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Recent Developments in Conventional Mango Breeding

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Abstract

The Mango (*Mangifera indica* L.), member of family Anacardiaceae, is amongst the most important tropical fruit of the world. The opportunity for breeding improvement in mango is significant and challenging. There is a lot more varietal wealth available but certain inherent constraints are involved like: long juvenility, high clonal heterozygocity, one seed per fruit, recalcitrant seeds, polyembryony, early post-zygotic auto-incompatibility and large area requirement for assessment of hybrids. On the other hand, wide range of diversity and ease of vegetative propagation of hybrid are the advantages for the breeders. Inspite of the difficulties encountered in the mango hybridization with controlled pollination, significant progress has been made in the recent past with regard to release of new hybrid varieties from many countries. Many countries have initiated mango breeding programmes with well-defined objectives in many countries, large number of hybrids are under intense screening and it is expected that many more hybrid cultivars will be released in the near future. Comprehensive knowledge about the phenology, inheritance patterns of mango and advanced techniques for hybridization have been quite helpful to overcome the problems like irregular bearing, susceptibility to diseases and pests, poor eating and keeping quality. The development of the genetic markers has further reduced the uncertainty in breeding of mango and maintaining the hybrid populations in a better way. Clonal selection within many cultivars has high yielding valuable results and hence appears to be worth pursuing particularly in countries where certain cultivars are in cultivation for long time. Use of genetic markers in mango improvement is increasingly appreciated and using this technique parentage of cultivars of unknown mango parentages are being identified. Also varietal development done by using conventional mango breeding technique by Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli has developed the hybrids like Ratna, Sindhu, Konkan Ruchi, Suvarna, Konkan Raja and Konkan Samrat. Depending on mango scion and rootstock breeding has to be taken up. Fruit specific breeding is more difficult inspite of the recent introduction of genetic/ molecular markers. There is need to generate information on genotype x environment interaction effect with screening of different cultivars under different agro-climatic zones; Phenology of flowering so that choosing the parent becomes easier. Biometrical tools like half sib or full sib analysis can generate information on combining ability and inheritance pattern. Raising large progeny population is the key for a selection of good recombinant. Evaluation of local types for biotic and abiotic stress which could be source of resistant genes. Development of suitable genotypes under varying agro climatic conditions, development of salt tolerant, rootstocks, dwarfening rootstock is the need and challenge for future breeding programme.

Key words: Conventional breeding, hybridization, clonal selection, genotype, environment x interaction

Introduction

The Mango (*Mangifera indica* L.), one of the 73 genera of the family Anacardiaceae in order Sapindales, is amongst the most important tropical fruits of the world. It is also called as king of the fruits. It originated in the South East Asian or Indo-Burma Region having 41 recognised species of mango originating as forest trees with fibrous and resinous fruits. Mango has been cultivated for thousands of years in India and its cultivation is as old as Indian civilisation. Its development and culture in the subcontinent is mainly contributed by the Mughal Emperors especially Akbar who planted Lakhibag, amateur gardeners, nurserymen and farmers by means of selection and subsequent cloning. Now, it is an integral part of history and culture of Indian subcontinent. Though soil and climatic conditions are highly suitable for mango production, India is still far behind in yield per hectare than the major mango producing countries of the world. At present, world is producing 23,455 (MT) of mangoes. India is the largest producer (12,000

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MT) of mango followed by China (2,142 MT), Mexico and Thailand with about 50 commercial producers of mango worldwide. Among mango exporters, Mexico is the largest one (209.4 thousand tons) followed by India and Philippine. Main importers from India and Pakistan involve Dubai, U.K. and Saudi Arabia. Exports can be lifted up rapidly by facilitating the growers and provide them incentives for production and subsequent expo.

Mango (*Mangifera indica* L.) exhibits a wide variation in flowering and fruiting habit due to varietal differences and diversity in agroclimatic conditions. Flowering is decisive factor in the productivity of mango. The process associated with mango involves shoot initiation followed by floral differentiation of apical bud and panicle emergence. All these developmental events occur in most of the mango cultivars sometimes during October to December. The induction of floral bud formation has strong links to prevailing environmental conditions and age of terminal resting shoots as under tropical locations, the flower induction occurs in response to age of previous years shoot, while cool inductive conditions are vital to floral induction under sub-tropical conditions.

Inspite of the innumerable problems that are faced in the breeding of mango crop, some of the success stories reported have evoked increasing attention to mango breeding in recent years. Among the major problems that are often met while undertaking a deliberate hybridization programme in mango *inter alia* are (i) poor fruit set in crosses, (ii) long juvenile phase, (iii) unpredictable outcome owing to parents being highly heterozygous, (iv) polyembryony in some popular cultivars and (v) extensive land area required and the maintenance of hybrid population for a long time. While some of these problems are inherent to the crop itself and very little can be done to totally eliminate them with our existing knowledge, it is now possible to realise a better outcome. Now many countries have taken up breeding programmes with definite objectives pertaining to the country and region. Valuable results have also emerged from programmes to select new genotypes from population raised by regulated open pollination, by clonal selection and from chance seedlings.

Barring a few hybrid cultivars resulting from planned hybridization programmes which are slowly gaining attention, most of the other mango cultivars have resulted from selection from chance seedlings resulting from natural cross- pollinations. However, in Florida, large numbers of cultivars have resulted from screening of seedling from known mother plants. In the Indian sub-continent, inspite of the existence of more than 1300 cultivars, there is still need to develop new cultivars since the old cultivars have been selected purely on the basis of fruit quality with very little emphasis on modern horticultural or industrial requirements. These requirements include dwarf frame work, regular bearing, absence of physiological disorders, good shipping quality and resistance to major diseases and pest.

