16 Mango

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16.1 Introduction

Mango (*Mangifera indica* L.) is popularly known as the 'king of fruits'. Believed to have originated in Eastern India (Knight 1980), it is an allotetraploid that evolved after interspecific hybridization and subsequent doubling of the chromosome number (Mukherjee 1953). The mango genome is of 4.39×10^8 bp size (Arumuganathan and Earle 1991) and has 20 chromosomes, most of them small. Mango is commercially grown in over 103 countries of the world but nowhere is it so greatly valued as in India. Apart from India the other major mango growing countries in the world are China, Mexico, Pakistan, Indonesia, Thailand, Philippines, Brazil, Australia, Nigeria and Egypt. According to Galan (1993) between 1971 and 1993, the production of mango worldwide, has increased by nearly 50 per cent. Much of this new production has occurred outside traditional centers of mango culture, in South and Central America, Africa and Australia. Mango lends itself to a variety of uses in one form or another, as every part of the tree is valuable and the fruits are rich sources of vitamin A and C. There is a good demand for mango fruits and their processed products in both the national and international market.

There are more than a thousand varieties of mango under cultivation, but only a few of them are grown on a commercial scale. Both polyembryonic and monoembryonic mango cultivars are grown. According to Leroy (1947), adventive embryony is probably due to the effect of one or more recessive genes. Polyembryonic cultivars are grown in Southeast Asia and in tropical Latin America, whereas monoembryonic cultivars are major contributors to commercial production in India and in Florida. Polyembryonic mango cultivars are seed-propagated and exhibit little variability in seedling populations. Monoembryonic mango cultivars are propagated either by inarching or by grafting bud wood onto seedling rootstock.

Not all varieties have been commercially exploited. There are varieties which have some unique characteristics while lacking in others. Therefore, it is necessary to study all the varieties/accessions in the germplasm in order to cope up with the mango crop improvement program. Several crop improvement measures have been taken up in mango with respect to high yield, regular bearing and resistance to certain physiological disorders. Although conventional breeding methods have yielded quite a good number of hybrids and varieties, they are laborious and time consuming. It is necessary that one should know the desirable characteristics of a variety before taking up conventional or molecular breeding. There is very limited work on molecular breeding and mapping of the genotypes in mango as compared to other fruit crops.

16.1.1 Origin and Distribution

The genus *Mangifera*, which belongs to the family 'Anacardiaceae', originated in the Indo-Burma region. According to Mukherjee (1958), the natural spread of the genus is limited to the Indo-Malaysian region, stretching from India to the Philippines and New Guinea in the East. Evidence based on morphological, phytogeographical, cytological, anatomical and pollen studies have indicated that the genus had its origin in the continental region of (i) Burma, Thailand, Indo-China and (ii) the Malayan Peninsula since these happen to be the main centers of species forma-

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tion; the Sunda Islands (Java, Sumatra and Borneo), the Philippines and the Celebes- Timor group forming the secondary centers of development. The highest concentration of *Mangifera* species is reported to be in the Malayan Peninsula, followed by the Sunda Islands and the Eastern Peninsula with many of the species being common between them (Mukherjee 1949a).

The genus which has been sub-divided into two sections, on the basis of the presence or absence of the disc in the flowers (Mukherjee 1949a), is reported to contain 41 species in all but almost all the edible cultivars of mango belong to the single species *Mangifera indica* Linn which originated in the Indian subcontinent. Occurrence of wild forms of *M. indica*, allied species *M. sylvatica* and *M. caloneura*, fossil leaf impressions of *M. pentandra* (a species similar to *indica*) and presence of numerous cultivated and wild varieties in India have been cited as some of the major reasons in favor of *M. indica* having originated in the Indo-Burma region through allopolyploidy, possibly amphidiploidy (Mukherjee 1958). The few other species which contribute edible fruits (though of relatively inferior fruit quality) are *M. caesia, M. foetida* and *M. odorata*, which are confined to the Malaysian region.

The mango, though well-known to the people of the Indian subcontinent for several centuries, was virtually unknown to any botanist until 1605 when Carol Clusius first mentioned about it in his writings (Mukherjee 1949b). Bauhin (1623, 1650), cited in Bose (1985) subsequently, referred to it under the names "Mangas" and "Amba". Bontius (1658) cited in Bose (1985) gave *Mangifera* the name for the first time and he referred to this plant as *arbor Mangifera* (the tree-producing mango). Later, it was mentioned in the literature as *Mangifera indica* Ray, *Mangus dornestica* Hermann or *Mangas sylvatica* Rheede. Linnaeus also referred to it as *Mangifera arbor* in 1747, prior to changing the name to its present form *Mangifera indica* L. in 1753, in his much quoted book 'Species Plantarum' (Mukherjee 1949b).

Besides the Indian subcontinent, the mango is now found in several countries of the tropical and subtropical world where Muslim missionaries, Spanish voyagers and Portuguese explorers introduced it to several places during 15th to 18th century. According to Hayes (1957) mango was being cultivated at the head of the Persian Gulf by the 16th century. It was introduced to Philippines after 1600, to Moluccas in 1665 and to Yemen in the later part of the 18th century (Burns and Prayag 1921). It is also reported that the mango was being grown in England under glass-house conditions as early as 1690 and that the trees at Kew were in fruiting during 1818. In Mexico, it was introduced before 1778 by the Spanish travelers from the Philippines. The Portuguese, who carried mango to South Africa in the 16th century from Goa, were also responsible for introducing it in Brazil by 1700 (Popenoe 1920). It was in cultivation in Barbados in 1742 and in Jamaica in 1782. According to Pope (1929) it was introduced in Florida and the east coast in 1860s and the west in 1870s (Hayes 1957). However, it was only in 1889 that the United State Department of Agriculture (USDA) successfully introduced the grafted plants from India. It reached Azores in 1865 and Queensland in 1870 (Burns and Prayag 1921).

Presently, besides India, it is being cultivated in Pakistan, Bangladesh, Burma, Sri Lanka, Thailand, Vietnam, Malaysia, the Philippines, Indonesia, the Fiji Islands, Tropical Australia, Egypt, Israel, Sudan, Somalia, Kenya, Uganda, Tanzania, South Africa, Niger, Nigeria, Zaire, Madagascar, Mauritius, the USA (Florida, Hawaii, Puerto Rico), Venezuela, Mexico, Brazil and the West Indies Islands.

16.1.2 Taxonomy

Mango *(Mangifera indica* L.) belongs to the dicotyledonous family 'Anacardiaceae'. The nomenclature of *Mangifera* species and mango cultivars has been complicated by the widespread use of synonyms (Lakshminarayana, 1980). This family consists of sixty-four genera, mostly of trees or shrubs, often containing milky or acrid juice, some of which are even poisonous. The leaves are exstipulate, usually alternate and simple. The inflorescence is generally an axillary or terminal panicle or spike bearing small and regular flowers. They may be unisexual (usually male) or bisexual, borne on the same or different trees. Mango possesses a very long taproot. The fruit is usually a drupe and seed is exalbuminous and is located inside the stony endocarp.

The tree is large, spreading, and evergreen, with a dense rounded or globular crown. The trunk is erect, thick, without furrows or buttresses, when old. The bark is thick, sometimes with longitudinal bursts containing a little yellowish transparent gum resin like juice. The wood is reddish grey, often streaked, moderately hard, coarse grained and soft in a young

tree. It is somewhat harder and darker brown on the older trees. Branches are very numerous, the lower ones spreading horizontally to a great extent, the upper ones gradually ascending till they become nearly erect in the center; branchlets are rather thick and robust, often with alternating groups of long and short internodes, glabrous, yellowish green when young, with slightly prominent scars of the fallen leaves.

16.1.3 Crop Improvement

The main objectives of mango breeding are to achieve regular bearing, high yield and resistance to certain physiological disorders like spongy tissue.

16.1.3.1 Mango Breeding

Hybridization, clonal selection, isolation of chance seedlings and mutation breeding are some of the conventional crop improvement methods in mango. After the initiation of hybridization in mango during 1911 at Pune, Maharashtra by Burns and Prayag, till today at least 30 hybrids have been released from different parts of India and abroad.

A clone is one of the basic categories of cultivar (Bricknell et al. 1980) with extremely important applications in horticulture. 'Cloning' may be defined as the vegetative regeneration of a single genotype as represented by a single plant, single growing point, single meristem or single explant. Cloning is a powerful procedure both as a plant selection tool for breeding and as a plant propagation tool for reproduction (Kester 1983). The exploitation of cloning for the selection of superior individuals followed by vegetative propagation has been one of the consistent themes throughout horticultural history. Clones exist in nature and with some species, vegetative multiplication is a major strategy for their adaptation.

16.1.3.2 Crop Improvement in Mango by Hybridization

In India, work on inter-varietal hybridization was initiated in 1911 at Pune, Maharashtra (Burns and Prayag 1921). Subsequently hybridization work was undertaken at many fruit research centers in the country, *viz*., Sabour in Bihar, Anantharajupet and Kodur in Andhra Pradesh, Saharanpur and Central Institute of Subtropical Horticulture, Lucknow in Uttar Pradesh, Krishnanagar in West Bengal, Quaidian in Punjab, IARI (Indian Agriculture Research Institute) in New Delhi, Periyakulam in Tamil Nadu, Paria in Gujarat, Vengurla in Maharashtra and Indian Institute of Horticultural Research, Hessaraghatta, Bangalore in Karnataka. Initial success in hybridization program was very poor because the technique of hybridization was traditional and cumbersome.

