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The Contribution of Biotechnology in Commercial Fruit Production

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India is the world's second-largest producer of fruit crops after China in the world. India's main fruit crops are mango, banana, citrus, guava, grape, pineapple and apple. Plant tissue culture and other biotechnological advances have made it possible to produce high-quality, disease-free, and true-to-type planting material quickly. The method is helpful for managing genetic resources and controlling viruses in addition to providing a worthwhile substitute in fruit tree propagation research.

Somatic embryogenesis, somatic hybridization, virus elimination, in-vitro mutagenesis, anther or microspore culture production of haploids, embryo rescue technique or embryo culture, protoplast culture, and somatic fusion are now included in the list of common plant tissue culture technologies. Micropropagation is the most widely used method among the as above mentioned techniques for producing fruit plants in large quantities. Meristem culture and micrografting methods have been standardized in various fruit plants to create virus-free plants. Plant species, variety, and culture conditions all affect success. Recently, focus has shifted to the potential advantages of using microorganisms in plant cultures grown in-vitro conditions.

Keywords: Plant tissue culture, somatic embryogenesis, somatic hybridization, in-vitro mutagenesis, anther culture, embryo culture, microspore culture, micropropogation, meristem culture.

Introduction

After China, India is the world's second-largest producer of fruit crops. The main fruit crops grown in India are mango, banana, citrus, guava, grape, pineapple, and apple. In addition to these, a remarkable area is home to other fruits such as peach, apricot, pear, almond, walnut, and strawberry in the temperate group and papaya, ber, phalsa, sapota, annona, jackfruit, and pomegranate in the tropical and subtropical group. While fruit is grown all over the nation, the states that produce the most fruit are Gujarat, Maharashtra, Tamil Nadu, Karnataka, Andhra Pradesh, Bihar, and Uttar Pradesh. Few important fruit crops are mentioned below.

Mango is the most important fruit, making up 22% of the nation's total fruit production, the highest in the world, with India accounting for roughly 54% of the total. The largest variety of mango cultivars is found in India. The states of Uttar Pradesh, Bihar, Andhra Pradesh, Orissa, West Bengal, Maharashtra, Gujarat, Karnataka, Kerala, and Tamil Nadu are the main producers of mangoes. Some important varieties are Alphonso, Dashehari, Langra, Fazli, Chausa, Totapuri, Neelum etc.

Banana ranks second in terms of production, making up around 38% of all fruits. In terms of banana production, India ranks first globally. Maharashtra has the highest productivity of 65.70 metric tonnes per hectare against national average of 30.5 tonnes/ha, while Tamil Nadu leads the other states with a 19% share. The states of Karnataka, Gujarat, Andhra Pradesh, and Assam are the other main producers of bananas. Dwarf Cavendish,

Bhusaval Keli, Basrai, Poovan, Harichhal, Nendran, Safed Velchi are some important varieties of banana.

Citrus ranks third in terms of production, making up 13% of total production. It is grown primarily in Maharashtra, Andhra Pradesh, Karnataka, the northeastern states, Punjab, Orissa, and Madhya Pradesh. Lime, lemons, sweet oranges, and mandarins make up the majority of the land under it.

Guava is the fourth most popular crop in India. The important varieties of guava are Allahabad Safeda, Lucknow-49, Nagpur seedless, Lalit etc. Uttar Pradesh is the leading state in gu

Grape ranks fifth in term of production. Important varieties of grape in India include Bangalore blue, Thompson seedless, Sonaka, Anab-e-Shahi, Perlette, Pusa seedless, Beautiful seedless etc. Maharashtra holds the top spot in terms of production, contributing over 67% of the nation's total output and having the highest productivity in 2023–2024. With a 28% share in 2023–24, Karnataka is the second-largest producer of grapes. Tamil Nadu, Andhra Pradesh, and Punjab are the other states that cultivate grapes.

Propagation of fruit crops by Sexual method: The main purpose of seed propagation in fruit crops is to produce rootstocks which are necessary for grafted plants. Papaya is one of the fruit crop that is typically propagated from seeds. Some important events that could occur during seed propagation are mentioned below.

Dormancy: Hard seed coats, gas and water impermeability, embryo immaturity, lack of certain endogenous growth promoters or an excess of endogenous growth inhibitors can all contribute to dormancy in seeds. To improve germination, various techniques such as chemical treatment, stratification, and scarification can be used to break dormancy in seeds.

Germination : This is the process by which a new plant grows from an established seed. Seed viability and suitable environmental conditions are prerequisites for germination.

Apomixis : In this process embryo is created from a single saprophyte cell rather than through the fertilization of two gametes, this is known as apomixis. This process produces vegetative embryos rather than zygotic or sexual embryos.