Identification of more gene donors for improvement of specific characters has also been made in many mango research centres. In addition, the use of isozymes as genetic markers (Degani *et al*. 1993) and the use of RAPD molecular markers to determine phylogenetic relationship (Schnell and Knight 1993) are all expected to offer valuable information to assist to conventional breeding programmes. The objectives of this paper is to present recent information on the results obtained in mango improvement at various research centres in different countries.

The improvement of mango rather any crop needs explore new recombinants primarily by means of exploiting the breeding methodologies. Diversity or heterogeneity the main character desired for breeding either natural manmade. It is required to have vast genetic pool to get new combinations of desired nature and developing new hybrid. It has been mentioned earlier that the development mango in the area (Indian subcontinent) is result selections from the amateur gardeners. Breeding has yet play its role in the development of this crop as it has been effectively manipulated in the distant past. Now, the scientists have developed certain hybrids of mango. To go for such strategies, a comprehensive knowledge of physiology of the reproductive parts, their breeding behaviour and cytological information of the crop is needed. This will eventually help to improve mango production status.

Breeding objectives

The objectives set for the mango breeding programmes in different countries vary greatly depending on the environment under which the crop is grown and the local market requirements, in addition to the general aims of obtaining high yielding and with better quality hybrids.

In the Indo-Gangetic plains of Northern India under the sub-tropical conditions where most of the commercial orchards are situated, the low temperatures during the winter months induce a severe growth check resulting in profuse flowering (Chacko 1991, Malshe *et al*. 2015). However, the popular commercial varieties in this region are unable to put forth new vegetative growth immediately after such a heavy fruiting and consequently tend to be 'irregular bearers' (Malshe *et al*. 2016). Hence one of the major objectives in this zone is to develop cultivars which are capable of initiating vegetative growth in the fruited stumps and such shoots to attain the physiological status to flower in the next season (regular bearing) (Salvi *et al*. 2013). The cultivar 'Neelum' which fits in very well with this requirement is hence used as a gene donor in this region. However, in the southern states of India where the winter temperature rarely goes less than 12-15^{0}c there is no cold stress on the trees and hence the trees give moderate yields every year and the branches in the same tree bear on alternate years (Salvi *et al*. 2012). Hence the requirement under such a climate is to have trees with more branches. In the extreme south under purely tropical conditions as in Kerala, the trees tend to be vegetative and flower erratically under conditions of high temperature (>30-25^{0}c day/night), high relative humidity and soil moisture. Trees grown in the wet tropics experiencing such climatic conditions often produce vegetative flushes and flowers and fruits of varying ages in different branches. Hence to stabilise yield in this zone genotypes like 'Prior' (polyembryonic) which are capable of giving good yields even under such climatic conditions may have to be used as one of the parents since considerable differences among mango cultivars have been observed in their flowering response to environmental stress.

The objectives of the mango breeding programme varies from region to region in India since some of the major diseases and physiological disorders are specific to certain climatic zones and cultivars, respectively. Since one of the most damaging diseases of mango, namely, mango malformation is more widespread in the sub-tropical Indo- Gangetic plains this is one of the important aspects that is taken care off while screening hybrids. However, this disease is not widespread in southern and western parts of India. Similarly, 'internal breakdown' is serious only in 'Alphonso', a very popular cultivar in western and southern India and hence hybrids involving 'Alphonso' are to be screened against this malady since Iyer (1991) has shown that this is genetically controlled.

The other major objective in most of the mango breeding programmes is the development of attractive skin colour in the hybrids to make the fruits more attractive and export-worthy. This is also one of the objectives in the Bangladesh mango breeding programmes since the majority of the commercial mango cultivars in the Indian sub-continent have green skin colour. Development of dwarf mango hybrids has also been one of the objectives in many breeding programmes in India since there is lot of interest for high density planting. In addition, no rootstock has shown consistent dwarfing effect on the scions in mango.

The Israel mango breeding programme is aimed at producing cultivars of better quality, appearance and yield with longer harvest periods (Lavi *et al*. 1993). Mango rootstock breeding which at present is taken up in Israel aims at developing rootstocks resistant/tolerant to the stress induced by calcareous soils, saline water and heavy non-aerated soils.

The mango breeding programme in Australia in essence is the improvement of the cultivar 'Kensington' since this is the dominant industry cultivar. Despite industry commitment to this cultivar, 'Kensington' bears irregularly, is susceptible to anthracnose and bacterial black spot and has a comparatively short post-harvest life compared to other mango cultivars. Opportunities exist through breeding to develop a new cultivar which retains the characteristics flavour of 'Kensington' but has improved productivity, greater disease resistance, enhanced skin colour, less sap exudation and better post-harvest performance (Whiley and Bartley 1993). Whiley *et al*. (1989) have shown that 'Kensington' has a strong vegetative bias under tropical conditions which most likely contributes to its irregular bearing pattern in these regions. In contrast, cultivars from Florida are highly productive in both tropical and sub-tropical environments. In addition, these cultivars usually have highly coloured fruit, some have resistance to bacterial black spot and others have improved post-harvest life compared to 'Kensington'. Hence the Australian breeding programme aims at developing hybrids retaining the 'Kensington' flavour along with other desirable features from Florida and Indian cultivars. The dominant leaf and fruit volatile in 'Kensington' responsible for its unique flavour is terpinolene (Bartley 1988) and this finding has helped in the early identification of progenies possessing typical 'Kensington' flavour.

The Brazilian Breeding Project aims at developing hybrids with improved fruit yield and quality, consistency in bearing and small tree size (Pinto and Byrne 1993) particularly suited or the tropical Savannah. The parents involved in the hybridization are from local selected cultivars and from North India and Florida.