Based on a genetic marker, Singh et al. (1980) have shown that the percentage of fruit set from crosses in mango can be doubled simply by doing away with cumbersome process of bagging the panicles again after cross-pollination by hand. This will facilitate the raising of large hybrid populations for selection. As a result of hybridization at least 30 hybrid mango varieties were developed since the initiation of hybridization program in the country. The following are some of the salient research findings with respect to crop improvement in mango by hybridization in different parts of India and Israel.

The hybridization work carried out at the Indian Agricultural Research Institute, New Delhi under the leadership of Dr. R. N. Singh led to the evolution of two improved, regular bearing hybrids namely 'Mallika' (Neelum × Dashehari) and 'Amrapali' (Dashehari × Neelum). The tree of Mallika is semi-vigorous, it is medium to heavy cropper (15 ton ha^{-1}) and has a strong tendency to bear regularly. The fruits have an attractive appearance and the average fruit weight is 307 g. The pulp recovery is high (75%) and it is fiberless and firm and the stone is very thin. Total soluble solids (TSS) is high (25°Brix), and keeping quality of the fruit is better (Singh et al. 1972; Singh 1990).

Amrapali, the other hybrid released from IARI, New Delhi is distinctly dwarf, precocious, highly regular and high yielding (Sharma et al. 1980). It has been utilized for a high-density plantation. An orchard with a density of 1,600 plants ha^{-1} can yield about 11.50 tons in the 4th year and yield reaches about 22.20 tons in the 7th year (Majumder et al. 1982). Besides, Amrapali is very high in vitamin A content (β-carotenoid pigment) and its flesh is deep orange in color. The deep flesh color is important in fruit preservation industry, since addition of artificial color is not required (Singh 1990).

Thirty-nine mango hybrids developed at IARI, New Delhi were studied in order to identify promising plant types on the basis of dwarf growth habit, regularity and prolificacy of bearing. Out of these, two hybrids, *viz*., Hybrid No. 427 and Hybrid No. 411 both of Neelum × Himsagar parentage were found to be dwarf, regular and prolific in bearing. Eight-year-old

hybrid plants of 427 and 411 attained the height of 2.27 and 3.70 m, respectively. The yield per unit volume was 1,433.96 g per cubic meter in Hybrid No. 427 seedling and it was 1,251 g in Hybrid No. 411 (Rangith 1984).

Intensive mango hybridization program was initiated at the Agricultural Experiment Station, Gujarat Agricultural University, Paria, Gujarat in 1968. Thirty hybrids of various parental combinations were assessed for high yielding, regular bearing varieties of mango with improved quality. Of these, three mango hybrids, *viz*., Neelphanso (Neelum × Alphonso), Neeleshan Gujarat (Neelum × Baneshan) and Neeleshwari (Neelum × Dashehari) were found promising and released for commercial cultivation (Sachan et al. 1989). 'Neeleshan' was found to be highly suited for table and juice purposes since fruit quality was excellent (TSS 19.06%; total sugar 13.13%).

'Neeleshan Gujarat' is moderately regular bearer and late maturing type. Fruits are of medium size weighing 318 g with an average yield of 47.75 kg tree^{-1}. Fruit quality is very good (TSS 16.26%; total sugars 11.51%; acidity 0.12%; Vitamin C 16.09 mg 100 g^{-1} pulp). 'Neeleshwari' is dwarf in nature, bearing is moderate and late maturing type. Fruits are medium in size (228 g) and the average yield per tree is 34.95 kg fruits. Fruit quality is very good (TSS 20.53%; total sugars 10.78%; acidity 0.11%; Vitamin C 36.04 mg 100 g^{-1} pulp). Fruit pulp is moderately firm, non-fibrous and suited for table and juice making (Sachan et al. 1989).

Work was initiated at the Regional Fruit Research Station, Vengurla, Maharashtra, in order to evolve a regular bearing variety having Alphonso like qualities. Crosses of Alphonso and Neelum were made since 1970. Out of 40,000 crosses made, nearly 300 hybrid seedlings were obtained and evaluated for desirable characters. Out of the hybrids planted in 1971, No. 13, a cross between Neelum × Alphonso, was found to be the most promising and later this hybrid was released for commercial cultivation under the name 'Ratna' (Limaye et al. 1984). 'Ratna' is a regular bearer with most of the fruit qualities of Alphonso. The fruit is large with good sugar/acid blend, pleasing aroma, long shelf-life, early maturing and free from spongy tissue (Salvi 1983; Salvi and Gunjate 1989). This hybrid is a high yielder and 8, 9, 10, 11 and 12 year old tree has yielded 41.52, 34.66, 30.00, 34.09 and 32.24 kg fruits per tree respectively (Salvi and Gunjate 1989).

Although, 'Ratna' unlike 'Alphonso' is a regular bearer, a good yielder with good fruit qualities and free from spongy tissue, this quality lacks an attractive blush, fruit shape and a typical 'Alphonso' flavor. Hence, intensive work involving back-crossing between 'Ratna' and 'Alphonso' was undertaken with an objective to bring about the desired improvement in 'Ratna'. Parthenocarpic mango Hybrid-117 perhaps the first of its kind was obtained as a result of intensive back-crossing between the hybrid 'Ratna' (Neelum × Alphonso) and Alphonso at RFRS, Vengurla, Maharashtra. This work resulted in the development of Hybrid 117 that was released for cultivation in the Konkan region of Maharashtra with the name 'Sindhu' (Gunjate and Burondkar 1993). 'Sindhu' is comparatively dwarf and a regular bearer having medium sized fruit (215 g), with very high pulp to stone ratio (26:1) and very thin (30 mm) and small stone (6.72 g). Fruits are deep orange in the flesh, fiberless and free from spongy tissue disorder. The fruit pulp is very rich in ascorbic acid (52.22 mg 100 g^{-1} of pulp) and β-carotene (11,850 mg 100 g^{-1}). The non-viable cotyledon free stone makes up only 3.1 per cent of the total fruit weight. The striking character of this hybrid is seedlessness (Gunjate and Burondkar 1993).

Work at Fruit Research Station, Sabour, Bihar on hybridization in mango was initiated as early as 1942 and in the first batch as many as 63 hybrid progenies were obtained. The difficulties faced in hybridization program and probable remedies have also been suggested (Sen et al. 1946). Naresh Kumar (1997) described Sundar Langra (Langra × Sundar Pasand) and Alfazli (Alphonso × Fazli) hybrids developed from Sabour. Sundar Langra hybrid is semi-vigorous, spreading tree, moderate but regular bearer, fruits are medium in size (240 g), fruit pulp was similar to Langra and skin was similar to that of Sundar Pasand.

Work was initiated at the Central Institute for Sub-Tropical Horticulture, Lucknow, UP on mango breeding and two hybrids, *viz*., CISHM-1 and CISHM-2 were released. CISHM-1 is a cross between Amrapali × Janardhan Pasand. Fruits of CISHM-1 are medium in size (225 g), oblong in shape and with slight sinus. Fruit skin is attractive, pulp is firm, high TSS (21%) and suitable for export purpose (Negi et al. 1996). CISHM-2 is a cross between Dashehari × Chausa. Fruits of this hybrid are medium sized, weighing 220 g and oblong in shape. Skin is smooth, tough, yellowish green when ripe. Flesh is firm with scanty fibers and dark yellow in color. TSS of pulp is high (23%). This hybrid has good potential because of its sooty mould free fruit surface even after exposure to heavy rains.

The fruits are similar to Dashehari but mature 15 days later than Dashehari (Negi et al. 2000).

Mango breeding at the Horticultural Research Station, Periyakulam, TNAU resulted in isolation of the Hybrid 2/7 which was later released as PKM-1 in the year 1980. This hybrid is a cross between Chinnasuvernarekha × Neelum. A mean yield of 536 fruits with 102.70 kg was obtained per tree per year with a maximum yield of 1420 fruits weighing 284 kg. The yield has been steady and regular. The hybrid bears fruits on clusters and the fruits are fairly big and very sweet in taste (Shanmugavelu et al. 1987).

An evaluation of four mango hybrids at the Horticultural Research Station, Periyakulam, revealed that a hybrid between Neelum × Mulgoa was high yielder. As many as 48.4 fruits per tree were obtained weighing 25.22 kg. It is a regular bearer with excellent fruit quality. Later this hybrid was released under the name PKM-2 (Shanmugavelu et al. 1987).

Work at the Regional Fruit Research Station, Anantharajupet, Cuddapah district, AP resulted in the release of hybrid, *viz*., Au-Rumani and the Fruit Research Station, Sangareddi released another hybrid, *viz*., Mangera. 'Au-Rumani' is a cross between Rumani × Mulgoa. This hybrid is high yielder and regular bearer. Fruit is apple shaped and resembles 'Rumani' with prominent shoulders. Fruit pulp is firm, sweet and good flavored (Swamy et al. 1970). Mangera is also a cross between Rumani × Neelum varieties. The tree of Mangera is dwarf and hence suitable for high density planting. It is a precocious, regular bearer and high yielder. The fruit is large, roundish oblique in shape. The fruit skin is uniformly yellow with red blush on the shoulders. Quality of fruit is very good (Swamy et al. 1972).

