Role of Biotechnology for Commercial Production of Fruit Crops

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Abstract

India is the second largest producer of fruit plants after China. Mango, banana, citrus, guava, grape, pineapple, and apple are the major fruit plants grown in India. Apart from these, other fruits like papaya, ber, phalsa, sapota, annona, jackfruit, and pomegranate in the tropical and subtropical group and peach, apricot, pear, almond, walnut, and strawberry in the temperate group are also grown in a remarkable area. Propagation in fruit plants through seed is primarily done to raise rootstocks, which are required for grafted plants. Some of the fruit plants like papaya are propagated through seeds, but dormancy, poor germination, low seed viability, and adverse environment conditions are major limiting factors. Advances in biotechnological techniques like plant tissue culture provided new methods for rapid production of high-quality, disease-free, and true-to-type planting material. The technique not only offers a valuable alternative in fruit trees propagation studies but is also useful for virus control and management of genetic resources. Nowadays the range of routine technologies of plant tissue culture has expanded to include 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. Out of the abovementioned techniques, the most exploited one for mass production of fruit plants is micropropagation. To produce virus-free plants, meristem culture and micrografting techniques have been

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standardized in different fruit plants. The success varies with the plant species, variety, and the culture environment. Recently, attention has turned to the possible beneficial effects of microorganism's in vitro plant cultures. It has been observed that mycorrhiza enhances the survival percentage of in vitro-raised plant.

3.1 Introduction

India is the second largest producer of fruit plants after China. Mango, banana, citrus, guava, grape, pineapple, and apple are the major fruit plants grown in India. Apart from these, other fruits like papaya, ber, phalsa, sapota, annona, jackfruit, and pomegranate in the tropical and subtropical group and peach, apricot, pear, almond, walnut and strawberry in the temperate group are also grown in a remarkable area. Although fruit is grown throughout the country, the major fruit-growing states are Maharashtra, Tamil Nadu, Karnataka, Andhra Pradesh, Bihar, Uttar Pradesh, and Gujarat. The major fruit crops of India are given below.

Mango is the most important fruit accounting for 22 % production of total fruits in the country, which is highest in the world with India's share of about 54 %. India has the richest collection of mango cultivars. Major mango-growing states are Uttar Pradesh, Bihar, Andhra Pradesh, Orissa, West Bengal, Maharashtra, Gujarat, Karnataka, Kerala, and Tamil Nadu. The main varieties of mango grown in the country are Alphonso, Dashehari, Langra, Fazli, Chausa, Totapuri, Neelum, etc.

Banana comes next in rank accounting for about 38 % of the total production of fruits. India has the first position in the world in banana production. While Tamil Nadu leads other states with a share of 19 %, Maharashtra has highest productivity of 58.6 metric tonnes against India's average of 32.5 metric tonnes per ha. The other major banana-growing states are Karnataka, Gujarat, Andhra Pradesh, and Assam. The main varieties of banana are Dwarf Cavendish, Bhusaval Keli, Basrai, Poovan, Harichhal, Nendran, Safed Velchi, etc.

Citrus fruits rank 3rd accounting for 13 % of total production. Lime, lemons, sweet oranges, and mandarin cover bulk of the area under these fruits and are grown mainly in Maharashtra, Andhra Pradesh, Karnataka, northeastern states, Punjab, Orissa, and Madhya Pradesh.

Guava is the fourth most widely grown fruit crop in India accounting for 4 % of total production. The popular varieties of guava are Allahabad Safeda, Lucknow-49, Nagpur seedless, Dharwar, etc. Bihar is the leading state in guava production with 0.30 MT, followed by Andhra Pradesh and Uttar Pradesh. The other states where guava is grown widely are Gujarat, Karnataka, Punjab, and Tamil Nadu.

Grapes occupy the fifth position among fruit crops. The major varieties of grapes grown in India are: Thompson seedless, Sonaka, Anab-e-Shahi, Perlette, Bangalore blue, Pusa seedless, Beauty seedless, etc. Maharashtra occupies the first position with a production of 0.68 MT of grapes, followed by Karnataka. The other states growing grapes are Punjab, Andhra Pradesh, and Tamil Nadu.

3.2 Propagation Techniques

Plant propagation is the method of production of more than one plant from the mother plant or the tissue over a specific time period. True-to-type plant production from the mother plant is one of the major objectives of propagation. Plants can be propagated either by sexual or vegetative means. In sexual reproduction method, the progeny gets characteristics from both of its parents. It will not grow "true" to either parent and has an unpredictable combination of characteristics. Plant propagation has been a useful tool since centuries, for fruit plants since time immemorial. Plant propagation depends on the plant species, variety, method of propagation, and climatic and

growth conditions. It is better to propagate fruit cultivars by vegetative propagation methods in order to ensure genetic reliability.

Plant propagation is primarily done by conventional methods, which include sexual and asexual means. However, in the recent past commercial plant propagation through biotechnological applications has made great contributions towards production of plants.

3.2.1 Sexual Method of Propagation

This is the method of raising the plants through seeds. The method is used for evolution of new varieties through breeding and production of hybrids.