Breeding of new mango cultivars and rootstocks are two main research objectives. The first one originated from the well-known disadvantage of the currently available cultivars and the second is the result of the specific conditions of mango growth in Israel.

The combinations of very good quality (taste, aroma and long shelf life) together with beautiful appearance are the breeding objectives of the cultivars' project. Although this combination is not found in any of the current commercial cultivars, these characteristics are seen in mango trees and that they could be combined into single cultivars. The reasons are that mango breeding projects aiming at the above mentioned goals are in their infancy, thus, producing cultivars which are better than the current available ones should be achievable. More than that, the cold winter in Israel, alleviates a major bottleneck of mango breeding namely the long juvenile period.

The suitable climatic conditions for mango growth in Israel, exist mainly along the Jordan valley, from the lake of Galilee through the Dead Sea to the Red Sea. Unfortunately the soil in these areas is quite calcareous and the water are saline. Mango growth in these areas is characterized by iron deficiency and the common horticultural practice to alleviate it is to add iron chelates to the irrigating water (as well as spray of Fe-Sulfate). The problem is that these treatments are both expensive and not always efficient. Thus, a breeding project aiming at the selection of mango rootstocks tolerant to these adverse soil conditions was initiated.

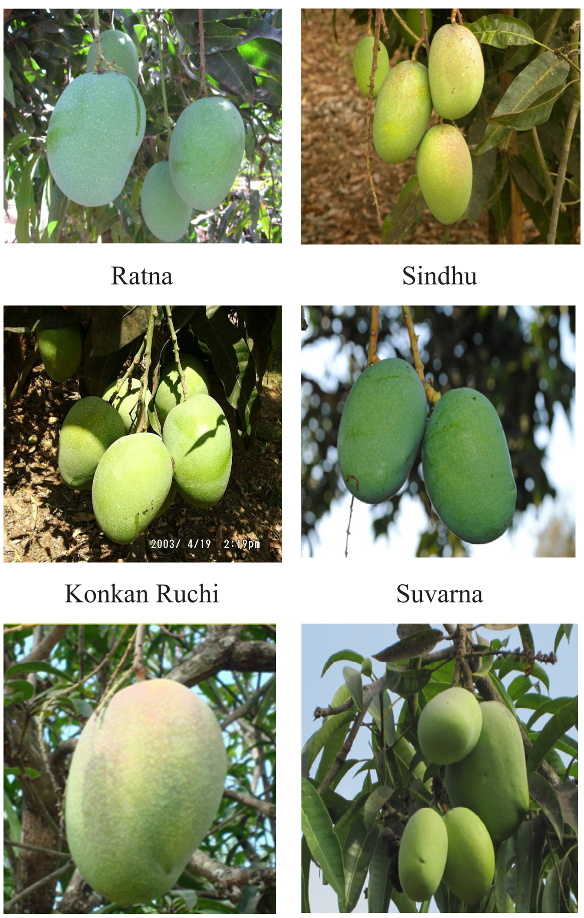

Fig. 1. Mango varieties developed through conventional breeding at Regional Fruit Research Station, Vengurle, Maharashtra.

Molecular biology has the potential for major improvement of fruit trees breeding projects including mango. Gene transfer is no doubt a major tool in this field and some research work is applied to mango too. Detailed reviews regarding mango breeding and the use of DNA markers are in the Mango: botany production and uses (Litz 1997).

Achievements in Breeding

Mango hybridization is in progress in various Research centres in India during the last 4-5 decades in an intense way. Many new hybrid cultivars have been released from some of these centres. However, the adoption of these new cultivars are fairly slow owing to various reasons. These include (i) apprehension among growers about the marketability of large quantities of new cultivars since consumers are aware only about the old existing cultivars, (ii) middlemen hesitant to take risk on marketing new cultivars, (iii) unknowingly marketing new cultivars under the brand of 'unknown varieties' and hence fetching less price and (iv) susceptibility of some of the new hybrid cultivars to certain diseases and problems in keeping quality owing to inadequate pre-release screening. In spite of these problems many new hybrids are getting popular and the acreage is increasing under new hybrids.

At the Indian Institute of Horticultural Research, Bengaluru, India two more hybrids have been released, in addition to the two which were released earlier. The hybrid 'Arka Anmol' ('Alphonso' x Janardan Pasand') is regular bearing, free from spongy tissue and with good keeping quality and sugar acid blend. The second hybrid, 'Arka Neelkiran' (Alphonso' x 'Neelum') is regular bearing with medium sized fruits, free from spongy tissue, good pulp colour, excellent skin colour and the tree is semi vigorous and consequently suitable for close planting. In addition, studies by Gowda et al. (1994) showed that the hybrid cultivars 'Arka Puneet' and 'Arka Anmol' were comparable with 'Alphonso' for fruit weight, TSS, acidity, reducing sugars, total sugars and quality of canned juice. Compared to the cultivars all the hybrids had a marginally higher pulp yield and possessed lower peel, stone and fibre contents (Iyer and Dinesh 1996).

In a programme at the Indian Agricultural Research Institute, New Delhi, designed to transfer the attractive skin colour to the otherwise suitable commercial cultivars, it was found that some hybrids involving ‘Sensation’ as one the parents had excellent skin colour combined with good fruit quality. At the Regional Fruit Research Station, Vengurle, Maharashtra, India, a parthenocarpic mango cultivar ‘Sindhu’ has been evolved as a result of back crossing ‘Ratna’ (Neelum x Alphonso) with ‘Alphonso’. ‘Sindhu’ has medium sized fruits (215 g) with a high pulp to stone ratio (26:1) and very thin (30mm) and small stone (6.7 g). The non-viable cotyledon-free stone which makes up only 3.1 per cent of the total fruit weight encloses a small degenerated ovule (1.1 g) inside the very soft endocarp (Gunjate and Burondkar 1993, Salvi *et al*. 2012).