Works on mango breeding at the Regional Fruit Research Station, Kodur, AP resulted in the development of four hybrids, *viz*., Neeluddin, Neelgoa, Neeleshan and Swarnjahangir. 'Neeleshan' is a cross between Neelum × Baneshan. It is a mid-season variety with regular bearing in habit. The fruit size is large and almost similar to Baneshan in shape. It bears in clusters and some trees bear a second crop. Fruits are medium to large in size (300 g). Pulp recovery is high (69%), it is fiberless, fragrant and suitable for canning. Neeluddin (Neelum × Himayuddin) is a regular bearer, but fruits are small in size (220 g). The fruit is pulpy, fiberless, juicy and aromatic.

Work was initiated at the Indian Institute of Horticultural Research, Hessaraghatta, Bangalore in Karnataka during 1970 to develop varieties with regular bearing, high yield, good fruit quality and free from spongy tissue. About 100 F_1 hybrids of different parental combinations were raised and evaluated. Out of which, four hybrids, viz., Arka Aruna (Banganapalli × Alphonso), Arka Puneet (Alphonso × Banganapalli), Arka Anmol (Alphonso × Janardhan Pasand) and Arka Neelkiran (Neelum × Alphonso) have been selected (Iyer and Subramanyam 1993).

Arka Aruna is dwarf in stature, regular, precocious and medium yielder. It bears large fruits (450 g) having attractive peel color with red blush, cream color and fiberless pulp of good flavor, high TSS (22°Brix) and small stone. It has high pulp percentage (78.5) and free from spongy tissue. This is suitable for homestead planting. Arka Puneet is of vigorous plant type with regular and prolific bearing of medium size fruits (225 g) having attractive peel color, good aroma, high TSS (22°Brix), fiberless pulp about 75%, free from spongy tissue and good keeping quality. This hybrid is suitable for table purpose and processing. It is free from fruit fly incidence. The fruits can be stored at room temperature for more than 15 days. Arka Anmol is a semi-vigorous plant type with regular, prolific bearing habit, fruits weighing about 250 g with good table and keeping quality. Attractive golden yellow peel color makes it suitable for export. Arka Neelkiran is a regular bearer, fruits are medium in size, attractive and quality of fruit is better than Neelum.

In Israel mango breeding is in progress at the Agricultural Research Organisation (ARO), Bet Degan from where two hybrids, *viz*., 'Tango' and 'Shelly' have been released. 'Tango' is a cross between Naomi × Keitt. Tango tree is medium in size, the inflorescence is pyramidal, 35 cm long and is densely flowered. The flowering season begins in late February to early March. Fruits are oblong with pointed beak and shallow sinus. The dorsal shoulder is higher than the ventral one and the apex is tombed. The fruit size is uniform with an average weight of 380 g. The skin of the mature fruit is thin and smooth and its color is orange, blushed with a brilliant red. The orange colored pulp is juicy and tender with scarce fibers. It has a mild, sweet and sour flavor with a mild, pleasant aroma. The fruit can be stored for 13 days at 20 °C and 21 days at 14 °C. Preliminary data suggest that the yield of 'Tango' is slightly above average of parents (Lavi et al. 1997b).

'Shelly' was selected from a cross between 'Tomy Atkins' and 'Keitt' and is a late ripening hybrid. Fruits are round with no beak or sinus, apex is rounded. Average fruit weight is 500 g (ranging from 350 to 700 g).

The peel of the mature fruit is of medium thickness and smooth and its color is orange with a red blush. The pulp is orange with a juicy, firm texture and scarce fibers. The flavor is mild and slightly sweet. Mature fruit have remained firm and attractive up to 30 days after picking at room temperature. Preliminary data reveals that the yield of Shelly hybrid is well above average of parents (Lavi et al. 1997a).

16.1.3.3 Crop Improvement in Mango by Clonal Selection

Clonal selection within cultivars has yielded valuable results and hence appears to be worth pursuing particularly in countries where certain cultivars are in cultivation for a long time. When a cultivar is grown for a long period, though originated through vegetative propagation, variation may occur due to mutation at micro or macro level. This mutation is a possibility in a variety when cultivated for a prolonged period.

Alphonso mango is an export quality cultivar of India. Pandey (1998) studied different clones of this cultivar, *viz*., 'Alphonso of Behat' in Saharanpur (UP), 'Alphonso Batli' of Kirkee, Pune (Maharashtra), 'Alphonso Punjab', 'Alphonso White' of North Kanara district of Karnataka, and found that they differ from one another in more than one character. Pandey (1984, 1998) described seven different clones in Alphonso mango, *viz*., Alphonso Behat, Alphonso Batli, Alphonso Bihar, Alphonso Black, Alphonso Bombay, Alphonso Punjab and Alphonso White. The origins of all these clones are not known but these clones are indigenous to different parts of India.

In perennial trees like mango, asexual propagation renders preservation of accumulated mutations (both macro and micro) which normally would be sieved out by sexual propagation. This offers the scope for the selection of good clones within a cultivar (Iyer and Mukunda 1998). Exploitation of natural variability through selection of superior clones of commercial mango cultivar has been undertaken by several workers. Naik (1948) observed significant variation among the trees of the same variety in an orchard with regard to fruit shape, size, color and quality of the fruits which was ascribed to bud mutations. Oppenheinmer (1956) based on a survey in many mango orchards in India reported wide variability in the performance of the trees of the same variety in the same orchard. Singh (1971) and Naik (1971) have emphasized on the role of clonal selection in mango improvement based on their extensive observations. Iyer and Dinesh (1997) and Iyer and Degani (1997) have emphasized on the need for great caution while identifying new clones. It is necessary to test the new clones under replicated trials to compare them against standard commercial varieties to confirm the distinctiveness and superiority.

Work on clonal selection at the University of Agricultural Sciences, Bangalore has led to the identification of two superior clones in the cultivars Alphonso and Raspuri (Mukunda 1996; Anon 1998). HSA-4 (Alphonso clone) has fruits of larger size (410 g) with attractive fruit color. NPP-5 (Raspuri clone) is a high yielder, regular bearer, has better fruit shape and TSS of 21% (Mukunda 1996; Iyer and Mukunda 1998).

Studies carried out on the evaluation of certain clones of mango cv. Alphonso at the University of Agricultural sciences, Bangalore have revealed that 'MA-1' clone was an outstanding progeny, as the plants were vigorous in growth habit; panicle density was highest and produced largest sized panicles (Mukunda 2004). Sex ratio in this clone was highest and as such fruit set was also highest. Fruits of 'MA-1' clone were medium to large in size, firm pulp, highest pulp recovery, thin stone and medium peel. Highest TSS, moderate acidity and excellent TSS/acid blend was noticed in the fruit pulp of 'MA-1' clone. Fruits of 'MA-1' clone had attractive skin color with red blush on the shoulders; flavor was delightful and superb in taste. Fruits of 'MA-1' were tolerant to spongy tissue.

A regular bearing and high yielding mango clone, "Dashehari-51" has been released by the Central Institute for Sub-tropical Horticulture, Lucknow, UP. This clone produces good crop every year without off-bearing rhythm, the per year productivity being 38.8% more than "Dashehari". Even in an 'off' year, 'Dashehari-51' clone produced an average yield of 43.4 kg fruits per plant per year while Dashehari tree produced very poor fruits (Chadha et al. 1993; Rajput et al. 1996; Ghosh 1997; Anon 1999; Negi 2000).

Desai and Dhandar (2000) studied a large number of mango varieties of Goa state which are important either from commercial or from breeding point of view, for their physico-chemical and morphogenetic variations. These varieties differ in respect of bearing habit, fruit size, fruit color, flesh contents, pulp color and quality. Pulp contents depending on fruit size varied from 67.56% in Bemcorado to 83.21% in RC-MS-1 a clonal selection of Bemcorado. In general, the varieties namely Mankurad selection and Bomcarado selection were observed to be promising as

these clones possessed most of the desired commercial traits meeting international standard.

Chaikiattiyos et al. (2000) conducted the progeny test of '320' Kaew clones collected from the North, North-east and Central Thailand, and selected a number of clones with superior horticultural traits for further evaluation. Of these, the 'SK007' clone produced the highest fruit yield of 65.4 kg per tree at the age of 7–8 years. A subsequent comparison yield trial of the previously selected clones indicated that the 'SK007', officially recommended by the Department of Agriculture, Bankok, Thailand and currently known as 'Kaew Sisaket', has an average yield of 25.5 kg per tree at year 5–6 with the average fruit weight of 252 g, 81% flesh and 0.049% citric acid. The fine texture, low fiber content and firm flesh offer good quality for pickling.

Jintanawongse et al. (1999) made an attempt to improve the existing commercial mango cultivars in Thailand by the clonal selection in Nam Dok Mai, Khiew Sawoey, Rad and Nang Klang Wan cultivars. Since 1990, the growth behavior, yield and quality of fruit characteristics such as fruit size, flesh color, sweetness, aroma and texture were evaluated. The data showed 'Clone No. 01' of Nam Dok Mai, 'Clone No. 04' and '05' of Khiew Sawoey, Clone No. 03 of Rad and 'Clone No. 03' of Nang Klang Wan clones were superior over the other clones. In addition, DNA fingerprinting was also made for all these five clones. These clones are to be released as recommended and certified cultivars by the Department of Agriculture in the near future.

