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Biotechnological Developments in Mango and Their Role in Crop Improvement

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Abstract: -

Biotechnology offers valuable support to conventional breeding methods and can accelerate mango improvement programs. Research efforts worldwide focus on various biotechnological approaches, including *in vitro* culture, micropropagation, embryo rescue, genetic transformation, marker-assisted selection, and DNA fingerprinting. Significant progress has been made in *in vitro* culture and somatic embryogenesis across different mango genotypes. The optimal explant for inducing embryogenic cultures is the nucellus excised from immature fruitlets. While high-frequency somatic embryogenesis has been achieved in certain genotypes, abnormalities can sometimes occur during embryo germination. Additionally, embryo rescue from young or aborted fruitlets can enhance hybridization success within a limited flowering period. Protocols for protoplast culture and regeneration have also been established. Furthermore, *in vitro* selection techniques have shown promise for screening germplasm for antibiotic tolerance and fungal toxin resistance. Genetic transformation using *Agrobacterium tumefaciens* has been reported, with efforts made to introduce genes associated with fruit ripening into plants. Studies on DNA fingerprinting and genetic diversity of mango cultivars and *Mangifera* species are also being conducted. This review highlights recent advancements in mango biotechnology and explores solutions to challenges encountered in *in vitro* propagation.

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Introduction

The mango (*Mangifera indica* L.) is one of the most prized fruit crops in tropical and subtropical regions, particularly in Asia. Its significance is evident from its reputation as the "King of Fruits" in the tropical world. Despite its popularity, mango production faces several challenges on a global scale. As a highly cross-pollinated species, many superior mango clones, including 'Indian' and 'Floridan' cultivars, are monoembryonic, making sexual propagation unreliable for producing true-to-type plants. This result in an extended juvenile phase before fruiting begins. Production issues arise in both scion and rootstock. Scion-related problems include biennial bearing, large tree size, susceptibility to major pests and diseases, short post-harvest shelf life, and physiological disorders such as malformation and spongy tissue. For rootstock breeding, Iyer and Degani (1997) emphasized the need for tolerance to soil-related stresses, induction of dwarfing traits, and a high degree of polyembryony to facilitate rapid clonal propagation. Most cultivated mango varieties, except for a few hybrids, are naturally occurring seedlings from cross-pollination. While hybrids with desirable traits are gaining popularity, there remains a severe shortage of such material. Additionally, many traditional mango varieties are not well-suited to modern horticultural practices, as they lack traits such as early fruiting, dwarf stature, consistent high yields, and resistance to diseases, pests, and physiological disorders. The perishability of mangoes further limits global trade. As a climacteric fruit, mangoes pose transportation challenges, and anthracnose (*Colletotrichum* sp.) is one of the most serious threats to production and post-harvest quality. There is an urgent need for a mango variety that possesses an ideal combination of desirable traits. Singh (1996) proposed that an ideal mango should be dwarf, a regular bearer, and produce medium-sized fruit (250–300 g) with high tolerance to fungal and bacterial diseases. Additionally, the fruit should have a stable, pleasant flavor and excellent storage quality. However, conventional breeding alone cannot meet these demands. The challenges of breeding woody perennial fruit crops like mango include a prolonged juvenile phase, self-incompatibility, low fruit set, high fruit drop, single-seeded fruits, high cross-pollination rates, polyembryony, polyploidy, and heterozygosity. Moreover, limited knowledge of the inheritance of key quantitative traits further complicates breeding efforts. Integrating biotechnology into breeding programs can accelerate the development of improved cultivars by addressing genetic limitations. Micropropagation in mango has not achieved the same commercial success as in crops like

pineapple, banana, and strawberry due to several obstacles, including latent microbial infections, excessive polyphenol exudation, and early explant necrosis. However, biotechnology offers solutions to some of the major challenges faced by the mango industry. Molecular techniques can aid in taxonomic classification, provide insights into gene regulation and expression, and contribute to the development of improved mango varieties with superior traits.

In vitro propagation

Mango genetic engineering relies on an efficient in vitro regeneration system. Additionally, in vitro propagation enables the rapid multiplication of superior clones within a short period. Polyembryonic mango genotypes, especially those used as rootstocks for their desirable traits, are primarily propagated through seeds, which produce a limited number of clonal seedlings identical to the mother plant. This presents an opportunity to leverage micropropagation to overcome the challenges of clonal rootstock production. However, mango tissues tend to darken rapidly in vitro due to the enzymatic activity of polyphenol oxidase, posing a significant challenge to successful tissue culture.