From the Bihar Agricultural College, Sabour, two hybrids, ‘Sundarlangra’ (Langra x Sundar Pasand) and ‘Alfazli’ (Alphonso x Fazli) have been released in 1980. The former has fruit quality very similar to ‘Langra’ and has regular bearing tendency. ‘Alfazli’ has plant and fruit characters similar to ‘Fazli’ but fruit matures much earlier than ‘Fazli’. In 1989, two more hybrids, namely ‘Sabri’ (‘Gulabkhas’ x ‘Bombai’) and ‘Jawahar’ (‘Gulabkhas’ x ‘Mahmood Bahar’) have been recommended for release. ‘Sabri’ has the shape of ‘Bombai’ with the attractive colour of ‘Gulabkhas’ with pulp having deep orange colour and is a regular bearer. ‘Jawahar’ has attractive shape, high pulp content, fibreless and is precocious in bearing (Hoda and Ramkumar 1993).

Among the other recent release are ‘A. U. Rumani’ (‘Rumani’ x ‘Mulgoa’) from Fruit Research Station, Kodur and ‘Manjeera’ (‘Rumani’ x ‘Neelum’) from Fruit Research Station, Sangareddy in Andhra Pradesh, India and ‘Neelphonso’ (‘Neelum’ x ‘Alphonso’), ‘Neeleshan Gujrat’ (‘Neelum’ x ‘Baneshan’) and ‘Neeleshwari’ (‘Neelum’ x ‘Dashehari’) from Paria Research Station, Gujarat. All these hybrids are reported to be of excellent quality. It is interesting to note that ‘Neelum’ happens to be one of the parents in most of the promising hybrids in India.

The mango breeding programme initiated in Israel has resulted in the isolation of 15 hybrid seedlings based on peel colour, fruit quality and favourable harvesting season. With regard to rootstock breeding, 9 seedlings have been identified based on their performance in calcareous soils. These are being compared with the well-known 13/1 (Lavi *et al.* 1993). A new cultivar ‘Naomi’ has been released which has smooth skin with red pigmentation weighing about 450g and a mid-season bearer under Israeli conditions. However, isoenzyme analysis of leaves could only indicate the origin of ;Naomi’ as originated from cv. ‘Palmer’ either by self-pollination or by cross-pollination with ‘Maya’, ‘Haden’, ‘Nimrod’ or ‘Irwin’ (Tomer *et al.* 1993).

The hybridization work at Maroochy Horticultural Research Station, Nambour, Australia has yielded very promising hybrids among the progenies of ‘Sensation’ x ‘Kensington’. The hybrids have very good skin colour combined with excellent fruit quality.

Thus, it would be apparent that a good number of hybrids are in the pipeline in different countries to be released for cultivation soon.

The investigations were carried out at the Regional Fruit Research Station, Vengurle. The breeding programme started from 1975 till 2015. The ‘Ratna’ (Hybrid No. 13) was a cross between Neelum tree no. 287 x Alphonso tree no. 900. This hybrid was released on 9th May 1981. The second hybrid ‘Sindhu’ (Hybrid No. 117) is a cross between ‘Ratna’ (Hybrid-13) x Alphonso 900 having parthenocarpic nature of fruit was released in September, 1991 during mango workshop held at Hyderabad. The third hybrid Konkan Ruchi (Hybrid-268) is a cross between Neelum tree no. 287 x Alphonso tree no. 900; this hybrid was released during the year 1999, particularly for pickle purpose as recommended by Joint Agresco. The fourth hybrid i.e. ‘Suvarna’(Hybrid-311) was released during June, 2009 as recommended by Joint Agresco (Salvi *et al*. 2012). The hybrid Konkan Raja (Hybrid 7/1) is a cross between Banglora x Himayuddin. This hybrid was released during the year 2010. The unripe fruits of Konkan Raja are used for salad purpose. The new hybrid Konkan Samrat, it is a cross between Alphonso x Tommy Atkins, released during the year 2014 (Salvi *et al*. 2014) (Fig. 1).

Clonal selection

Somatic mutations accumulated over the years get preserved in vegetatively propagated plants since there is no gametic sieve and hence offers scope for selecting desirable clones within a cultivar. Mango has proved to be no exception to this generalisation. Ever since Naik (1948) reported the existence of intra-clonal variation in mango with regard to many fruit characters, there has been many reports on the subject. Oppenheimer

(1956) after a survey of many orchards in India, reported wide variability in the performance of the trees of the same variety in the same orchard not ascribable to environment. Singh and Chadha (1981) assembled some distinct clones of the major commercial cultivar 'Dashehari' and their study for a period of thirteen years showed that Line No. '51' was distinctly superior to all other clones in terms of regularity in bearing, yield and free from 'Malformation'. Singh *et al.* (1985) isolated two clones in the cultivar 'Langra' based on higher yield and better fruit quality.

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

It is, however, important in such studies to put the new clones under replicated trials to compare against standard commercial varieties to confirm their distinctiveness and superiority. It would also be worthwhile to use the DNA finger printing and other sophisticated techniques to establish that the new clones are genetically distinct from the original cultivar (Iyer and Dinesh 1996).

