



A Recent Approach in Breeding Technology in Mango

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Historical overview

Historically, the majority of cultivars that are currently cultivated globally have their origins in the selection of open-pollinated seedlings that were either the result of deliberate breeding programmes or random seedlings. Techniques like regulated open and closed pollination, as well as monoembryonic and polyembryonic seedling selection, are part of the systematic breeding methods in use.

The reproductive physiology of the mango affects each of these methods, which poses serious obstacles and constraints for some traditional breeding methods. Several characteristics, including the large number of flowers per panicle, polyembryony, juvenility, polyploidy, and intrinsic heterozygosity, as well as the perennial flowering habit (Mathew and Dhandar 1997; Villegas 2002) can all have an impact on how well mango breeding programmes are run and managed. However, any genetic gain can be captured in a new cultivar due to the ease with which clone trees can be vegetatively reproduced. Below is a discussion of each technique's benefits and drawbacks.

Mutation breeding

Mango cultivars that have undergone spontaneous natural mutations include Davis-Haden, which originated as a sport of Haden, and Alphonso and Puthi, which originated as chimaeras. (Singh 1960). γ radiation and chemical induction of mutations in mango for breeding purposes has been reported to induce dwarfness, firmer flesh, higher T.S.S and better sugar acid blends in a few of the treated plants (Sharma 1987).

Monoembryonic seedling selection

Numerous significant cultivars found worldwide originated from natural seedlings through selections that took advantage of the spontaneous out-crossing that occurs in open-pollinated seedlings. Many of the cultivars that came from Florida and India in the previous century were the result of systematic monoembryonic selection initiatives. The seedling selection programme that produced the Australian cultivar "R2E2" was modelled after "Kent." (Bally 1998). In monoembryonic selection programs large numbers of seedlings from one or more desired monoembryonic maternal parents are grown and evaluated and screened for improved genotypes. Polyembryonic maternal parents are not suitable as their seedlings are genetically similar to the maternal parent.

The method of monoembryonic selection has various drawbacks. Because many seedlings self-pollinate, there is typically little diversity among the seedlings. Before suitable

improved selections can be identified, a large number of seedlings must be screened in the field. This is because self-pollinated seedlings are difficult to identify until they start to crop. The breeder has no control over the hybrid seedlings' paternal (pollen) parent, in addition to the low percentage of selectable seedlings. The ease of producing large numbers of hybrids for testing outweighs these drawbacks, but modern mango breeders are less fond of this technique due to its high effort to reward ratio and lack of genetic control.

Polyembryonic seedling selection

The goal of polyembryonic seedling selection is to take advantage of the diversity found in polyembryonic populations that result from out-crossed zygotic seedlings or natural mutation. The majority of polyembryonic seedlings are known to be true to their maternal parent type and nucellar in origin. However, in a number of polyembryonic cultivars, zygotic off-type seedlings have been reported to range from 2% to 47%. (Beal 1981; Truscott *et al.* 1993). The ability to identify the zygotic embryos in the seed is the primary issue with this method, which is also the reason plant breeders have not traditionally preferred it. Rather than selecting and raising zygotic seedlings for selection, polyembryonic selection programmes have typically relied on identifying superior types within sizable commercial populations. This method has been reported to produce some interesting cultivars in Florida such as 'Alica', 'Herman' and 'Florigon', all which originated from seedlings of 'Saigon' (Knight and Schnell 1993), as well as some variations in the cultivar 'Kensington Pride' (Johnson 1995).

Controlled open pollination

With steps taken to promote out-crossing with pollen from a desired source, this technique takes advantage of the natural out-crossing of monoembryonic cultivars.

To maximise out-crossing to the desired maternal (pollen) parent, various strategies can be applied. Frequently, a sizable insect-proof cage containing pollinating insects encloses the two parent trees. In order to create the high-quality hybrids "Mallika" and "Amrapali," Sharma and Singh (1970) employed this technique with the self-incompatible cultivar "Dashehari" and other cultivars. Israel has additionally used this method. (Degain *et al.* 1993). The requirement for the caged parent trees to flower at the same time is one of the technique's main limitations. An alternate technique is to graft a female parent's branch onto a male parent tree, increasing the likelihood of out-crossing to the desired male parent, or to plant lone female parent trees close together and encircled by male pollinating parents. This technique has been successfully been used in Australia by Whiley *et al.* (1993), who produced the cultivar 'Calypso' from a cross between 'Sensation' ♀ and 'Kensington Pride' ♂.

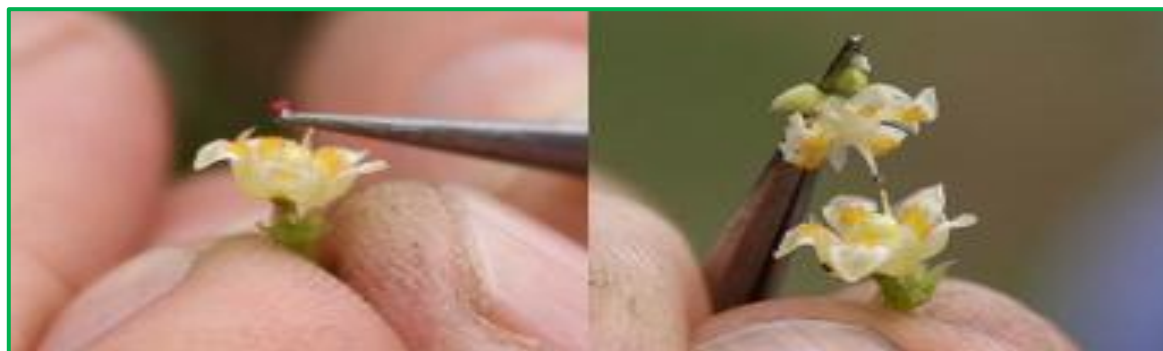
Over the past 25 years, controlled open pollination has become one of the more widely used breeding techniques; however, it has a number of drawbacks. Similar to all open-pollinated methods, numerous self-pollinated offspring are frequently generated. (Lavi *et al.* 1998) which take up valuable space and time because they must be field grown and assessed for several years before being identified and eliminated. Without DNA fingerprinting, identifying the male or pollen parent is challenging, which complicates statistical analyses of segregation patterns in the breeding population and adds little to our knowledge of mango genetics.