Somatic embryogenesis

Compared to other horticultural crops, mango has proven to be a challenging species for tissue culture. The nucellus has been the primary explant used in mango tissue culture, as nucellus-derived plants are typically free from viruses and other endophytic pathogens due to the lack of vascular connections with surrounding maternal tissue. This makes the efficient recovery of somatic embryos, especially in monoembryonic mango cultivars, a valuable approach to eliminating systemic diseases and reducing losses caused by environmental stress in tropical conditions. However, initial attempts at somatic embryogenesis in mango have reported relatively low plant recovery rates. Various developmental anomalies, including polycotyledony, fasciation, lack of bipolarity, and secondary embryo formation from hypocotyls, have been observed, preventing embryos from maturing properly. Additionally, issues such as precocious germination and progressive necrosis have further hindered the successful recovery of mango plantlets. To address these challenges, researchers have focused on optimizing conditions for embryogenic culture induction, maintenance, embryo maturation, and somatic embryo germination. Studies have found that adding 3.0 µM ABA and 6.0% (w/v) sucrose can help minimize precocious germination. Dewald et al. (1989b) proposed the use of liquid shake culture for maintaining embryogenic cultures, with modified B5 medium supplemented with 5–6% sucrose and

20% (v/v) coconut water (CW) proving to be optimal for somatic embryo development. Despite the high embryogenic potential of nucellar tissues, success has been limited in cultivars such as Chaunsa and Anwar Rataul, mainly due to excessive phenolic exudation from the nucellus explant into the culture medium.

Organogenesis

Rooting field-grown mango shoots presents significant challenges due to high phenolic exudation and systemic contamination during culture initiation. The first successful attempt at organogenesis was reported by Rao et al. (1981), who induced root formation from callus derived from mango cotyledons cultured on MS medium supplemented with kinetin and NAA. However, shoot development was not observed. Later, Singh et al. (1991) induced callus from various explants, including epicotyl segments, leaf petioles, and shoot tips from aseptically germinated embryos, with the highest callus formation occurring in epicotyl segments. Direct root organogenesis was noted in cultures of epicotyls and shoot tips treated with low concentrations of 2,4-D.

Thomas and Ravindra (1997) explored shoot tip culture in several mango genotypes and highlighted interrelated issues such as phenolic exudation, medium discoloration, and explant browning. These problems were influenced by factors including the culture medium, genotype, explant type, season, and decontamination treatments. While media additives helped mitigate browning, deep-seated contamination persisted, and repeated decontamination treatments often exacerbated phenolic exudation. To overcome the difficulties associated with field-grown shoots, in vitro-grown shoots were found to be more responsive to culture conditions. Ara et al. (1998) developed a two-step protocol for in vitro rooting of microshoots obtained from nucellar somatic embryos. Among the auxins tested, IBA proved most effective for root induction and growth, with optimal results achieved through a 24-hour pulse treatment using 5.0 mg L^{-1} IBA, followed by transfer to an auxin-free medium. However, initiating rooting from microshoots derived from somatic embryos may limit the commercial viability of this approach. Greenhouse-grown shoots have been proposed as an alternative, offering a balance between field-derived and in vitro-grown shoots. Reuveni and Golubowicz (1997) attempted to culture small internodes from greenhouse-grown mango trees but did not achieve shoot formation. Similarly, Hare Krishna (2006) used glasshouse-raised shoot segments from the 'Amrapali' cultivar, resulting in only callusing at the cut ends and axillary bud sprouting, with no further shoot proliferation. Cultures