Introduction of cultivars

The role of introduction in crop improvement is well known and in case of mango it is well documented particularly with regard to the evolution of cultivars in Florida. The large number of diverse genotypes introduced together with subsequent breeding and adaptation has made Florida a secondary centre of diversity for mango germplasm and enabled to make a unique contribution to the fruit industry (Knight and Schnell 1994). However, recent experience has shown that extreme caution will have to be taken while introducing cultivars from one region to another. For example, it was observed by the senior author in Bangladesh among the recent introductions many accessions were heavily infested with Red Rust caused by an alga, *Cephaleuros virescens* Kunze. after growing for 3-4 years although this was not a serious problem either in the country from which it was introduced or in Bangladesh itself. This indicates that certain pathogen could manifest in an alarming way in a totally new environment. Similarly, it was observed by the authors that some plants of the cultivar 'Amrapali' when introduced to Bengaluru (South India) from New Delhi (North India), (a distance of nearly 2500 km) the vegetative malformation appeared after a period of three years although South India is known to be fairly free of this malady. Such examples point to the need for longer quarantine requirements for certain diseases and the need for generation more information on major disease.

The incompatibility system also appears to operate only under the certain environment. The cultivars "Irwin" and "Nam Dok Mai", through are not reported to be self-incompatible. do not set fruits in the wet tropics of Northen Australia in the absences of flowering of any other mango cultivar. This indicated that these two cultivars are self-incompatible atleast under the particular environment under question (Elias Chacko, Pers. Comm.). It is, therefore, necessary that well designed studies on pollination are required before recommending any new introduction for large scale commercial planting.

The need for undertaking more studies into the genotype x enviornment interaction in mango has come to the forefront in the past particularly in view of some interesting reports. Recent research has demonstrated the existence of strong genotypic x environmental responses within and between the embryonic groups of mango cultivars which can largely account for their relative performance (Whiley 1993). There is a general agreement that day/night temperature below 20/15^{0}C trigger flower induction. In sub-tropical areas, however, cold night temperature of <10^{0}C during anthesis were found to the cause of poor fruitset while in the tropics low mango yields are often attributed to the failure of flower induction. Issarakraisila and Considine (1994) have shown that in the "Kensington" night temperature below 10^{0}C resulted in pollen grains with low viability (<50%). A temperature between 15 and 33^{0}C during the phase from meiosis to the prevacuolate microspore was optimum for pollen development (70-80% viability). Temperature sensitive phase was estimated to begin 155 degree days (base temperature 10^{0}C) before anthesis and to end 78 degree days after anthesis. Calculations of such values should be useful for potential fruitset, date of maturity and yield. This may also aid in the prediction of performances of major cultivars in certain climatic zones based on studies in controlled environment before mass introductions are made.

Experience gained with introduction of cultivars within India from one agroclimatic region to another has indicated that for certain characters while some genotypes are stable others are highly influenced by enviorment .The cultivars "Latra", "Creeping", "Kerala Dwarf", "Ellachi", and "Meghlonton" have dwarf growth habits under a wide range of environmental conditions while "Amrapali"("Dashehari" x "Neelum") which is dwarf under North Indian conditions does not remain so in South Indian environment. Similarly, "Ladavio", a variety of Goa (Western India) and Ratna (Alphonso x Neelum) developed at Vengurla (Western India) remain very dwarf under North Indian conditions with plant height not more than 1.2m even after 6-8 years. Each cultivar appears to have its own optimum environmental requirement in order to flower and fruit well. North Indian (sub-tropical) cultivars like "Dashehari", "Langra" and "Mallika" ("Neelum x Dashehari") though flowered initially for 3 years went out of flowering subsequently. "Alphonso", the most important cultivar of western India fails to perform well under North Indian conditions (Yadav and Rajan 1993).

Time of flowering in mango is also a function of genotype - environment interaction. Some of the off-season bearing cultivars like "Baramasia", "Royal Special", "Amino-Do-Phasla", "Teenphasla" flower more than once a year under a wide range of environment. However, under the equatorial climate prevailing in Kanyakumari most of the cultivars flower more than once a year. Such examples indicate the need for obtaining a series of background information while introducing cultivars (Iyer and Dinesh 1996).

Methodology for hybridisation

Considering the tedium encountered during emasculation, hand pollination and the post-pollination care that are required for successful hybridisation efforts are being made to simplify the procedures. Singh et al. (1980) have observed that there is no need of bagging the crosses panicles after pollination since the pollen grains germinate fast and effect fertilization and that there was no chance of any other contaminating once the pollination is effected. The senior author during his work in 1994 in Australia has observed that the covering of the stigma with gelatinous capsule soon after pollination is simple and effective to avoid pollen contamination.

In view of the findings that there are considerable differences among cultivars with regard to time of receptivity of stigma, it has become essential to obtain this information for all the cultivars which are being utilised as a female parent in the hybridisation programme. Robbertse *et al.* (1994) have shown that there are clear indications to show that differences in receptivity existing among the cultivars. In "Tommy Atkins", "Sensation" and "Kent", pollination success rate was higher in morning that in the afternoon while in "Isis" and "Keitt", the reverse was true. Time of pollination and female parent both played an important role in pollination success, although the interaction between female parent and the time of pollination had the largest effect.

Whiley *et al.* (1993) instead of resorting to hand pollination interplanted the monoembryonic Florida cultivars ("Sensation", "Tommy Atkins", "Irwin and Palmer") within rows and close proximity to the "Kensington var. Grosszmann". The seeds obtained from the monoembryonic cultivars were assumed to be hybrids with "Kensington". Thus they were able to screen large numbers of seedlings are of the desired parentage.

The handling of hybrid seedlings consists essentially of primary and secondary selection. Primary selection from hybrid seedlings progeny is based on (i) precocity, (ii) fruit size and shape, (iii) fruit skin colour, (iv) fruit quality-physical and chemical and (v) disease and pest susceptibility. The selected seedlings are then used as scions and at least 10 grafted plants of each of these are tested against the standard check particularly with regard to regularity in cropping, yield, fruit quality and susceptibility to diseases and pests.

