



Suresh Kumar et al.; International Journal of Pharmamedix India, 2013, 1(2), 398-414.

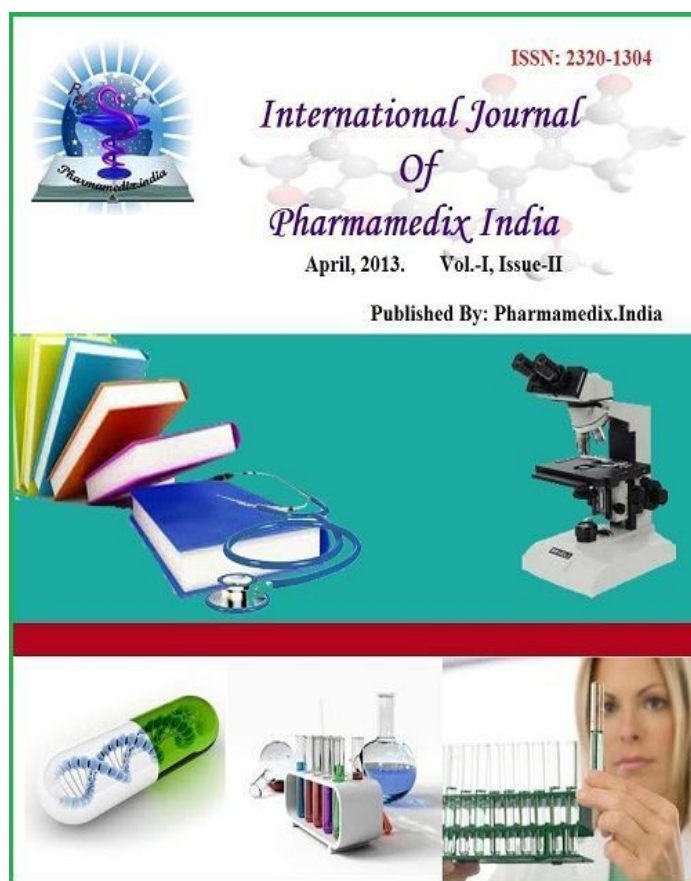
“Clonal Variability Studies in ‘Alphonso’ Mango (*Mangifera Indica* L.) by Phenotypic Characters and Molecular Markers’

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Abstract:

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. This present review gives information about the clonal variability in alphonso mango.

Keywords: Clonal Variations, Somatic Mutations, Markers.

Introduction:

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 elsewhere. 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 ^[1]. In Maharashtra, mango is cultivated in an area of 0.43 million ha and produced at 0.6 million metric tonnes, while in Karnataka, the area and production are 0.12 million ha and 1.11 million metric tonnes respectively ^[1].

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 phylogenetic 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^[2]. 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^[3]. 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.

2.1 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^[4]. 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^[5]. Mukherjee *et al.* (1985) conducted a survey of mangoes in eastern India and identified some superior clones^[6]. Singh and Chadha (1981) in a study of mango orchards of 'Dashehari' located four clones which were superior in

performance^[7]. Singh *et al.* (1985) isolated two high yielding clones from the orchards of 'Langra' with improved resistance to bacterial black spot^[8]. Whiley *et al.* (1993) identified certain strains within 'Kensington' clone, having improved resistance to bacterial black spot disease^[9].

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^[10] 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^[11]. Gan *et al.* (1981) observed considerable variations within the vegetatively propagated mango cultivars based on polymorphism of four enzymes isolated from leaf tissues^[12]. 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 ^[13].

2.3 MARKER ASSISTED CHARACTERIZATION 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 ^[14].

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 ^[13]. 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 transferase 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 transferase 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 ^[15]. Degani *et al.* (1993) and Schnell and Knight (1992) demonstrated the

feasibility of using isozymes as biochemical markers in mango ^[16, 17]. Degani *et al.* (1992) analysed leaf phosphoglucosomerase (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 ^[18]. 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, leucine amino peptidase, phosphoglucose isomerase. They found that there were significant differences for the frequency of occurrence of nucellar and zygotic seedlings ^[17]. Degani *et al.* (1993) distinguished the zygotic seedlings and nucellar seedlings of two polyembryonic mango cultivars viz. Tarapint and '13-1'. Leaf extracts of the seedlings were analysed for isozymes of isocitrate dehydrogenase, phosphoglucose isomerase, phosphoglucose mutase 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 heterozygous ^[16].

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, phosphoglucose 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 ^[18,19]. Results of the studies made by Jintanawongse and Changtragoon (2000) in the commercial Thai mango cultivars 'Kniew Sawoe', '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,

phosphoglucosyltransferase, malate dehydrogenase and glucose-6 phosphate dehydrogenase ^[20].

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 noncoding DNA that is not transcribed ^[21]. Species with larger genomes

normally have more repeated DNA and a higher proportion of repeated DNA to single copy DNA ^[22, 23]. 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: ^[24]

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 [25].

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 [26].

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 ^[27].

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 ^[28]. 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 ^[29]. 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 [30].

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 [31]. 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 nonadditive 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 [32]. At IIHR, Bangalore, 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 [33].

Hemantkumar *et al.* (2001) working at UAS, Bangalore, 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 [34].

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 carried out 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 F1 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 F1 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 versa. For the characters studied, PCV was higher than GCV [35]. Karihaloo *et al.* (2003) carried out 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 ^[36].

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 ^[37]. 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 ^[38].

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 ^[39]. 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 Lattice 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 ^[40]. 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 ^[41].

CONCLUSION:

This review giving an information about the clonal variability in alphonso mango leading to crop variability and also gives a which clonal propagation cause a high yield of crop, how to improve the crop.

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