survived for over six months. When cotyledonary nodes from in vitro-germinated 'Kurukkan' seedlings were used, both shoot initiation and callusing were observed, and cultures remained viable for more than six months. Yang and Ludders (1993) used shoot tips from greenhouse-grown rootstocks and found that shoot proliferation was superior in G medium supplemented with BA, zeatin, 2iP, IAA, and IBA, compared to B5 or Woody Plant Medium (WPM). Conversely, Shahin et al. (2003) reported that stem node explants were more effective than shoot tips for shoot proliferation. The highest shoot proliferation was observed in modified WPM supplemented with 20 g L^{-1} sucrose, 30 mg L^{-1} adenine, 2 mg L^{-1} iso-pentyladenine, 0.5 mg L^{-1} IBA, and casein hydrolysate. The best rooting response was obtained in modified WPM containing 30 mg L^{-1} adenine, 1.0 mg L^{-1} BA, and 4.0 mg L^{-1} IAA. Earlier, Raghuvanshi and Srivastava (1995) examined the morphogenic potential of mature leaf explants. They successfully induced caulogenic callus, which was later subcultured on MS medium for multiple shoot formation. Liquid shake culture was employed to minimize phenolic exudation and prevent explant necrosis. Multiple shoots were separated and transferred to rooting media, but only 20% of the cultured explants successfully developed roots on medium supplemented with 9.8 µM IBA.

Embryo culture

Embryo culture has the potential to enhance mango breeding efforts. Various factors contribute to fruit drop, including competition among developing fruitlets, nutritional deficiencies, moisture stress, hormonal imbalances, climatic factors such as high temperatures, rainfall during flowering, hailstorms, strong winds, varietal characteristics, fertilization failure, and attacks from diseases and pests. Research indicates that the formation of the abscission layer is predetermined. The separation of cells in the abscission zone occurs due to the dissolution of the middle lamella (Chadha, 1959), followed by cell wall breakdown triggered by an increase in hydrolyzing enzymes like cellulose, polygalacturonase, and uronic acid oxidase. As the process advances, the fruit's weight ultimately causes the tearing of xylem tissues. Ethylene has been definitively identified as a key promoter of fruit abscission. Changes in the levels of hydrolytic enzymes and ethylene are likely regulated by mRNA associated with abscission-specific genes. However, no such genes have been identified in mango. A promising genetic approach to controlling abscission involves transgene technology, where transgenic plants are engineered to express antisense versions of genes that encode enzymes responsible for cell wall degradation and ethylene biosynthesis.

These genetically modified plants would produce lower levels of hydrolytic enzymes and ethylene-related enzymes, reducing fruit drop. Another potential approach involves introducing ethylene-resistant mutations, such as Arabidopsis mutants etr1 and ein2, which are insensitive to ethylene. These mutants, when controlled by their own promoters, could significantly reduce mango fruit drop. Mango breeding efficiency has been significantly improved by in vitro regeneration techniques using immature fruitlets (35–45 days old). Chandra et al. (2003a, b) successfully regenerated immature mango embryos into complete plantlets using MS basal medium supplemented with 9 mg L^{-1} BA, 3 mg L^{-1} kinetin, 400 mg L^{-1} glutamine, 500 mg L^{-1} activated charcoal, and 60 g L^{-1} sucrose. This approach resulted in a 72% success rate for complete plantlet formation. Sahijram et al. (2005) recommended collecting mango fruitlets 6–8 weeks post-pollination for embryo culture. Hybrid embryos were aseptically excised from immature ovules and cultured in vitro on semi-solid half-strength modified MS medium containing 1.25 g L^{-1} casein hydrolysate and 4.5% sucrose. After 12–14 weeks, well-developed seedlings were transferred to non-sterile conditions using tap water in parent culture vessels for initial hardening.

Somaclonal variation/in vitro selection

Somaclonal variations can be highly beneficial to breeders, serving as an effective tool for generating genetic diversity. A single gene mutation has the potential to modify an essential horticultural trait, potentially leading to the development of an improved variety with enhanced characteristics through in vitro techniques. However, despite its advantages, somaclonal variation has not made a substantial impact on mango breeding by producing beneficial off-types of existing selections that may be lacking certain desirable traits. To obtain useful off-types from established cultivars, Litz (2001) exposed embryogenic cultures of three mango cultivars- Hindi, Keitt, and Tommy Atkins-to gamma irradiation ranging from 0 to 200 Gy using a cobalt-60 (^60Co) source. The median lethal doses (LD50) for Keitt and Tommy Atkins were found to be approximately 125 Gy and 100 Gy, respectively, whereas the LD50 for Hindi remained undetermined within the tested dosage range. In vitro selection using fungal toxins has also been explored in mango. Jayasankar (1995) subjected embryogenic cultures of Hindi and Carabao to purified culture filtrates of *Colletotrichum gloeosporioides*, and after multiple subcultures under stress conditions, tolerant lines were developed. The resistant embryogenic cultures of Hindi and Carabao were repeatedly selected using either progressively increasing

concentrations of culture filtrate or continuous exposure to a fixed concentration of filtrate or phytotoxin. When co-cultured with the resistant embryogenic mango cultures, the pathogen's mycelial growth was inhibited. Furthermore, conditioned plant growth medium containing macerated resistant embryogenic cultures did not hinder fungal growth, suggesting that extracellular antifungal compounds played a role in the plant's defense mechanism.