In order to hasten the progress in mango breeding, the approach taken by the Australian National Mango Breeding Programme appears to be very practical. The programme which is initiated jointly by the CSIRO, Queensland Department of Primary Industry, Western Australia Department of Agriculture and Northern Territory Department of Primary Industries and Fisheries apportions the crosses to different centres to avoid duplication during each year. The hybrids thus obtained at various centres after the primary selection at the place of origin are sent for testing to all other centres. For each cross-combination a total of atleast 50 hybrids of the same targeted which should decide about the utility of producing more hybrids of the same parental combination. The ownership of the ultimately released cultivars rests with all the co-operating agencies (Iyer 1994).

Reproductive physiology

Floral biology: Mango inflorescence is terminal with frequent emergence of the multiple axillary panicles. Both perfect (2-70%) and hermaphrodite flowers occur on the same panicle. Total number of panicles 1000-6000 depending upon the variety. Anthesis starts early in the morning and completes at noon. Stigma receptivity remains for 72 h but most receptive period is for the first 6 h. Minimum pollen germination time is 1.5 h (Spencer and Kennard 1955). Initial fruit set depend upon the ratio of the perfect to male flowers (Iyer *et al.* 1989). Proportion of perfect flowers required for optimum fruit set must not be less than 1%.

Pollination: Mango is self-fertile but cross pollination increases fruit set. Some self-unfruitful cultivars may get benefit from cross-pollination. There is almost no air-borne pollen since it is heavy and adherent. The eye irritation (dermatitis) may result from volatile oils from flowers, mangiferol (sesquiterpene alcohol) and mangiferone (ketone). Pollination of 'Haden' mango in Florida and found no significant difference between percentages of set in selfed and cross-pollinated flowers. Naturally more than 50% flowers don't receive any pollen.

Self-pollination may also occur in some cultivars. Though the ratio of hermaphrodite to male flowers is cultivar related, cool temperatures may also influence sex expression to favour majority of male flowers. There are several hundred flowers in a panicle and less than 1% only develops fruits because of pollination failure and premature fruit drop. Crossed flowers set fruit; whereas, selfed ones did not, indicating self-sterility. The actual degree of self-fertility and sterility in individual cultivars has not been determined, but there is some variation. Though self-sterility is not a major problem in fruit set, but within cultivar, there is a definite need for a pollinating agent. Some of the embryos are capable of development without fertilization, however, Naik obtained no parthenocarpic fruit set of more than 100,000 flowers studied. The effect of cool weather adversely affects pollen tube growth, but this was not considered to be of major importance. Getting flowers to set fruit was more of a problem than getting trees to bloom. The studies indicate that the need for cross-pollination between mango cultivars is not critical, at least for most cultivars, but pollinating insects are needed to pollinate within cultivar to get satisfactory crop.

Perspective of breeding in mango: The opportunity for breeding improvement in mango is significant and challenging. There is a lot more varietal wealth available but certain inherent constraints are involved like: long juvenility, high clonal heterozygocity one seed per fruit, recalcitrant seeds, polyembryony, early post-zygotic auto-incompatibility and large area requirement for assessment of hybrids. On the other hand, wide range of diversity and ease of vegetative hybrid propagation are the advantages for the breeders. There are very few man made commercially important hybrids. Mango development is mostly based on the selection of clones/ chance seedlings. These selections were made for fruit quality only. Seedling screening from known mother plants is another way of selection for better lines. Modern age requirements of a good cultivar involve: dwarfness, precocity, profuse and regular bearing, attractive, good sized and quality fruit, absence of physiological disorders, disease and pest resistance and improved shelf life etc. As far as the improvement of the rootstock is concerned, the main features desired are polyembryony, dwarfness, tolerance to adverse soil (high pH & soil type etc.) and climatic conditions and scion compatibility.

Now, more comprehensive knowledge about the phenology, inheritance patterns of mango and advanced techniques for hybridization is available. Many environmental and physiological factors related to the undesirable character of mango varieties (irregular bearing, susceptibility to diseases and pests, poor eating and keeping quality, etc.) are closely controlled by genes. To overcome these, plant breeding can play an important role and work should be done in three directions as introduction, selection and hybridization. Hybrid populations can be managed in a better way and the development of the genetic markers has further reduced the uncertainty in breeding mango.

Cultivars Breeding: Both controlled crosses and open pollination were used to produce the seedlings. About 100 cultivars were used as parents, of which 30 participated in the controlled crosses. These crosses were performed by caging one (selfing) or two trees under a net in the presence of a beehive. Selfing progeny were obtained from five cultivars. Over 15,000 seedlings were planted during the last 10 years, of which 8500 seedlings still exist. Every year, 1500-2000 new ones are planted and during the last three year about 1000 seedlings are discarded annually. Evaluation is done in two steps: first in the orchard, where fruits are assessed for weight, colours, taste and storage capability. Selected trees, (about 0.25%) are being grafted in three to four locations around the country to test their yield, shelf life, and adaptation to the various climatic and soil condition.

Rootstocks Breeding: In the first stage of this project, all the available poly-embryonic cultivars were tested for tolerance to iron deficiency and salinity. Since this search did not reveal anything better than the current available rootstock ('13/1'), the second stage is based on mono-embryonic seedlings. Several thousands of these seedlings were sawed in several selection plots which are characterized by either calcareous soil or saline water. The survivor's good-looking seedlings (about 0.1 %) were grafted on small rootstocks in the greenhouse to serve as mother orchard. Bud-wood from these selected seedlings treated with rooting hormone (1 % Potassium salt of IBA + 1 % Benlate) was rooted under mist for several weeks and then hardened. These plants serve as rootstocks to be grafted with commercial cultivars and tested in the orchard.

