harvest treatment for ripening changes and quality of mango fruits cv. Kesar and Amrapali, *Acta Hort.,* **291:** 472-478.

Singh, H. and Chadha, K. L., 1981, Evaluation of some mango cultivars on the basis of their bio-chemical composition. *Ind. J. of Hort.,* **38** (1-4): 70-73.

Singh, J., Singh, R. R. and Yadav, G. S., 2004a, Genetic divergence in mango. *J. Applied Biology*, **14** (1): 36-38.

Singh, J., Singh, R. R., Yadav, G. S. and Singh, U. K., 2004b, Studies on genetic variability in mango (*Mangifera indica* L). *J. Applied Biology*. **14** (1): 34-35.

Singh, J., Singh, R. R., Yadav, G. S. and Singh, U. K., 2004c, Studies on correlation and path analysis in mango. *J. Applied Biology*, **14**(2): 34-36.

*Singh, L. B., 1960, Botany, cultivation and utilization. *In: The Mango, Botany, Cultivation and Utilization*, Leonard Hill, London. pp. 123-128.

*Singh, P.K. and Choudhary, R.D., 1977, *Biometrical Methods in Quantitative Genetic Analysis*, Kalyani Publishers, New Delhi, pp.178-185.

Singh, R. N., Gorakh, S. Rao O.P. and Mishra, J. S. C., 1985, Improvement of ‘Banarasi’, ‘Langra’ through clonal selection. *Prog. Hort*., **17**: 273-277.

Souza, V. A. B., Lima, P. S. Da. C., Pinto, A. C., Pereira, M. E. C. and Alves, R. E., 2004, Genetic variability in mango genotypes detected by RAPD markers. *Acta Hort.,* **645**: 303-310.

Srivastava, A.P., Ramesh Chandra and Ranade A.S., 2004, Applicability of PCR based molecular markers for parentage analysis of mango (*Mangifera Indica* L.) Hybrid. *Indian J Genetics,* **64**(4): 275-280.

Staub, J. E and Serquen, F. C., 1996, Genetic markers map construction and their application in plant breeding. *Hort. Sci.,* **31** (5): 729-741.

*Tanksley, S. D. and Pichersky, E., 1988, Organization and evolution of sequences in the plant nuclear genome, *In: Plant Evolutionary Biology* (Eds. Gottleib, L. D and Jain, S. K.) Chapmann and Hall Ltd., pp. 55-79.

Varalakshmi, B., Reddy N. Y. and Reddy B. M. M., 1994, Genetic divergence in ridge gourd (*Luffa acutangula* Roxb) *J. Genetic and Plant Breeding,* **48**: 131-134.

Wahab, A. M. and Gopalkrishnan, P. K., 1993, Genetic divergence in bittergourd (*Momordica charantia* L). *South Ind. Hort.,* **41** (4): 232-234.

Waskar, D. P. and Dhemre, J. K. 2004, Effects of postharvest treatments on shelf life and quality of Kesar mango fruits during storage. *J. Maharashtra Agric. Univ.,* **29** (2): 159-163.

*Welsh, J.C.P., and Mc-clelland, M., 1990, Polymorphisms generated by arbitrarily primed PCR in the mouse: Application to strain identification and genetic mapping. *Nucl. Acids Res.,* **19**: 303-306.

Whiley, A. W., Mayers, P. E., Saranah, J. and Bartley, R. P., 1993, Breeding mangoes for Australian conditions. *Acta Hort.,* **341**: 136-145.

Williams, J. G. K.., Kuber, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V., 1990, DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*, **18**: 6531-6535.

*Williams, J. G. K., Rafalski, J. A. and Tingey, S. V., 1992, Genetic analysis using RAPD markers. *Method in Enzymology,* **56:** 254–260.

Yadav, S. S., Prasad, A. and Abidi, A. B., 1984, Physico chemical characteristics of some mango varieties grown in Uttarpradesh, *Prog. Hort.,* **16**(3-4): 166-168.

*Young, T. W. and Ledin, R B., 1954, Mango breeding. *Proceedings of the Florida State Horticultural Society,* **67:** 241-244.