Genetic transformation

Genetic transformation offers a method to modify specific horticultural traits in perennial plants without affecting their overall phenotype. This approach is especially valuable for tree species, where breeding new cultivars is challenging due to long generation cycles, high heterozygosity, and nucellar embryony. The ability to introduce targeted genetic modifications depends on successfully regenerating elite tree selections from cell and tissue cultures. As a result, the genetic integrity of the original clone remains intact, except for the introduced trait. Significant progress has been made in genetically transforming mango embryogenic cultures using *Agrobacterium tumefaciens*. In transformation experiments, two engineered *Agrobacterium* strains were used: (i) C 58CI with plasmid pG3850::1103 carrying the selectable marker NPT II and (ii) A208 containing pTiT37-SE::pMON9749, a co-integrate vector encoding NPT-II and GUS genes. Studies found that kanamycin sulfate at 12.5 µg/ml was toxic to proembryonic masses in suspension cultures, whereas a concentration of 200 µg/ml was lethal in semi-solid medium. Putative transformants were confirmed through histochemical staining with X-GLUX and Southern hybridization. However, obtaining fully developed transgenic plantlets was unsuccessful due to embryo hyperhydricity. Cruz-Hernández et al. (1997) successfully transformed 'Hindi' mango cultures using a disarmed strain of *A. tumefaciens* (LBA 4404) carrying antisense genes for ACC oxidase, ACC synthase, and alternate oxidase, aiming to induce anti-ripening characteristics in fruits. Additionally, they were able to mature some transformed embryos. Gutierrez et al. (2001) identified a mango cDNA homolog of the ethylene receptor gene *ETR-1*, which showed transient expression during fruit ripening and tissue wounding. Further advancements by Cruz-Hernández et al. (2000) optimized particle bombardment parameters, leading to transient expression of the beta-glucuronidase gene in polyembryonic masses. Stable transgene expression was later achieved using green fluorescent protein as a reporter gene.

Conclusion and future thrusts

Biotechnology offers significant potential for mango improvement. Tissue culture methods such as anther and ovary culture can be utilized to develop homozygous lines, while genetic transformation is increasingly being explored to create stable transformants with desirable traits. Genetic markers are particularly valuable as they complement conventional breeding strategies in *Mangifera* species. Substantial progress has been made in establishing regeneration protocols for various mango cultivars, and transformation via repetitive somatic embryogenesis has been successfully achieved. However, despite the successful regeneration of different genotypes, the efficiency of converting somatic embryos into fully developed plantlets remains low. Future research should prioritize improving the conversion rate of somatic embryos and enhancing plantlet regeneration from shoot and nodal segments. Many of the dominant mango varieties in global trade, such as Haden, Kent, Sensation, and Alphonso, have large canopies that make them unsuitable for high-density planting. Introducing dwarfing genes from Indian cultivars like Amrapali, Kerala Dwarf, and Manjeera could help induce dwarfing in these otherwise vigorous cultivars. Another significant challenge in mango cultivation is alternate bearing. This issue could potentially be addressed by introducing flower-meristem-activity genes such as *AGAMOUS-LIKE 20* (AGL20), *APETALA1* (AP1), and *LEAFY* from *Arabidopsis*. AGL20 plays a key role in floral initiation by integrating signals from multiple pathways, responding to both environmental and internal factors. Once activated, AGL20 induces the expression of *LFY*, which subsequently triggers *AP1* expression. Furthermore, advances in biotechnology can help address abiotic and biotic stress challenges in mango cultivation. As an invaluable tool for breeders, biotechnology holds the potential to revolutionize the mango industry by enabling the precise genetic modification of varieties for specific purposes-achievements that were previously unattainable through conventional breeding.

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