Mutations: Bud mutants occur frequently and are a valuable source of variation. Albino mutant occurrence is quite frequent in mango seedlings and in certain shoots in mature trees. There are only two cases of somatic mutants yielding new cvs. 'The Davis' sport of 'Haden' (Young and Ledin 1954) and 'Rosica' bud mutant of Peruvian cultivar 'Rosado de Ica'. The mutants are high yielding, regular bearing and seedy.

Dormant buds of Langra to gamma radiations and grafted on one-year-old seedlings. Bud graft exposed to 3.0 kR of radiations bore heavier and larger fruits than control. Singh and Chadha (1981) located four superior clones from orchards of Dashehari while Singh *et al.* (1985) isolated high yielding clones from 'Langra' orchards. Sharma and Majumdar (1988b) irradiated bud sticks of Dashehari with chemical mutagens (EMS and NMU) and top worked them on to 10 years old seedlings. The mutants showed dwarfness, changes in shape, leaf serration and TSS. Mutations can be successfully propagated asexually. The techniques used need to be perfected in mango to make mutation breeding more purposeful.

Genetic marker

Mango cultivars are currently identified on the basis of distinct morphological characters. However, morphological traits cannot serve as unambiguous markers since they may vary with environmental conditions (Tanksely *et al*. 1989) In addition this type of cultivar identification usually requires growing plants to maturity and it often lacks decisiveness and objectivity. Recently, reliable genetic markers have been developed and introduced for mango cultivar identification. These include isozymes, RAPDs (random amplified polymorphic DNAs) and VNTRs (variable number tandem repeats).

In mango, the feasibility of using isozymes to detect possible genetic variation among individuals of so-called clones was shown by Gan *et al*. (1981). Recently, Degani *et al.* (1990, 1992) developed several polymorphic enzyme for mango and employed them for systematic characterization and parentage analysis of mango cultivars.

They showed that morphologically very similar cultivars such as "Pico" and "Carabao" can be easily distinguished by isozyme analysis. Their isozyme banding patterns supported the origin of some cultivars such as "Haden" from "Mulgoba", "Zill" from "Haden" (Campbell 1992) and "Tahar" from Irwin (Slor and Gazit 1982). In addition, the accepted hybrid origin of some other mango cultivars was refuted by the isozyme analysis of Degani *et al*. (1990, 1992): Thus, "Edward" is commonly accepted to be a hybrid of "Haden" and "Carabao" (Campbell 1992). However, isozyme evidence showed that "Carabao" cannot be the male parent of "Edward". In addition it was shown that, contrary to common assumption, "Mulgoba" cannot be a parent of "Keitt" and "Givataim" cannot be an offspring of the Polyembryonic cultivar "13-1".

Schnell *et al.* (1995) studied the application of RAPDs to the identification of mango cultivars. Among 11 primers selected, no single primer gave a unique banding pattern for any of the 25 accessions examined; however several different combinations of two primer banding patterns produces unique finger prints for each accession.

Adato *et al*. (1995) evaluated the usefulness of 10 different mini- and microsatellite probes for the identification of mango cultivars. Jeffrey's mini satellite probe 33.6 was the most informative, yielding well-resolved band patterns, representing highly polymorphic loci. Genetic markers can serve to detect linkages with agriculturally important traits. If the gene(s) to be transferred is marked by a tightly linked genetic marker, the segregating progeny can be screened at the seedling stage before the trait is expressed (Iyer and Degani, In press).

DNA fingerprinting was done by hybridization of digested genomic DNA to mini micro satellites according to Adato *et al*.1995. AFLP patterns were obtained using the Applied Bio systems (ABI) AFLP kit. The reaction products were run on the 377 Applied Bio systems (ABI), DNA Sequencer.

Even though DNA markers became a very popular tool, only a limited work has been done with this tool in mango. The application of mini satellite probes was documented by Adato *et al*. (1995). This work demonstrated the ability to individualize mango cultivars and rootstocks. Several mini and micro- satellites were used as probes in order to identify 40 various mango trees. The DNA fingerprints (DFP) detected by the mini satellite probe 33.6 were the most polymorphic. On the average, five to seven specific bands and 52-55% Band Sharing were observed pending on the restriction enzyme.

This system was also used to assess genetic relatedness between twenty various mango cultivars. The DFP patterns revealed after hybridization to the probes, were used to calculate the distance (d-values). The d values combined with available knowledge regarding the origin of these cultivars (Campbell 1992), allowed the drawing of a genetic relationship tree between these cultivars.

Genetic analysis carried out on the progeny of a cross between "Tommy Atkins" and "Keitt" revealed no pair of bands with complete linkage (partial linkage between bands was detected). Similarly, no band was found to be allelic to another band and no polymorphic band in the parents was homozygote.

A preliminary work on the application of AFLP to mango was carried out (Lavi *et al*. 1997, unpublished data). We have used both the fluorescent kit of Applied Bio systems (ABI) and the radioactive kit produced by Gibco. The DNA was restricted by both restriction enzymes *Eco* RI and *Mse* and adaptors were ligated to the DNA. DNA amplification was carried out by the PCR reaction in two steps using the right primers. The primers used in the second step are longer than those of the first step thus allowing the selections of a subset bands. In the ABI kit one primer was fluorescently labelled and in the Gibco kit it was radio-actively labelled. The band pattern obtained in this allowed the individualization of several mango cultivars (Lavi *et al*. 1996).