Controlled closed pollination (hand pollination)

In controlled closed pollination, two desired parents are crossed using manual pollination techniques, which transfer the desired pollen to the female parent flowers while keeping the unwanted pollen out.

Due to the significant natural fruit thinning of the initial fruit set per panicle, early attempts at hand pollination involved crossing a large number of flowers on a few panicles over several days, producing a very small number of hybrids. (Singh 1960). This technique

was substantially improved by Mukherjee *et al.* (1961) who crossed larger number of panicles and fewer flowers per panicle. A further modification to the technique of Mukherjee *et al.* (1961) was suggested by Singh *et al.* (1980) who, after pollination, did not re-bag the crossed panicles? They asserted that there was very little chance of unintentional pollen contamination because the re-bagging had harmed the crossed flowers' stigmas and styles, decreasing the proportion of fruit set. Another modification to the Mukherjee *et al.* (1961) technique has been to replace re-bagging step after crossing with application of gelatinous capsules to enclose the flowers (Bally *et al.* 2000). This technique is currently used in the Australian mango breeding program.



(a) Emasculation of hermaphrodite flowers prior to pollination by removing the anthers from the filaments with a fine pair of tweezers, and (b) Transfer of pollen from by gently touching the dehisced anther on the male flower on to the stigma of the female flower.



(A) and (B) Encapsulation of female flowers after pollination to exclude foreign pollen and retain humidity around the flower for pollen germination

The benefits of hand pollination include the ability to pollinate any desired female parent tree and a variety of pollen sources that aren't always at the same location, all without the need to set up intentionally grown crossing blocks. The identification of both parents of every hybrid produced by closed pollination methods enables statistical analysis of segregation patterns. Resources are not lost cultivating and assessing self-pollinated progeny, in contrast to open pollination methods. The biggest disadvantage of this technique is the relatively low number of hybrids generated per number of panicles crossed. However, success rates are dependent on the particular parental combination used in the cross. Kulkarni *et al.* (2002) Depending on the parents involved, reported success rates ranged from 0 to 122% (percentage of mature hybrid seed obtained per crossed panicle). Cultivars that bore bunches, like 'Creeping' and 'Willard,' exhibited the highest success rates (>100%). Although

the exact causes of the hand crossing technique's poor success rates are unknown, potential contributing factors include pollen viability issues and parent incompatibilities. (Dag *et al.* 2000; Sukhvibul *et al.* 2000), stigmatic receptivity and protogyny, (maturation of stigmas before anthers), (Spencer and Kennard 1956) and low natural fruit set in mango.

Biotechnology-Assisted Technology

Molecular biology and somatic cell genetics are applied to the improvement of plants through the process known as biotechnology-assisted breeding. (Litz and Lavi 1997). Many of the above-discussed limitations—such as low fruit retention, polyembryony, heterozygosity, polyploidy, long juvenility, large tree size, and long life cycle—may be overcome or lessened by using biotechnology-assisted breeding techniques.

Mango breeding and improvement can benefit from molecular biology (genetics) in a number of ways. Gene cloning can be used to modify current mango cultivars with genes crucial to horticulture, and marker assisted selection can be utilised in traditional breeding to identify parents and choose progeny. (Litz, 2004). Other biotechnological methodologies include the efficient somatic embryogenesis and plant recovery from elite (nucellar) material, induction of random mutations in embryogenic cultures and challenging for resistance to a specific selective agent, and transformation with a gene that mediates a desired horticultural trait. On the basis of past and current research, it is probable that resistance to abiotic soil stress and certain diseases can be addressed by mutation breeding and the control fruit ripening, seedlessness and certain diseases can be addressed by genetic transformation (Litz, 2004). Progresses have been made on controlling fruit ripening by inhibition of expression of genes encoding ethylene biosynthetic enzymes (Cruz-Hernandez *et al.* 1997), and selection of several embryogenic cultures of mango which indicate some resistant to anthracnose fungal pathogen (Jayasankar *et al.* 1998; Jayasankar *et al.* 1999; Litz 2004).

There are several reviews on the application of the modern biotechnology tools on mango cultivar improvement, to which the readers can refer to (Litz and Lavi 1997; Litz and Gomez-Lim 2002; Lavi *et al.* 2004; Litz 2004).

Conclusion

Mango conventional breeding has many limitations and takes a long time, but there are also many opportunities to create new cultivars with improved traits that will boost fruit quality, productivity, and the mango industries' ability to compete economically. Through the identification of the genetic linkage between the marker allele and the gene of interest, recent and future developments in molecular markers, particularly those appropriate for a high throughput MAS, should significantly increase the efficiency of existing conventional mango breeding methods. Finding and cloning horticultural interesting genes from the mango and its many relatives will improve the rate at which transgenic somatic embryos germinate and produce plants, thereby revolutionising the genetic improvement of mango cultivars. Biotechnological developments and statistical analysis of current breeding populations are also substantially improving our understanding of the genetic traits and their inheritance in mango.

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