Singh (1999) reported a new variety 'MDCH-1' (Madhulika) out of 41 species found in the world. Studies made from 1990 onwards at ICAR, Manipur Centre, Imphal showed that this new species was the most promising one of the region besides its resistance to stone-weevil which is the common pest for the region. Among the 17 genotypes studied in foothill conditions of Manipur, the genotypes MDCH-1 and STH-1 have been found to be very promising with most desirable characteristics such as regular bearer, dwarf, better fruit quality and resistance to stone-weevil and diseases. Thus Singh (1999) was of the opinion that the new genotypes, MDCH-1 and STH-1, would be 'wonder mangoes' of the 21st century, and may be able to revolutionize the mango production in India and abroad.

Survey of local mango genotypes at Goa state has enabled collection of 68 different varieties. Big-fruited clones of 'Hilario' and 'Udgo' with outstanding fruit quality were screened (Anon 1994). During 1985–1995, mango clone Cardozo Mankurad was selected from the Mankurad variety of Goa. This new clone was described in detail and compared with the parent variety Mankurad. Cardozo Mankurad is regular bearer and produces large fruits of attractive color, high quality and yield (Mathew and Dhandar 1997).

Intensive survey made by Ramaswamy (1989) in the North-Western region of Tamil Nadu comprising Salem and Dharmapuri districts led to the isolation of three selections of 'plus trees'. Parameters like regular bearing, dwarfness, high yield, good fruit quality and field tolerance to pests and diseases formed the basis of selection. Dwarf clonal selections, one each in Rumani and Bangalora, and a high yielding elite Neelum clone were isolated. The estimated bark phenol contents were from 4,300 to 4,800 µg per gram of fresh weight. This physiological parameter appears to be linked with dwarfness. The selected clones lend scope for high density planting at a density of 500 trees per ha. The average weight of fruit in these elite clones of Rumani (362 g), Neelum (404.20 g) and Bangalora (639.40 g) clones was high and the pulp recovery was the highest (89.26, 87.74 and 93.93%, respectively) and TSS of the fruit pulp was medium to good (16.85, 22.34 and 16.96%, respectively).

Vijayakumar et al. (1991) made an intensive survey in Dharmapuri district of Tamil Nadu and exploited the natural variability present in Rumani and Neelum varieties of mango. 'DPI 55' a clone of Rumani and 'DPI 45' a clone of Neelum were dwarf in nature and suitable for high density planting. These clones possess precocity, regular bearing, attractive good quality fruit with high productivity. Vijayakumar et al. (1992) developled "Paiyur 1" mango, a clonal selection from Neelum variety of Tamil Nadu. This new clone is a dwarf plant, low spreading in nature and thus suitable for high density planting at a close spacing of 5 m × 5 m accommodating 400 trees per hectare. During the ninth year of planting the fruit yield of 'Paiyur 1' clone was 22.30 kg per tree accounting for 8,920 kg of fruits per hectare. The mean fruit weight was 121 g with 68 per cent pulp recovery and good taste.

An assessment of various vegetative and fruit characters was made by Subramanyam and Iyer (1989) in mango germplasm collected from different regions of India, to select suitable parents for hybridization. Accordingly they identified two dwarf plants from collections made in Kerala state, which they named as 'Kerala Local' and 'Local Kalapady'. These selec-

tions have potential for incorporating dwarfness in vigorous growing commercial varieties as well as using as new clones to establish high density orchards.

Mukherjee et al. (1983) based on detailed surveys of mango orchards in eastern India have identified many superior clones. Based on their survey 23 elite clones were identified. Some of the clones which ranked excellent grade by them are: 'Dawadi', 'Emrat Bhog', 'Shah Pasand', 'Sadik Pasand', 'Kali Bhog' (all from Murshidabad) and 'Misrikanta' (from Maldah). They also suggested that it would be worth putting these clonal selections in a varietal trial for comparison with the standard commercial variety for releasing as new superior selections for commercial exploitation and for utilization in the hybridization program.

In Punjab, several selections of sucking mango with good sucking qualities, abundant juice, less fiber, small stone and red blush on cheeks have been made at the Regional Fruit Station, Gangian. These are numbered as GN 1, GN 2, GN 3, GN 4, GN 5, GN 6 and GN 7 (Sandhu et al. 1990).

The 'Davis-Haden' is an example of a bud sport originated in Florida (USA) from 'Haden' variety. The fruit of 'Davis-Haden' is somewhat larger than 'Haden' and its season of maturity is about a month later. Such mutations occur frequently in some plants though in mango nothing is known of the frequency with which spontaneous mutations occur (Young and Ledin 1954). One of the mutant varieties reported in mango from Peru is 'Rosica'. It is a bud mutant of the local Peruvian cultivar "Rosado de Ica". In trials it was found to be precocious and regular bearing, giving good yield of high quality fruit. Unlike other local cultivars, it did not produce small seedless fruit, and it was monoembryonic (Medina 1977). Li Gueiheng et al. (1996) reported that 'Hongmang 6' is a mutant derived from Zill variety of mango from China. Production of Hongmang 6 is very high, 7-year-old trees producing yields of 43.50 kg fruits per tree. Fruits are of good eating quality and red to purplish-red in color while flesh is dark yellow and juice with TSS of 15.8%. Roy and Sharma (1960) observed a bud-sport in the local variety Hirasonia at the Bihar Agricultural College, Sabour, which bore virtually fiberless fruits, larger than those of parent variety and differing in other respects. The mutant was considered to be of potential economic value. Sharma et al. (1981) obtained some very interesting plant types in the VM_1 generation by stabilizing the changes induced through the heading back technique. Amongst these are plants with very long and very short extension growths, very thick and very thin shoots; multiple branching shoots; very large and very small lanceolate leaves and small leaves with highly wavy margins and plants which appear to be compact in growth habit. A few of these induced plant types, both from the varieties Dashehari and Mallika, appear to be promising from the point of view of dwarfness and hence, may prove their immense value. Higher TSS and better sugar/acid blend, than the standard Dashehari (as a control), were also observed in few plants.

A clone of mango 'Totapuri' has been selected at the Gujart Agricultural University, Paria, Gujrat (Anon 1997). This clone was identified from a private farmer's mango orchard of Babubhoi Bhagvanbhai Patel of Goima village, Pardi taluk, Valsad district. The age of the mother plant is 35 years and vigorous in nature with irregularly spreading primary and secondary branches. This clone flowers and fruits twice in a year, first week of October and second week of March. It yields 24,000 fruits per tree per year weighing 700 kg per tree (Naik 2000).

In North-Eastern region, selections Manipur-I and Manipur-II have been identified. These clones are dwarf, precocious, polyembryonic and regular bearer (Chadha and Yadav 1996). Chadha (1998) also reported about clonal selection in mango ('Sunderraja') made at Rewa, Madhya Pradesh. Two clones one each of Banganapalli, 'Rati Banganapalli' and clone ('Nuzvid') selected earlier, performed well (Anon 1999). Further, one clone each of Alphonso, Totapuri and Banganapalli has been selected at the Regional Fruit Research Station, Vengurla, Maharashtra; the Fruit Research Station, Anantharajupet, Andhra Pradesh, and the Agricultural Experimental Station, Paria, Gujrat, respectively (Anon 1999).

Studies on evolving improved plant types through physical and chemical mutagens are, in general, lacking in fruit trees, more particularly in mango. The study conducted by Sharma et al. (1983) showed that the LD_{50} values (S-irradiation) for the mango cultivars Neelum, Dashehari, and Mallika were 3.9, 2.9 and 2.4 Krad respectively. The effective dosages of EMS and NM for cvs. Dashehari and Neelum were 1.5% and 0.05%, respectively. Primary effects of both physical and chemical mutagens were found to be more or less the same. Some interesting changes in vegetative characters have also been established. Out of these a few plants appeared to be promising for

dwarfness whereas in some others, fruit quality improved.

A survey was initiated by Singh et al. (1985) between 1982 and 1985, to find out relative superiority of selected clones from different orchards, *viz*., Pilkhini, Motijhil and Nawalpur around Varanasi. One of the selected mango clones of 'Banarasi Langra' mango on the basis of overall performance, the 'clone No. 2' from Pilkhini orchard and 'clone No. 1' from Motijhil orchard were found to be most promising clones because of less fruit drop, better yield, light bottle green color of the skin with fairly high fruit quality and negligible incidence of malformation.

Kher (1961) gave a detailed account of the morphology and anatomy of the leaf, stem and fruit of a variegated mango plant from Malihabad region. The variation appears to be genetically controlled and results from a mutation in the middle histogenic layer. Singh and Ranganath (1997) surveyed 85 locally available clones in South and North Andaman, and six clones having regular and early flowering habit and bearing good yield of quality fruits were selected for further study. Two years after planting of grafts in the field, it was noticed that clone No. 6-1-3 showed early flowering (early October), and in its first year of fruiting about 100 fruits were harvested in March. The fruits were of good quality with minimum thickness of peel and had minimum stone weight.

Rukayah Aman (1989) reported that mango clone MA-125 which is known to be difficult to flower can be induced chemically using flower inductants. Both paclobutrazol and uniconazole between 5 and 10 g a.i. per tree and 0.50 to 1.50 g a.i. per tree, respectively increased flowering and fruiting in this clone. However potassium nitrate was not effective for this clone.