Screening of germplasm collection

The cultivars originated in the Indian sub-continent have been described by various authors from time to time and this information has been used either to recommend them directly for cultivation or recommended them as sources of desirable genens in breeding programmes (Singh and Singh 1956; Singh 1978; Naik and Ganguly 1950; Saha 1972; Iyer and Subramanyan 1986). A check list of mango cultivars is now available (Pandey 1984). Most of these cultivars are conserved in ex situ collections maintained at various research centres and being screened from time to time.

Screening for reduced tree size has become very essential since none of the rootstocks tried have been able to consistently dwarf the scion in mango coupled with high yields per unit area. "Kerala Dwarf" and "Creeping" have been found to be dwarf and are now being used in breeding programme as one of the donor parents. With regard to resistance to powdery mildew field resistance was observed in "Azam-us-Samar", "Chambeliwali", "Chausa", "Karnuli Mulgoa", "Totapuri" and Vanraj at Fruit Research Station, Sangareddy, India (Hashmath Unnissa 1979). Under Punjab conditions "Totapuri Red Small" was found to be tolerant to powdery mildew (Singh and Kapur 1979). In screening test in Uttar Pradesh, Gupta (1976) noted that "Neelum". "Zardulu", "Bombay", "Totapuri", "Khurd" and "Janardan Pasand" were resistant under the North Indain sub-tropical conditions whereas Datar (1983) observed only "Totapuri" to be resistant such. Such information on varietal evaluation are proving to be valuable.

Role of wild species in mango breeding:

Fairchild (194 observed that crosses between five stamened and Indian mango could produce hybrids with better pollination quality. Bompard (1993) stated that *M. laurina* could used to incorporate resistance to anthracnose. There are certain wild cultivars of mango i.e., *M. orophila* and *M. dongnaiensis* both described from Malaysia and Vietnam respectively, that are restricted to mountain forests about sea level. These could help to start mango cultivation even in the Mediterranean areas. Other wild species have certain specific characters like *M. mangifica* is fibreless, *M rufocostat* and *M. swintonioides* have off-season bearin habit, *M. pajang* and *M. foetida* have good quality fruits an *M. casturi* from *S. kalimanta* is prolific bearer with small beak sweet fruit. These species may be helpful to enhance the existing gene pool and to develop new hybrids in mango (Bompard 1993; Kostermans & Bompard 1993). *M. altissima* unaffected by hoppers, tip and seed borers (Angeles 1991).

Breeding problems

Breeding problems can be minimized by minimizing the high fruit drop, shortening juvenility and polyembryony dilemma for the breeder and asset in rootstock propagation. Isozymes are used to identify the zygotic seedling from the nucellar one's as the nucellar seedling should have same isozyme alleles as that of the maternal parent. (Schnell and Knight 1992; Truscott 1992; Degani *et al.* 1992; 1993). The mango fruiting season for South Florida is very short (from mid-June to mid-August). Extending the fruit season could provide opportunities for increased production and more favourable marketing conditions for growers. One-way to accomplish this is to modify flowering time. Schnell *et al*. (1999) studied flowering in mango to prolong the harvest season. Three variables (days to bloom, days in bloom and days in bloom and fruit) were measured on eight varieties for six years.

Replicate trees of the same variety reacted very similarly within a given year indicating that large replicated plantings are not necessary to study these variables. This is important as many of the mango varieties in the germplasm collection in South Florida are represented by one or two mature trees only. Repeatability of the flower phenology characters was high, indicating that much of the variation is heritable and useful for further breeding. In future, better efficiency in mango breeding will have to rely on planned hybridization assisted by the new tools offered by biotechnologies. The recent emergence of molecular markers and the application of somatic embryogenesis to genetic transformation will enable the integration of specific genes from cultivated varieties or wild species into popular current cultivars.

Conclusion

Fruit trees in general and mango in particular, are lagging behind several field crop plants in which significant amount of genetic information have been gathered. Moreover, only a few mango breeding projects exist and they are quite limited in size. On the other hand, even the few studies mentioned above have contributed significantly to our knowledge probably due to their limited number.

Mango cultivars include a large number of local cultivars that are quite popular in several Asian countries including India, and which are characterized usually by the green peel and relatively sweet taste. On the other hand, the Floridian cultivars (such as 'Tommy Atkins' 'Keitt' and others) are the popular cultivars found in western markets including the US and Europe. Our breeding efforts were aimed towards the selection of new cultivars with high quality, beautiful appearance, good yield and long shelf life. Our preliminary data, suggest that such cultivars could be selected.

There is an interest in mango rootstocks in most mango growing areas as a tool to control tree size. Israel is different in to aspects, having a relative small mango tree (probably due to the climatic conditions) and facing major problems of soil and water quality. As a result, we are looking for a mango rootstock tolerant to calcareous soils and saline water. Our data suggest that such a rootstock might be found among the mono-embryonic types. Such a rootstock will then have to be propagated vegetatively probably by rooting of cuttings.

Quantitative genetic analysis, although not very common in fruit trees, might shed some light on the genetics of the crop. In the case of mango, the fact that the non-additive genetic variance is significant in most traits, similarly to what was found in avocado (Lavi *et al.* 1993), suggest that mango cultivars of low performance could also serve as parents for generating seedlings in the breeding programme if needed to widen the genetic background of these parents.

DNA markers are a very common tool for several purposes including identification and improvement of breeding projects. Unfortunately, only a limited work in this avenue has been carried out in mango. Based on this work, it seems to us that investment of research effort in the application of this tool to mango and especially using the AFLP will be quite fruitful for both cultivars identification, assessment of genetic variability (for example; collection of mango Gene-Banks), study of genetic relationship and improvement of breeding programs mainly by identification of linkage between DNA markers and horticulturally important traits using marker assisted selection (MAS).

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