Polyembroyny : The term "polyembryony" describes seeds that contain multiple embryos. One type of embryo, known as a gametic or sexual embryo, is created when male and female gametes unite. Without the assistance of a male gamete, the other embryos are created by the simple mitotic division of nucellus cells. In mangos and citrus, the nucellar embryo phenomenon is frequently observed.

Propagation of fruit crops by Asexual methods: Plants can be propagated vegetatively or asexually using a technique other than sexual propagation. It does not alter the new plant's genetic composition or quality. In terms of growth, ripening, yield, and fruit quality, the plants generated using this technique are true to type.
Various fruit plants are commercially multiplied through asexual or vegetative propagation. Cutting, layering, budding, and grafting are some of these techniques.

Cutting : It involves removal of stem with a few buds from the parent plant and placing it in an environment that will allow it to grow into a full plant. This technique is frequently applied to plants, causing easy and convenient roots, which makes plant reproduction quick and inexpensive. Fruit plants such as grapes, phalsa, and baramasi lemon are commercially cultivated using cuttings. Cuttings are made following pruning for deciduous fruit plants including grape, pomegranate, phalsa, and fig. However, cuttings from evergreen fruit plants, such as baramasi lemons can be made in the spring (February–March) and the rainy season (August–September).

Layering : It involves inducing roots in the shoots while they are still joined to the mother plants. For fruit crops that are difficult to root after being separated from their mother plants

i.e an alternate method of propagation. The three most popular layering techniques are mound, ground and air layering. Rooting is carried out on the shoot itself while it is still joined to the mother plant and air layering is carried out in the air. The following year, in February or September–October, these layers can be sown in the fields.

The ground layering method involves selecting a plant branch that is close to the ground and removing a ring of bark directly below the bud. The ground layering method involves selecting a plant branch that is close to the ground and removing a ring of bark directly below the bud. When still connected to the mother plant, this branch is then bent and buried in the ground. To keep the soil moist, it is watered frequently. The roots take shape and the new plant splits off from the mother plant in a matter of weeks. This technique is frequently used to propagate Baramasi lemons.

In the mound layering method, the plant returns in either July or February. From ground level, the new shoots emerge in April and September. These shoots have a ring of bark removed, and moist soil is placed over them. During the rainy season, the rooted stools from April stooling are separated, and the August stooling is removed the following spring. Following their separation from the parent plant, these stools are planted in nursery fields. This technique, also referred to as stool layering, is employed to propagate apple and guava rootstocks.

In the air layering method¸ firstly a healthy branch from the parent tree is chosen. A ring-shaped incision or slanting cut is typically used to carefully remove a small portion of the bark of pencil thickness. This wound encourages the growth of roots at that location by interfering with the branch's hormone and nutrient flow. To promote root development, apply rootex to cut.

It is then covered with a moist sphagnum moss to shield the injured area and create an environment that is favorable for the growth of roots. This supports the formation of new roots and also retains moisture for further root development. As the roots mature, they expand into the moist medium and form a solid bond with the parent tree. The best time to perform air layering in fruit crops is spring. It practiced mainly in fruit crops like guava,litchi etc.

Grafting : This technique involves two portions of the plant are linked together so that they grow together as a single plant. The scion twig in this manner has more than two buds on it. Fruits like pear, peach, plum, almond, mango, etc. are frequently grafted. Grafting is done when the plants are dormant in temperate fruits like peache plum and almond but it is done when the trees are actively growing in mango.

Budding : This technique involves inserting just one bud into the rootstock. Compared to grafting, this procedure is much simpler and quicker and requires less budwood. The ideal timing for budding is said to occur when the bark begins to slip on the scion as well as the stock. This demonstrates the activity of the cambium, which is made up of xylem and phloem and is responsible for union. This technique is typically used in the spring and during the monsoon season. There are three types of budding: chip, patch, and T-budding. T-budding occurs on most fruit trees during the monsoon season (July–September) or in the spring (March–April). This is the most often used technique for growing citrus plants. In guava, patch budding occurs in May and June. The chip budding method is typically used when the stock and scion are still dormant, right before the onset of fresh growth.