Siller Cepeda et al. (1994) studied the fruit quality and post-harvest behavior of four mango clones, *viz*., 'Osteen', 'Palmer', 'Fabian' and 'S1-25' in comparison with 'Kent' the commercial variety from Mexico. 'Osteen' and 'Kent' had similar fruit characteristics. 'Osteen' had the largest fruits and 'Fabian' the smallest (516 and 387.5 g per fruit, respectively). After storage 'Osteen' had the highest pulp percentage (83.2) and the lowest percentage of seeds (5.8) and peel (10.9). 'Osteen' and 'Fabian' had a yellowish pulp color while the other clones had a more orange colored pulp. It was concluded that 'Osteen' has potential as a commercial cultivar because when compared with 'Kent', it showed a comparable or higher values of fruit weight, pulp weight, sweetness and sugar/acid ratio. The post-harvest behavior of Palmer indicated that it could be used for longer storage periods, because it maintained high firmness and a low sugar/acid ratio after the storage. 'Fabian' had a high percentage of latent anthracnose which was expressed during storage.

Strains within 'Kensington Pride' have been identified in Australia and one of them, Grosszmann even having improved resistance to bacterial black spot (Mayers et al. 1988; Whiley et al. 1993).

16.1.3.4 Limitation of Traditional Breeding Methods

The main disadvantage of traditional breeding is that it is laborious and consumes lot of time. A superior mango type can be isolated through selection, clonal selection and by hybridization. It involves extensive field and paper work; and collection of at least ten years' data to confirm its quality. One can release a variety through these methods in his lifetime. The juvenile period present in most of the tropical fruits also affects quick assessment of a variety. Therefore, it is necessary to look for the important objectives in mango improvement program and use the available molecular approaches to address these problems. Selection of unique plants/genotypes/clones can be easily achievable using molecular approach. Markers can be used as a tag to isolate unique types using marker-assisted selection. Even linkage distance studies among genotypes can be taken up using these markers. Using molecular approach one can isolate desirable genes from one plant and incorporate into another. With this one can genetically manipulate a plant to have desirable characters. It can be easily achievable in less time. However, molecular studies in mango are limited when compared to other fruit crops.

16.2 Application of Molecular Markers for Genetic Analysis in Mangoes

There are hundreds of mango cultivars, of which only some 25 to 40 are of commercial importance (Chadha and Pal 1986). Commercially grown cultivars have been identified on the basis of leaf, panicle, fruit, and stone characteristics; however, these characters may change with environmental conditions (Laksh-

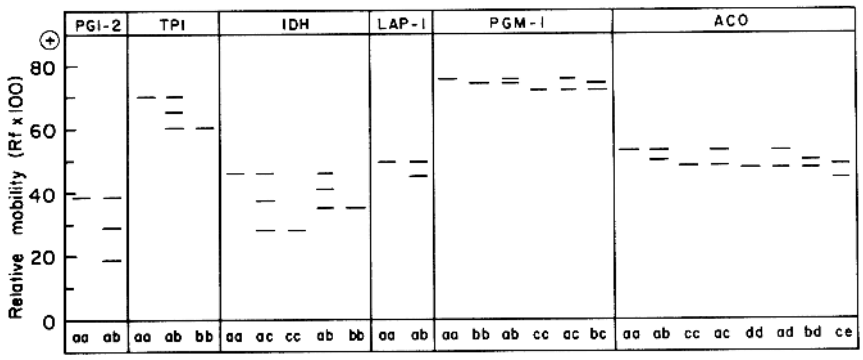

Fig. 1. Schematic zymograms of representative phenotypes for PGI, TPI, IDH, LAP, PGM And ACO isozymes in mango. Relative mobility ($R_f \times 100$) on the *left*; *O* = Origin. All enzymes migrated anodally (toward the top of the figure) (With permission from J Am Soc Hort Sci)

minarayana 1980). Furthermore, the actual identity of some cultivars is still in question, because similar cultivars grown in different areas often have different names (Lakshminarayana 1980). Having reliable means of cultivars identification and verification is important. Therefore, identification of genetic markers is of great value in this regard.

16.2.1 Isozymes

In the recent years, enzyme polymorphism has been used successfully to identify cultivars in various fruit species. However, isozymes can be affected by stage of development and tissue used for extraction (Feret and Bergmann 1976). Leaf isozymes of esterases, aspartate amino transferase, acid phosphatases, and alkaline phosphatases were used to detect possible genetic variation among individual mango clones (Gan et al. 1981). Degani et al. (1990) have used isozyme systems aconitase, isocitrate dehydrogenase, leucine aminopeptidase, phosphoglucose isomerase, phosphoglucomutase, and triosephosphate isomerase to characterize 41 mango (*Mangifera indica* L.) cultivars. The outcross origin of some of the mango cultivars was supported by the isozymic banding patterns (Fig. 1). Reported parentage of some other cultivars was not consistent with their isozymic banding patterns.

Isozyme systems were also used to detect zygotic seedlings from five polyembryonic cultivars of mango. Significant differences were found between cultivars for the percentage of zygotic and nucellar seedlings detected (Schnell and Knight 1992). They were able to determine the off-types using isozymes and concluded that this procedure can be used to help certify rootstock mother trees (Fig. 2).

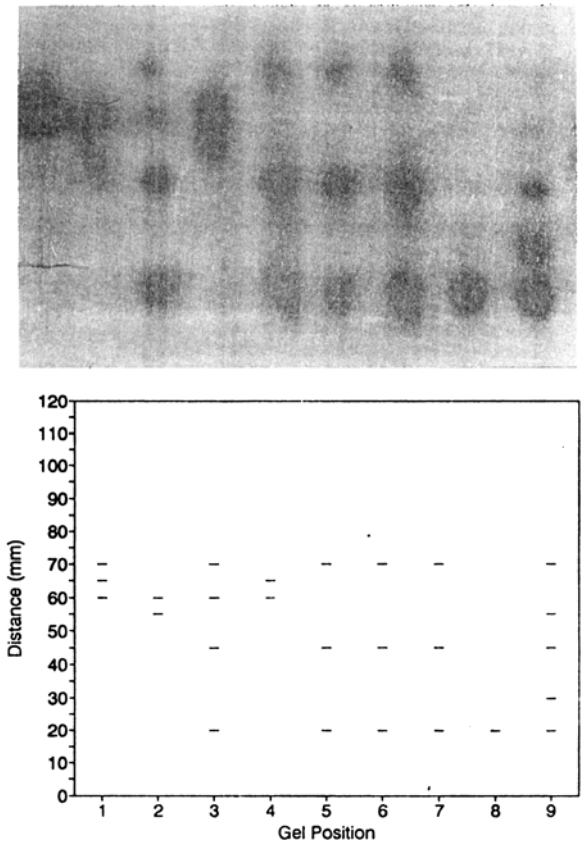

Fig. 2. Isozyme banding patterns (photograph and corresponding diagram) of glucose-6-phosphate iosmerase among rootstock cultivars and seedlings. Gel position: 1) Madoe, 2) Madoe RSP 1, 3) Madoe RSP 11, 4) Madoe RSP 18, 5) 13-1, 6) Turpentine, 7) Turpentine RSP 2, 8) Turpentine RSP 9 and 9) Golek RSP 13 (With permission from HortScience)

On the contrary, Gazit and Knight (1989) used one enzyme system, glucose-6-phosphate isomerase (GPI), and gas chromatography to detect zygotic plants among open-pollinated seedling populations from polyembryonic mango cultivars. Gas chro-

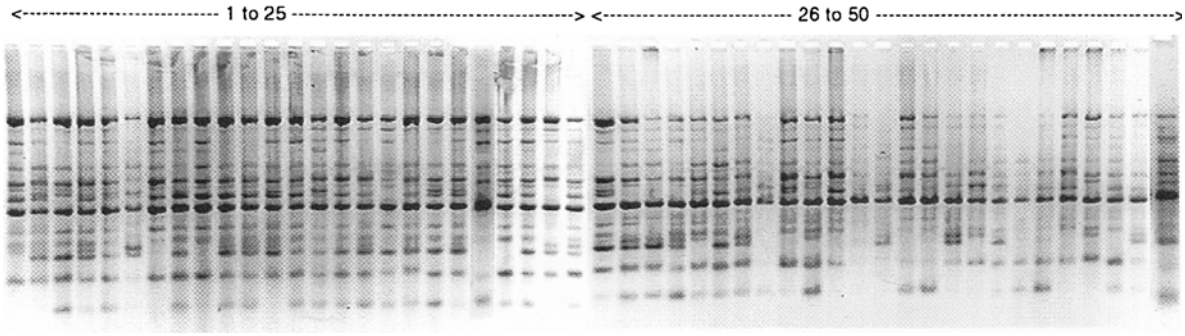

Fig. 3. RAPD gel profile of 50 mango cultivars using Operon primer D1. *Lanes* 1–10: Raspuri, Neelum, Baneshan, Ratna, Mulgoa, Dashehari, Hamlet, Alphonso and Totapuri, *Lanes* 11–20: PKM-1, Rumani, Sindhu, Mallika, Amrapali, Neeleshan, Neelgoa, Neeluddin, PKM-2 and Himayuddin, *Lanes* 21–30: Kesar, Goamunkur, Suvarnarekha, Vanraj, Cherukurasam, Arka Aruna, Jehangir, Svarna Jehangir, Kuddus and Kalapadu, *Lanes* 31–40: Arka Puneet, Vikrabad, Arka Anmol, Pulihora, Rajgira, Achar Pasand, Fazli, Khas-Ul-Khas, Nekkere and Bombay Green, *Lanes* 41–50: Langra, Janardhan Pasand, Willard, Allumpur Baneshan, Rajapuri, Zardalu, Kishen Bhog, Tenneru, Ratnagiri Alphonso and Dilpasand

matograms were too cumbersome for analysis of large populations; the isozymes system proved to be simple, repeatable, and cost effective. However, enzyme polymorphism in mango has not been examined systematically.