---

*Original not seen

APPENDIX I

Details of chemical composition

Buffers	Composition	Concentration
Extraction Buffer	Tris Base Na Cl EDTA CTAB	100 mM 1.4 M 20 mM 4%
Tris-EDTA (TE) buffer	Tris base EDTA	10 mM 20 mM
Running Buffer [Tris Borate-EDTA(TBE)]	Tris Base Boric acid EDTA	5x: 54 g litre^{-1} 5x: 27.5 g litre^{-1} 5x: 20ml (0.5M,Ph-8.0) per litre
Loading buffer	Bromophenol blue Sucrose in water	0.25% 40%(w/v)

APPENDIX II

Monthly mean weather data recorded during the experimental period from May, 2007 to April, 2008 (Dharwad, Karnataka)

Month	**Temperature(℃)**		**Relative Humidity (%)**		**Rainfall (mm)**
	Max.	**Min.**	**Max.**	**Min.**	
May-07	34.41	21.39	81.59	40.77	1.43
June-07	29.70	21.40	90.60	68.80	7.33
July-07	27.00	21.10	90.70	79.12	6.98
August-07	27.10	20.50	91.80	76.70	5.67
September-07	27.20	20.60	92.30	48.40	6.02
October-07	29.60	20.60	77.20	45.80	2.41
November-07	29.50	15.10	68.20	40.00	1.79
December-07	29.00	14.60	81.40	49.16	0.0
January-08	29.75	12.90	85.13	46.11	0.0
February-08	31.12	16.30	90.23	49.41	0.0
March-08	32.42	18.87	85.56	53.42	3.58
April-08	34.68	20.43	84.22	56.35	0.96

APPENDIX III

Monthly mean weather data recorded during the experimental period from May, 2007 to April, 2008 (Dapoli, Maharashtra)

Months	Temperature(℃)		Relative Humidity (%)		Rainfall (mm)
	Max.	Min.	Max.	Min.	
May-07	33.40	23.30	87.00	62.20	0.09
June-07	30.50	23.32	95.00	86.20	10.97
July-07	28.87	23.82	95.75	91.75	9.53
August-07	27.92	23.20	96.80	88.40	8.20
September-07	28.70	22.90	98.00	90.75	7.24
October-07	32.02	20.95	90.00	69.75	0.09
November-07	32.44	16.14	87.40	70.40	0.24
December-07	32.12	13.10	94.75	60.25	0.00
January-08	31.67	21.32	86.75	70.40	0.00
February-08	29.25	19.00	92.25	66.07	0.00
March-08	30.10	20.12	94.42	68.53	0.00
April-08	29.85	23.32	87.45	64.74	0.00

CLONAL VARIABILITY STUDIES IN ALPHONSO MANGO (*Mangifera indica* L.) BY PHENOTYPIC CHARACTERS AND MOLECULAR MARKERS

MAKARAND DHONDU MANCHEKAR 2008 Dr. A. N. MOKASHI
MAJOR ADVISOR

ABSTRACT

The present investigation on "Clonal variability studies in Alphonso mango (*Mangifera indica* L.) by phenotypic characters and molecular markers" was conducted at the Department of Horticulture, College of Agriculture, University of Agricultural Sciences, Dharwad during the year 2006-2008. The investigation comprised of two experiments. The first experiment was conducted to assess the genetic divergence among nine Alphonso clones of mango, collected from different pickets of both Maharashtra and Karnataka states in India, using Mahalanobis D^2 statistic over physico-chemical parameters of fruits. Analysis of variance revealed significant differences among the clones for all characters except stone thickness. The clones were grouped into two clusters. Cluster I, which consist of the clones DPL-I, RTN-I, DEV-I, VEN-I, DWR-I, DWR-II, DWR-III and BGM-II had the highest length of fruit, stone thickness, per cent of pulp, pulp to stone ratio, pulp to peel ratio and shelf life. Cluster-II, comprising the clone BGM-I recorded significantly highest total fruit weight, diameter of fruit, weight of peel, weight of stone and weight of pulp. Regarding quality parameters, the highest values were observed for total soluble solids, ascorbic acid and total sugar in cluster-I. Similarly in cluster-II, highest values were recorded in titrable acidity and reducing sugar. No relationship between geographical origin and genetic diversity was established.

In the second experiment nine Alphonso mango clones were examined using Random Amplified Polymorphic DNA (RAPD) markers with decamer primers of arbitrary sequence. Seven of the eight primers screened were informative and 36 amplified DNA bands were selected as RAPD markers. Clusters analyzed based on seven RAPD markers produced a dendrogram of the genetic relatedness among the nine Alphonso clones. The clones *viz.,* DEV-I, RTN-I, VEN-I, DPL-I, DWR-I, DWR-II and DWR-III were most similar and formed into one cluster, whereas BGM-I and BGM-II formed another.