Some special techniques of propagation: Utilizing biotechnological methods, such as plant tissue culture, to quickly multiply and regenerate commercially significant plants is a relatively new and innovative discovery. New techniques for producing high-quality, disease-free, and true-to-type planting material quickly were made possible by biotechnology advancements. In addition to providing a viable alternative in fruit tree propagation studies, biotechnological technologies like in vitro culture and micropropagation are also helpful for managing genetic resources and controlling viruses. The process of in vitro micropropagation

was used to quickly propagate under aseptic conditions, eliminate viruses from certain plant species through meristem culture, and preserve plant germplasm in vitro. These days, the uses of plant tissue culture extend far beyond micro and clonal propagation. Some useful techniques are mentioned below:

Micropropagation - It generates millions of identical plants also known as "clones"in a comparatively short amount of time, without regard to seasonal limitations and in aseptic circumstances. In order to provide healthy propagules, tissue culture plant propagation which includes advanced methods such as meristem culture and molecular disease indexing is incredibly helpful. In addition to these benefits, micropropagation also helps with import and export of germplasm, removing issues associated with quarantine. The meristem tissue utilized in micropropagation has a size of 0.1–0.5 mm and contains either one or two leaf primordia. For the first time, virus-free plants were grown from shoot meristems in 1952. The field of micropropagation was later expanded to include a vast array of plant species, including fruit and plantation crops, after **Murashige and Skoog (1962)** discovered the hormonal control of organogenesis and identified the most widely used tissue culture medium. The method of micropropagation for many horticultural plants has also been standardized with the growth of science and technology. The ways of regeneration of whole plant from small plant parts include the following.

- Regeneration from existing mersitems- This is referred to as proliferation of axillary shoots. The extant meristems, like shoot tips and nodal buds, are cultivated on diverse media that have been supplemented with a range of plant growth regulators, either separately or in combination.
- Regeneration from adventitious meristems- By using mature plant components like leaves, internodes/stems, and roots, adventitious shoot development can be used to produce shoot multiplication either directly or through the formation of callus. Generally speaking, when there is a high ratio of cytokinin to auxin, shoots grow and viceversa.
- Regeneration by somatic embryogenesis - The somatic embryos are bipolar structures that develop from somatic or vegetative cells and have both a shoot and a root meristem. For induction, a high concentration of auxin in the culture medium must be followed by a low concentration of auxin and cytokinin. Somatic embryos can develop directly on explants, through liquid suspension cultures, or by callus development. After encapsulation, the somatic embryos may function as artificial seeds, which is a compelling substitute for plant multiplication.

Steps involved are enlisted below :

1. Establishment of explant - It depends on source of explant ,size of explant and type of explant (like leaf ,root etc.) ,surface sterilants and in vitro culture conditions (like temp, light , humidity etc).
2. Shoot multiplication - Shoot multiplication media is used to cultivate the established explants. These growth regulators and hormones strengthen the medium, preventing callus development and promoting the expansion of existing cultures. Therefore, multiplication is the outcome of the right hormonal combinations. As a result, cytokinins like BAP and kinetin as well as auxins like IAA, NAA, and 2,4-D are used carefully in culture media. Cytokinins are known to promote the growth of new shoots.
3. Rooting of shoots - The medium containing auxins such as IAA, NAA, and IBA is where the in vitro regenerated shoots are rooted. Stress situations might also cause the rooting to occur on a hormone-free media. In order to prevent somaclonal variations, the roots should ideally not develop callus.

4. Hardening and transfer to the main field - Before moving to the field, the resulting in vitro rooted plantlets are hardened and acclimatized. High humidity in a greenhouse is needed for hardening of plants.

Factors affecting in vitro multiplication :

- **Selection of explant** - In micropropagation, choosing a healthy explant is crucial **(Williams and Maheshwaran, 1986).**
- **The organ i.e to serve as tissue source** - Numerous researchers have described well-established methods of using different types of explants as tissue sources, such as in vitro propagation of banana cultivars through the culture of excised shoot apices **(Doreswamy et al. 1983 ; Vuylsteke and De Langhe 1985 ; Banerjee et al. 1986 ; Pandey et al. 1993 ; Sudhavani and Reddy 1997 ; Kotecha and Kadam 1998 ; Kumar et al. 2005 ; Choudhary et al. 2013).** Male inflorescence has been used in plant regeneration research in significant banana cultivars **(Krikorian et al., 1993; Escalant and Teisson, 1994**). When compared to other plant components, the shoot tip is thought to be the most receptive and superior explant.
- **Size of explant and overall quality of parent palnt** - Numerous researchers have confirmed that the formation of in vitro cultures is mostly determined by the quality of the transplants **(John and Murray, 1981; Kim et al., 1981; Keathley, 1983)**. **Madhulata et al. (2004)** found that whereas banana shoot explants with too short shoots did not regenerate, larger explants did.
- **Physiological state** - An essential component of the micropropagation process is the physiological condition of the plant from which the transplant is taken. Compared to older trees, younger trees offer better explants for regeneration **(Bonga 1982; Sommer and Wetzstein 1984)**. The careful selection of explants is crucial to the effectiveness of micropropagation from mature trees **(Murashige 1974; Sommer and Caldas 1981)**

Problems occuring during micropropagation

Contamination – It does not allow the multiplication of culture. In vitro culture requires the successful disinfection of explants, which frequently entails a defined set of treatments that differ depending on the species and type of explant in question **(Thorpe and Patel, 1984)**. Two possible sources of contamination in tissue culture are endophytic microbes found in the tissue itself or microorganisms that are carried over from the explant's surface are carry over microbes. Bacterial contamination is a major issue for banana in vitro propagation. Endogenous bacteria cause a significant number of explants to be killed during banana micropropagation **(Hadiuzzaman et al., 2000)**.