16.2.2 DNA Markers

As the efficiency of a selection scheme or genetic analysis on phenotype is a function of heritability of the trait, factors like environment, traits of multigenic and quantitative inheritance, or partial and complete dominance often confound the expression of genetic traits. Many of these complications of a phenotype-based assay can be overcome through direct identification of genotypes with DNA (deoxyribose nucleic acid) based diagnostic assay. Genomic fingerprinting has been accomplished traditionally through the use of isozymes, and more recently through restriction fragment length polymorphism (RFLP), variable number tandem repeats (VNTRs), or a combination of both. While these methods have been very useful in cultivars identification, they have a number of disadvantages, including a limited number of isozyme loci and the time, expense, and use of [^{32}P] for labeling with RFLPs and VNTRs. Polymerase Chain Reaction (PCR) based method overcomes these disadvantages and is used in several crops. For this reason, DNA-based genetic markers are being integrated into several plant systems and are expected to play an important role in future plant improvement programs in mango.

16.2.2.1 Random Amplified Polymorphic DNA (RAPD)

PCR technology has led to the development of several novel genetic assays based on selective DNA amplification (Krawtez 1989; Innis et al. 1990). A genetic assay was developed independently by two laboratories (Welsh and McClelland 1990; Williams et al. 1990). RAPD assay detects nucleotide sequence of polymorphisms in DNA using only a single primer of arbitrary nucleotide sequence. The protocol is also relatively quick and easy to perform and uses fluorescence instead of radioactivity. Because the RAPD technique is an amplified-based assay, only nano-gram quantities of DNA are required. One of the strengths of this new assay is that they are more amenable to automation than conventional techniques. It is simple to perform and is preferable to experiments where the genotypes of large number of individuals are to be determined at a few genetic loci.

The use of RAPDs to determine genetic relationships has been demonstrated in several crops. Within *Mangifera* (mango) species, RAPDs have been used to determine phylogenetic relationships (Schnell and Knight 1993). RAPD generated clusters did not agree with the taxonomic classification in mango based on phenotypic traits (Kostermans and Bompard 1993) into *Mangifera* and *Limus*. When the two subsections of the genus were analyzed separately, the classification agreed more closely with the traditional taxonomic analysis. This technique has been successfully used to identify 25 accessions of mango and to validate their genetic relationships (Schnell et al. 1995). Genetic relatedness of traditional Indian mango cul-

tivars grown in commercial scale was studied using RAPD makers (Ravishankar et al. 2000). Results of the study indicated that cultivars from a particular geographical region were closely related. In India, it is very difficult to distinguish wild trees from cultivated ones as they spread all over Indian peninsula, the result clearly indicate that majority of the commercial cultivars evolved from local germplasm and later they were selected and propagated vegetatively. Fifty commercial mango cultivars were screened using RAPD markers to estimate the genetic diversity (Kumar et al. 2001). A high degree of genetic variation was observed among the cultivars and the variety 'Mulgoa' was found to be very distinct (Figs. 3 and 4).

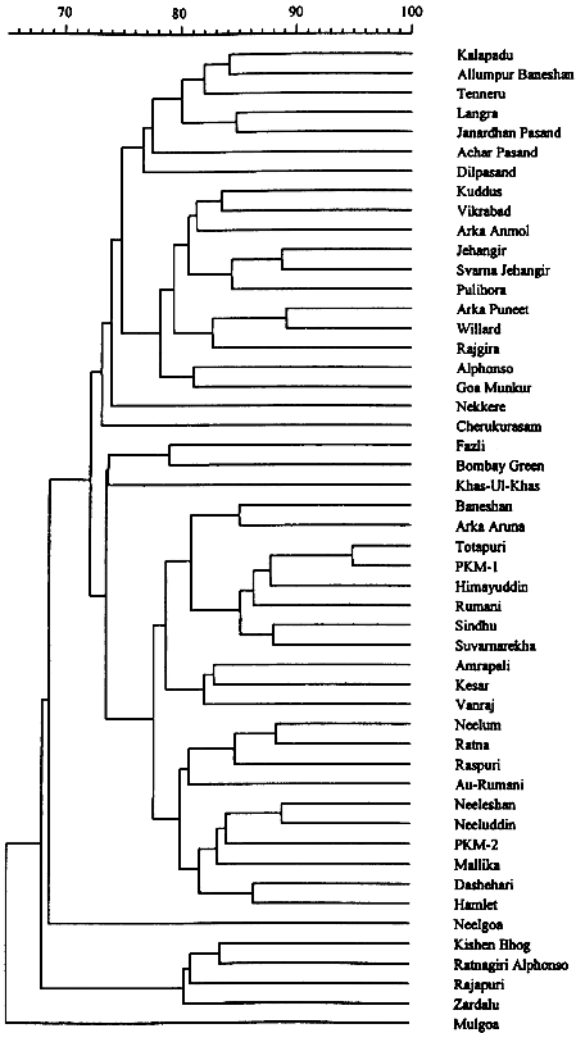

Fig. 4. Association among mango genotypes revealed by UPGMA cluster analysis from RAPD data of 139 amplification products generated by 10 primers

Along with this paternity analysis of 14 mango hybrids was also carried out. The cluster analysis revealed the relationship of hybrids with their parents. The progenies were placed close to one of their parents whose characters resemble with it.

Lopez-Valenzuela et al. (1997) reported that RAPD marker can distinguish mangoes based on embryonic types and their geographical origin. The genetics of polyembryony in mango (*Mangifera indica* L.) was studied by Arnon et al. (1998) and they suggested that it is determined single dominant gene. The segregation pattern obtained by RAPD of individuals originating from selfing of several monoembryonic cultivars and one polyembryonic line indicated that the polyembryony in mango was of genetic nature. All the plants originating from monoembryonic bear monoembryonic fruit. An one-monoembryonic to three-polyembryonic segregation pattern was observed among individuals originating from the polyembryonic line, indicating that polyembryony in mango is under the control of a single dominant gene. This was also proved by Ravishankar et al. (2004) where they used both RAPD and chloroplast DNA for PCR-RFLP analysis to detect the genetic bases of Indian polyembryonic and monoembryonic mango cultivars. The cluster analysis of both markers revealed that eventhough the embryonic types are intercrossable, the polyembryonic types group separately indicating diverse genetic base. This suggested that polyembryonic types might have been introduced from other parts of Southeast Asia and is unlikely to have originated from India.

The long juvenility period of mangoes (up to 5 years) would make RAPD markers an extremely useful tool for the identification of cultivars during propagation and growth. The ability to identify mango cultivars using RAPD markers would also aid in the management of germplasm collections as identical cultivars often have different names. However, RAPD markers suffer from low reproducibility between laboratories.

16.2.2.2 Simple Sequence Repeats (SSRs)

This is widely used as a versatile tool in plant breeding programs as well as in evolutionary studies because of their ability for showing diversity among cultivars (Adato et al. 1995; Mhameed et al. 1996; Levi and Rowland 1997). SSRs, also known as microsatellites, are an efficient type of molecular marker based on tan-

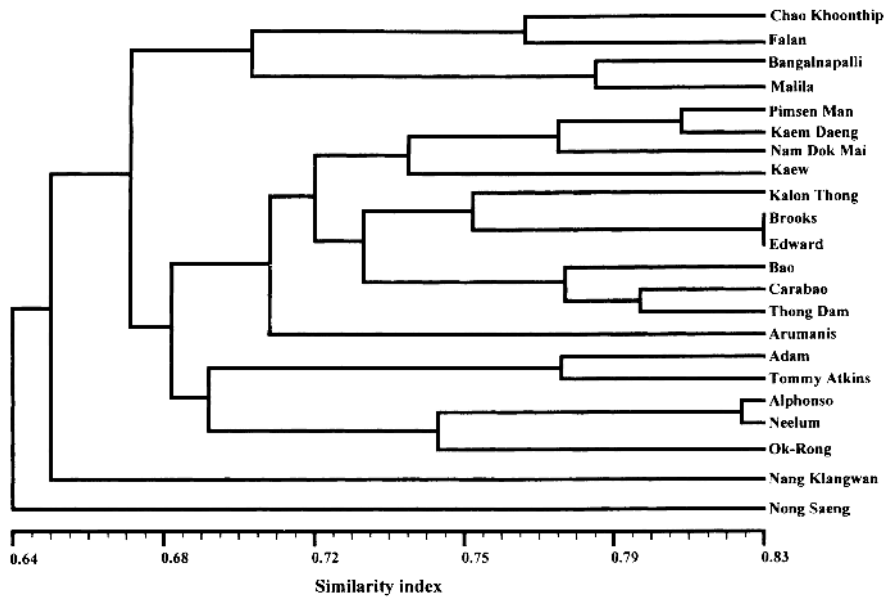

Fig. 5. Dendrogram illustrating the phylogenetic relationship among 22 mango cultivars based on UPGMA cluster analysis (With permission from Elsevier)

dem repeats of short (2–6 nucleotides) DNA sequence (Charters et al. 1996). These repeats are highly polymorphic, even among closely related cultivars, due to mutations causing variation in the number of repeating unit (Brown et al. 1996). SSRs, therefore, target highly variable and numerous loci.