3.2.1.1 Advantages

1. This is the most popular method of propagation in some fruit plants like papaya.
2. Seed propagated rootstocks are hardy and develop better root system.
3. Viruses do not transmit through seeds; thus mostly the seedlings are virus-free.
4. Occurrence of polyembryony in fruits leads to the development of uniform seedlings.

3.2.1.2 Disadvantages

1. Long juvenile period in seed-grown plants as compared to asexually raised plants.
2. The progeny is not true to type.
3. It is not economical to handle larger trees.

3.2.2 Asexual Method of Propagation

In this method, the plants are propagated from vegetative part of the mother plant instead of seeds.

3.2.2.1 Advantages

1. In some fruit plants, which do not bear seeds, this is the only propagation method.
2. The plants are generally true to type and uniform in growth, yield, and quality.
3. Short juvenile period as compared to seed-grown plants.
4. The advantages of rootstocks can be obtained by budding or grafting susceptible varieties on resistant/tolerant rootstocks.

3.2.2.2 Disadvantages

1. New variety or hybrid cannot be developed by this method.
2. Plants are not so vigorous and long lived as the seedling trees.
3. Conservation of these plants is expensive as compared to storage of seeds.

3.3 Propagation of Fruit Plant by Seed

Propagation in fruit plants through seed is primarily done to raise rootstocks, which are required for grafted plants. Some of the fruit plants like papaya are conventionally seed propagated. There may be following events may occur during seed propagation.

3.3.1 Dormancy

The dormancy in seeds may be due to hard seed coat, water and gas impermeability, immaturity of embryo, deficiency of some endogenous growth promoters, or excess of endogenous growth inhibitors. Different methods like stratification, scarification, and chemical treatment can be used for breaking of dormancy in seed to improve germination.

3.3.2 Germination

This refers to the emergence of a new plant from the mature seed. Germination requires seed viability and appropriate environmental conditions.

3.3.3 Apomixis

Apomixis occurs when an embryo is produced from a single cell of the saprophyte and does not

develop from fertilization of two gametes. This is a natural mechanism during which vegetative embryo is produced instead of sexual or zygotic embryo.

3.3.4 Polyembryony

Polyembryony refers to the seeds having more than one embryo in the seed. One of the embryos arises from the union of male and female gametes and is called gametic or sexual embryo. The other embryos are produced by simple mitotic division of cells of nucellus without the help of male gamete in their formation. The phenomenon of nucellar embryo is of common occurrence in citrus and mango.

3.4 Vegetative Propagation

Vegetative or asexual propagation is propagation of plants by the method other than sexual propagation. It includes no change in genetic makeup or quality of the new plant. The plants produced by this method are true to type in growth, ripening, yield, and fruit quality.

Commercial multiplication of various fruit plants is done by using vegetative or asexual propagation. These methods include cutting, layering, budding, and grafting.

3.4.1 Cutting

It is the method of propagating fruit plants. In this method the stem (small part of plant) having at least few buds is detached from parent plant and placed under favorable conditions to develop into a complete plant. This method is commonly used in plants, which cause rooting easily and readily, thus multiplication of plants is very quick and cheap. The fruit plants like phalsa, baramasi lemon, and grapes are commercially propagated by cuttings. In case of deciduous fruit plants such as grape, pomegranate, phalsa, and fig, the cuttings are made after pruning. While in evergreen fruit plants like baramasi lemon, the cuttings can be prepared during the spring (February–March) and rainy season (August–September).

3.4.2 Layering

Layering is a vegetative propagation method, in which roots are induced on the shoots while they are still attached to the mother plants. This is an alternate propagation method in fruit plants which do not root easily when detached from the mother plants. Most commonly used methods of layering are air, ground, and mound layering. Air layering is done in air, and the rooting is done on the shoot itself when it is still attached to the mother plant. These layers can be planted in the fields during the following year in February or September–October. In ground layering method, a branch of plant, which is near the ground, is chosen, and a ring of bark is removed just below the bud. This branch is then bended and buried in soil when still attached to the mother plant. The soil is regularly watered to keep it moist. Within a few weeks, the roots are formed and new plant is separated from the mother plant. This method is commonly followed for propagation of baramasi lemon. In mound layering method, plant is headed back either in February or in July. The new shoots come out during April and September, from ground level. A ring of bark is removed from these shoots and they are covered with moist soil. The rooted stools of April stooling are separated during rainy season, and those of August are removed in the following spring. These stools, after separating from the parent plant are planted in the nursery fields. This method is also known as stool layering and is used for propagation of guava and apple rootstocks.

3.4.3 Budding

This is a method in which only one bud is inserted in the rootstock. The method is very easy and fast and saves budwood as compared to grafting. As soon as the bark starts slipping both on the stock and scion, this is considered to be the optimum time for budding. This shows that the cambium (xylem and phloem), which is the tissue responsible for union, is active. This method is generally employed during spring and rainy season. The budding may be T-budding, patch budding, and chip budding.