Release of Phenolic compounds- Certain plant species cultured explants release phenols into the medium which oxidize the phenols and generate quinones which are toxins that hinder the growth of the cultured explants. This results in browning. Browning can be avoided by using antioxidants such polyvinylpyrrolidone (PVP), citric acid/ascorbic acid, activated charcoal and polyvinylpolypyrrolidone (PVPP) in the culture medium and during sterilization. Fruit trees with the same issue include date palm, banana and guava.

Variation in tissue cultured raised plants - Callusing and plant regeneration from callus, rather than direct shoot induction and proliferation, are the causes of variability. Variability in plants grown in tissue culture is very undesirable. Compared to axillary meristems, plants regenerated via adventitious meristems are more vulnerable. to mutations since it originates from a single cell or a little collection of cells. This could make the regenerated plants different. By adding growth regulators that prevent callusing, such as triiodobenzoic acid (TIBA), phloroglucinol, and phloridzin, as well as by lowering the content of inorganic salt in the culture medium, the variation brought on by callusing can be prevented.

Mortality in greenhouse - Transplantation shock can affect tissue culture-raised plants because of their altered leaf morphology, low photosynthetic efficiency, open stomata, and

decreased epicuticular waxes. Therefore, it is imperative that these plants harden before being transplanted under field circumstances. In vitro-raised plants can be hardened by using anti- transpirants, partially defoliating the plants, and conserving moisture by maintaining a high humidity level around them.

Micrografting - It is used as an alternative to create virus-free plants because it is challenging to regenerate entire plants from meristem in woody species, such as the majority of fruit and forest plants. Scion preparation, rootstock preparation, in vitro grafting, and plant acclimatization/hardening are some of the several processes involved in micrografting. The nucellar seedlings cultivated in vitro serve as root stocks. The juvenile growth of field-grown trees, defoliated glasshouse-grown plants, or in vitro proliferating nodal segments derived from mature trees are the sources of the scion (meristem 0.1–0.4 mm). Under aseptic conditions, the grafting is carried out with the aid of a stereomicroscope. Citrus tristeza virus, peach latent mosaic virus, and pear vein yellow virus are just a few of the viruses that have been eradicated in fruit plants using micrografting.

Meristem tip culture - This technique involves cultivating the meristem tip, which is made up of one or two pairs of leaf primordia, in a medium. The plantlets regenerate after a few weeks, and once they have hardened, they are placed in the soil in an environment that is similar to their native habitat. Instead of producing diploid plants, meristem tip-cultured plants produce polyploid ones. Furthermore, the removal of viruses from contaminated plant material is greatly aided by meristem tip culture. Quick growth of It is also feasible to reproduce plants that are ordinarily difficult to do so using vegetative techniques via means of meristem cultivation. The resulting plants are pathogen-free and may be kept in smaller spaces and for longer periods of time.

In Vitro Myccorhhization -The potential advantages of microorganisms in in vitro plant cultivation have recently drawn attention. For instance, Pseudomonas species can lessen hyperhydricity **(Bela et al., 1998);** *Bacillus pumilus*, *Alcaligenes faecalis*, and *Pseudomonas* species enhance shoot multiplication **(Monier et al., 1998)**; and the root endophyte *Piriformospora indica* encourages explant hardening **(Sahay and Varma, 1999)**. Due to a proven improvement in the posttransplant performance of in vitro-grown plants, mycorrhization in micropropagation—specifically, the use of arbuscular mycorrhizal fungus (AMF)—is currently gaining traction **(Lovato et al., 1996; Rai, 2001).** The development of efficient AMF production methods, mycorrhization of in vitro plants, screening for efficient AMF strains, and improved nutrient uptake, water relations, aeration, soil pH balance, and their potential use as bioregulators **(Lovato et al., 1996)** have all contributed to the recent increase in research interest in AMF. A variety of instruments are now available to examine the viability of various AMFs for use in commercial micropropagation enterprises. Additionally, CPB has finished the mycorrhiza project. Mycorrhiza has been employed as a biohardening substance. Mycorrhiza has been found to increase the survival rate of plants grown in vitro. Research has been conducted on bananas.

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