Using Jeffrey's minisatellite probe Adato et al. (1995) identified and analyzed genetic variations among mango genotypes. They were able to trace back the offspring to one of the parents using banding pattern, demonstrating the reliable application of the system for paternity dilemmas. On the contrary SSR anchored primers were used to identify and to validate genetic variation among Thai cultivars (Eiadthong et al. 1999), but could not separate the cultivars according to their embryonic types, nor the types eaten as ripened fruit or unripe fruits (Fig. 5).

Recently, Sequence Tagged Microsattelite Sites (STMS) markers are being developed by many laboratories for mango. The main advantage of these markers are precise quantification of allele length and they are amenable for automation using automated DNA sequencer. Viruel et al. (2005) reported the sequence and variability parameters of 16 microsatellite primer pairs obtained from two mango genomic libraries after digestion of DNA of the cultivar Tommy Atkins with *Hae*III and *Rsa*I and enrichment in CT repeats. The polymorphism revealed by those microsatellites was evaluated in a collection of 28 mango cultivars of different origins. The SSRs studied allowed unambiguous identification of all the mango genotypes studied. They suggested that this discrimination can be carried out with just three selected microsatellites. UPGMA cluster analysis and principal coordinate analysis grouped the genotypes according to their origin and their classification as monoembryonic or polyembryonic types reflecting the pedigree of the cultivars and the movement of mango germplasm. A similar attempts is being made to develop SSR markers for mango in France (Duval et al 2005), Japan (Honso et al. 2005) and in India (Ravishankar 2006. Personal communication). These SSR markers are going to help extensively in mango genome mapping.

16.3 Linkage Mapping

Genetic linkage and QTL (Quantitative Trait Loci) mapping experiments involve large volumes of data. These include pedigree details, genotypes and trait data all of which must be combined in different forms to suit the nuances of each analysis program. Such experiments frequently also consist of collaborations between several groups making data sharing and concurrency a key concern. Many good software modules for statistical analysis of genomic data are offered in the public domain like MapMaker for linkage map construction, MapMaker/QTL for interval mapping for experimental crosses and others.

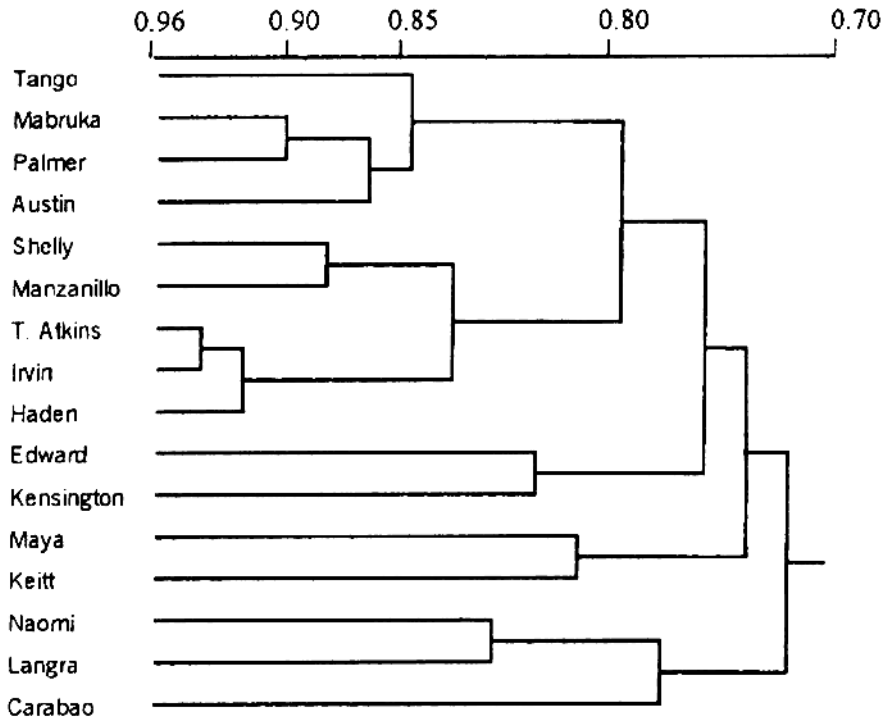

Fig. 6. Analysis of 16 mango cultivars using 42 various AFLP bands (With kind permission from Springer Science and Business Media)

An important development during the last decade in quantitative genetics was the ability to identify genome regions responsible for variation of a trait due to the advent of molecular markers (Paterson et al. 1988). The term QTL has come to refer to polygenes underlying a quantitative trait. In genetics, the distance between genes on the genome is assessed on the basis of the frequency of recombination of the genes, estimated from scoring genotypes of progeny of a cross (Kearsey and Pooni 1996). Mapping quantitative traits is difficult because the genotype is never unambiguously inferred from the phenotype. Classical quantitative genetics pursues a different approach, using statistical concepts such as means, variances, correlations, heritabilities (h^2), built on assumptions, e.g., that effects of individual genes on a trait are small and additive. This assumption sheds little light, if any, on the individual genes themselves (Prioul et al. 1997).

Major gene mutants, however, are scarce and may not exist in a population under study. Because QTL may occur throughout the genome, a large number of gene markers are required to locate them. Early studies of quantitative traits suffered from the lack of major-gene markers that could make a complete map. This problem was overcome with the realization that maps could be constructed using pieces of DNA as markers.

The advent of complete genetic linkage maps consisting of codominant DNA markers like RFLP, AFLP and SSR, that has stimulated interest in the systematic genetic dissection of discrete Mendelian factors underlying quantitative traits in plants. A marker linkage map can be used to localize QTL for a quantitative trait, as first demonstrated by Paterson et al. (1988). The basis of all QTL detection is the statistical analysis of associations between markers and trait values. Statistical techniques for using a marker map to detect QTL have reached a fairly high level of sophistication, but improvements are still being made(Kearsey and Farquhar 1998). A widely used method was intervalmapping (Lander and Botstein 1989). Other approaches, e.g., the multiple QTL method (Jansen 1995), were developed to detect multiple linked QTL. However, a QTL detected by any technique is not a true gene, only the indicated genome region that most likely contains gene(s) for the trait under study.

Two complementary uses of the QTL approach have emerged: the fundamental and the applied (Prioul et al. 1997). The first use, which is of interest to physiologists, targets QTL by determining their contribution to physiological components of macroscopic traits. Not only does the QTL approach provide unequivocal answers to a range of physiological questions, it also generates new insight into the causality

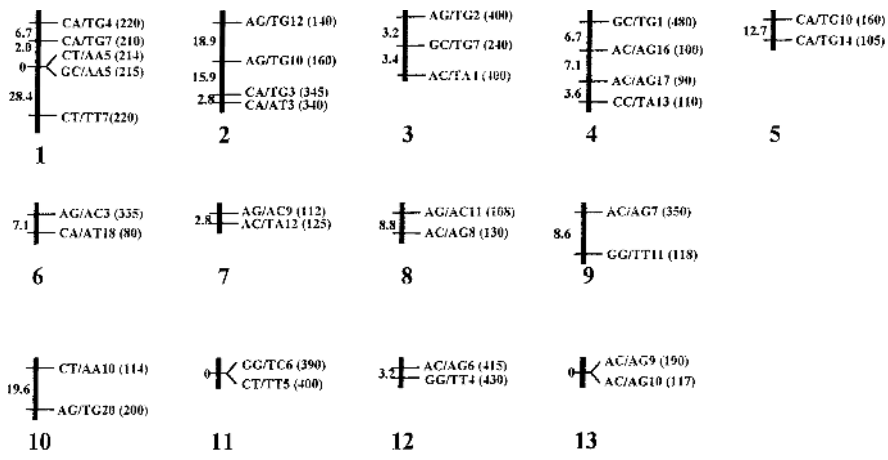

Fig. 7. Mango genetic linkage map. The various marker names refer to the primer combinations used for AFLP analysis. The numbers in parentheses indicate the size of the markers in base pairs (bp). Distances between markers are in centiMorgans. (With kind permission from Springer Science and Business Media)

between components that would have been difficult to obtain by conventional physiological approaches (Simko et al. 1997). The second use of the QTL studies, which is of interest to breeders, is marker-assisted breeding (MAB). This approach uses markers for tagging QTL of interest so as to pyramid favorable QTL alleles and break their linkage with undesirable alleles (Lee 1995; Ordon et al. 1998; Ribaut and Hoisington 1998).

The AFLP technique is based on the detection of genomic restriction fragments by PCR amplification, and has been applied to various plant species. This PCR-based technique permits inspection of polymorphism at a large number of loci within a very short period of time and requires very small amount of DNA. This method is robust for efficient DNA fingerprinting of the mango genome. A great majority of the AFLP markers (85%) are transmitted in a Mendelian fashion. Thus these markers could be used for genetic analysis.

Kashkush et al. (2001) used AFLP technique to identify mango cultivars, for studying the genetic relationship among 16 mango cultivars (Fig. 6) and seven mango rootstocks, and for the construction of a genetic linkage map. A preliminary genetic linkage map of the mango genome was constructed, based on the progeny of a cross between 'Keitt' and 'Tommy-Atkins'. Each of the two parents and 29 progeny were genotyped using 105 AFLP bands. The segregation of the alleles from each marker was examined for deviation from Mendelian expectation. The combined map consists of 13 linkage groups and 34 markers (Fig. 7). They reported that the genetic map consists only of markers that behave in a Mendelian fashion. Linkage analysis was carried out using both the MAPMAKER and LINKEM software. Both the programs provided identical results. They finally conclude that AFLP markers are suitable for cultivar identification, estimating genetic relationships and mapping QTLs in mango.

This is the only reported work on genetic linkage mapping in mango. Plant Genetics group at United States Department of Agriculture, Agricultural Research Service, Miami, Florida are working to solve the problems associated with the evaluation, enhancement, and preservation of subtropical/tropical fruit, using linkage maps. They have maintained germplasm repository. Their main objective is to develop and apply new or improved methods for elucidating the genetic bases for important horticultural traits, genetic marker-based approaches for genetic diversity assessment, and selection of improved germplasm and also identifying genes involved with horticulturally and agronomically important traits using Candidate Gene Approach (CGA). Mapping QTL for fruit characteristics, yield and disease in an F_2 population in mango in under progress. More focus has to be given towards this area of research that helps in tagging the genes of desirable traits.

16.4 Gene Isolation and Analysis

Gene expression studies in mangoes are limited. It is desirable to identify the genes uniquely expressed for particular traits. This would help in genetic manipulation of the plant to derive superior types having desirable characters. In mango, genes related to ripening have been characterized biochemically, but at molecular level only very few genes have been studied like

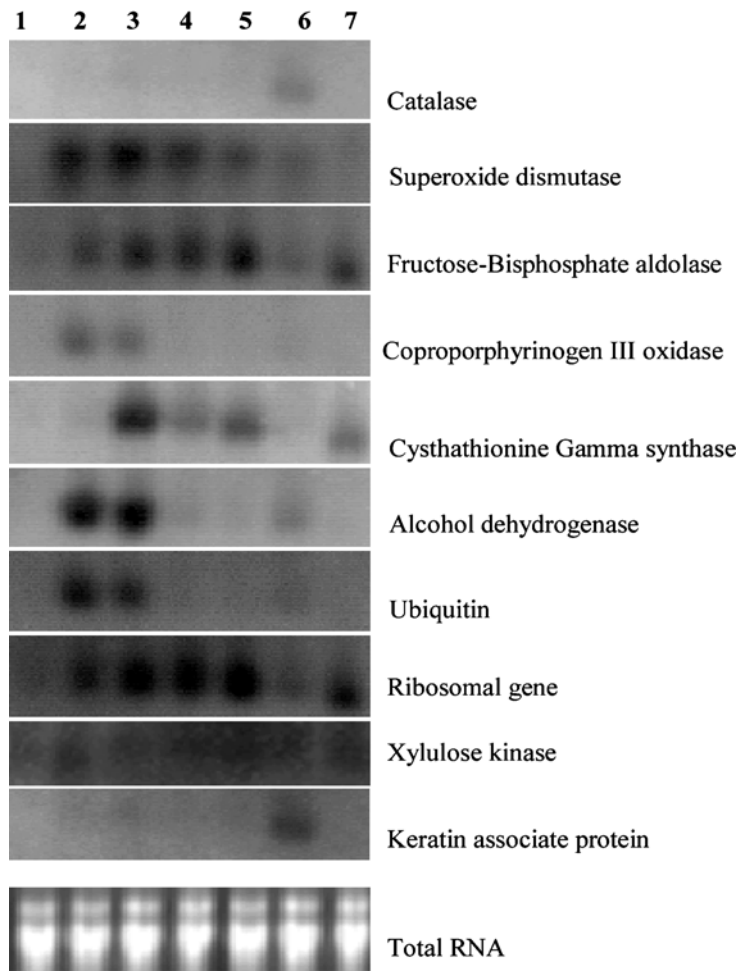

Fig. 8. Autoradiogram of northern analysis of the ten selected genes. 20 μg of total RNA from tissues of matured unripe fruites, RNA from 1, 2, 3 and 4 d fruits after ethylene treatment and from both spongy and healthy tissues. *Lane* 1–5 represent different stages of fruit ripening starting from matured fruits and *lane* 6–7 represent RNA from both spongy and healthy tissues

peroxisomal thiolase mRNA (Bojorquez and Gomez-Lim 1995), alternative oxidase (AOX) and uncoupling proteins (UCP) (Considine et al. 2001). AOX and UCP play an important role in post-climacteric senescent processes.

The next step in the molecular analysis of fruit is the construction of a gene library. This approach has already yielded valuable information in other fruit crops, where the genes coding for several ripening-related enzymes have been isolated. In mango, a cDNA library has been recently constructed and several ripening-related genes have been isolated (Gomez-Lim et al. unpublished data). Among the genes identified are those coding for cellulase, ACC synthase and the alternative oxidase, an enzyme involved in the cyanide-resistant respiration in fruits (Day et al. 1980). Preliminary expression studies of these genes show that they are predominantly expressed during fruit ripening, a fact consistent with their function and with studies in other fruits. In addition, other ripening related genes from mango have been cloned (Gomez-Lim et al. unpublished data) but their identity is still unknown. They also show predominant expression during ripening. Sequence analysis of these genes is in progress together with further studies to try and elucidate their function or identity.

At molecular level, no studies have been carried out to isolate genes involved in pest and disease resistance, and physiological disorders. This has been successfully isolated in several other crops. Very recently RAPD analysis was used to determine the genetic diversity on mango malformation pathogens (Zheng and Ploetz 2002). Vasanthaiah (2006) successfully isolated the differentially expressed genes specific to spongy tissue in Alphonso mango cultivar using subtractive hybridization. Thirty-seven genes from both spongy and healthy tissues were cloned and characterized. Higher expression of catalase, ubiquitin, coproporhyrinogen III oxidase and keratin genes were noticed in the tissue indicating

the existence of oxidative stress in spongy tissue (Fig. 8). It is also evident from the earlier studies that high temperature, high humidity, high respiration and low transpiration (Shivashankara and Mathai 1999) result in spongy tissue. These conditions elevate temperature and free radical levels in the fruit, which are toxic to the cell and resulting in cell membrane damage. Because of this the activity of enzymes like amylase, glutamate dehydrogenase, glutamate oxaloacetate transaminase, peroxidase were reduced. This influences sugar metabolism making the tissue hard and affecting the normal ripening. This study (Vasanthaiah 2006) indicated that oxidative stress is the probable cause for the spongy tissue formation.

Recently, in mango fruit cv Alphonso, 26 differentially regulated cDNAs from ripening tissue were isolated using PCR based subtraction at Indian Institute of Horticultural Research, Bangalore. These cDNA sequences were analysedwith NCBI database to assign putative identification. Expression patterns of major latex protein,cytokinin oxidase, omega-3-fatty acid desaturase, chitinase, putative flavanone 3-beta hydroxylase, putative auxin regulated protein, lipoxygenase, succinate dehydrogenase, protein phosphatase-2C and Acetyl-CoA acyltransferase were studied using RNA blot analysis at different stages of ripening. Majority of the identified genes were up-regulated during ripening process. A few of the identified genes have not been characterized in mango. The genes identified by differential expression are involved in changes associated with fruit ripening process like surge in respiration, ethylene biosynthesis, softening of mesocarp tissue, accumulation of pigments, development of characteristic aroma, change in color of the fruit and defense response (Ravishankar 2006 personal communication).

16.5 Gene Manipulation by Genetic Engineering

Limited reports exist on genetic manipulation in fruit crops. Recent experiments have shown that it is possible to turn-off the expression of certain genes in transgenic plants by introducing a gene constructed to generate antisense RNA (Eguchi et al. 1991). This allows expression of specific genes to be diminished, permitting their identification and assessment of their function during ripening. Genetic transformation has also been employed to achieve this goal. In mango a number of enzyme activities have been detected during mango fruit ripening (Selvaraj and Kumar 1995). Some of these may or may not be directly involved in the softening process. The results of the genetic transformation studies clearly indicated that correlative data linking enzyme activity and fruit softening might not accurately predict enzyme function.

The genetic transformation studies have also shown their potential for prolonging fruit shelf life. These procedures are universal and can be applied to many crops. Recent achievements in the transformation techniques will permit testing the function of specific hydrolytic enzymes for extending mango fruit shelf life. This development is particularly relevant because it will probably be the first tropical fruit whose ripening pattern may be genetically manipulated.

16.6 Future Scope

Mango has comparatively small haploid genome size and is about three times as large as *Arabidopsis* (Armuganathan and Earle 1991). This fact should be helpful for the application of other molecular techniques like Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), SSR, Differential Display RT-PCR (Reverse Transcriptase-PCR) and Subtractive Hybridization technique to assess genetic diversity, to construct linkage maps and to identify genetic markers linked to a trait of interest among different mango cultivars. This will help in isolating distinct types that are regular and precocious bearer. However, these techniques can also be used in isolating desirable genes responding to pest and disease incidence, and physiological disorders, which can be utilized to genetically manipulate mango plant to make it disease resistant. Very recently, Amplified Fragment Length Polymorphism (AFLP) information was used for identification of mango (*Mangifera indica* L.) cultivars to study genetic relationship among mango cultivars and rootstocks for the construction of a genetic linkage map (Kashkush et al. 2001). The development PCR based SSR markers from various laboratories will further strengthen molecular mapping activities and help in fine mapping

of mango genome. The molecular techniques also provide information on the genetic variability of mango species to build database on mango genetic diversity. Finally, all these molecular techniques have a potential for developing a superior genotypes with desirable characteristics in a shorter time when compared to conventional breeding methods.

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