In most fruit trees T-budding is performed either in the spring (March–April) or in the rainy season (July–September). This is the most common method of propagation of citrus plants. Patch budding is done in guava during May and June. Chip budding method is usually employed just before the start of new growth, i.e., when the stock and scion are still dormant.

3.4.4 Grafting

Grafting is a vegetative propagation method of fruits, where two plant parts are joined together in such a manner that they unite and continue their growth as one plant. In this method, the scion twig has more than two buds on it. Grafting is commonly done in pear, peach, plum, almond, mango, etc. In temperate fruits like peach, plum, and almond, grafting is done when the plants are dormant, while in mango it is done when the trees are in active growth. The different methods of grafting are tongue grafting, cleft grafting, approach grafting, and side grafting. Tongue grafting is commonly used when the stock and scion are of equal diameter. Cleft grafting/wedge grafting is done during dormant period. Approach grafting/inarching is commonly followed in mango. Side grafting can be carried out successfully from March to October, but success during May and October is rather low. This method of propagation is commonly used in mango.

3.5 Propagation of Fruit Plants Through Specialized Organs

Propagation of fruit plants through specialized organs like runners, suckers, and slips. Runner is a specialized stem that develops from the axil of a leaf at the crown of a plant. It grows horizontally along the ground and forms a new plant at one of the nodes, e.g., strawberry. A shoot arising on an old stem or underground part of the stem is known as sucker. The capacity of a plant to form suckers varies from plant to plant, variety to variety, and is even climate dependent. The sucker formation is common in fruit plants like pear and banana. In banana, sword suckers are commonly used for propagation of plants. Slips are shoots just arising below the crown but above the ground. Pineapple is commercially propagated through this method of propagation.

3.6 Special Techniques of Propagation

The use of biotechnological technique like plant tissue culture in the regeneration and fast multiplication of economically important plants is a comparative recent and radical development. Advances in biotechnology provided new methods for rapid production of high-quality, disease-free, and true-to-type planting material. Biotechnological tools like in vitro culture and micropropagation not only offer a valuable alternative in fruit trees propagation studies but are also useful for virus control and management of genetic resources. The technique of in vitro micropropagation was employed for virus elimination from certain plant species by meristem culture, rapid propagation under aseptic conditions, and in vitro preservation of plant germplasm. Today plant tissue culture applications encompass much more than clonal propagation and micropropagation. The range of routine technologies has expanded to include somatic embryogenesis, somatic hybridization, virus elimination, as well as the application of bioreactors to mass propagation (Aitken-Christie et al. 1995; Paek et al. 2001). These include:

- Micropropagation
- Meristem culture
- Somaclonal variations
- In vitro mutagenesis
- Anther or microspore culture production of haploids
- Embryo rescue technique or embryo culture
- Protoplast culture – somatic fusion

Out of the abovementioned techniques, the most exploited one for mass production of horticultural crop plants is micropropagation. Thus this technology is dealt with details as follow.

3.6.1 Micropropagation

The vegetative propagation of fruit plants has been practiced for centuries, and many improvements in conventional methods have been made over the years. Recently, the tissue culture technique, i.e., micropropagation, has expanded their scope and potential on commercial scale. Micropropagation is suitable for the rapid and large-scale clonal multiplication of elite germplasm. The technique has been referred as micropropagation because the size of the tissue in culture is very small as compared to conventional method. The size of meristem tissue used for micropropagation is about 0.1–0.5 mm size having only one or two leaf primordia. First time in 1952 virus-free plants were obtained by culturing shoot meristems. Later on with the discovery of the hormonal control of organogenesis and finding of most commonly used tissue culture media by Murashige and Skoog (1962), the scope of micropropagation was further extended to huge scale of plant species, including fruit and plantation crops. With the advancement in science and technology, micropropagation technique has also been standardized for many plants, and it is now widely used for multiplication of many horticultural plants. Now micropropagation is perhaps the most popular and widely commercialized global application of plant biotechnology in horticulture. A large number of plants are being cloned and exploited commercially worldwide. Novel germplasm in horticultural crops, created using various biotechnological tools, also needs to be multiplied rapidly for quick dissemination. This is possible only by integrating in vitro culture and molecular biology techniques.

Micropropagation is well known as a means of producing millions of identical plants ("clones") under aseptic conditions, in a relatively short period of time, independent of seasonal constraints. Propagation of plants through tissue culture, including sophisticated techniques of meristem culture and molecular indexing of diseases, is of immense use in making available healthy propagules. Besides these, micropropagation is also applied advantageously for import and export of germplasm, obviating quarantine-related problems.

3.6.1.1 Advantages of Micropropagation

Micropropagation techniques have several advantages over conventional propagation techniques. These include:

1. Year-round availability of plants irrespective of seasonal constraints
2. Fast multiplication of true-to-type planting material
3. Conservation of plant diversity
4. Multiplication of hybrids
5. Disease-free plant production
6. Highly beneficial in dioecious fruit plant species (date palm and papaya), where large-scale production of female plants is possible
7. Export and import of germplasm become easy requiring minimum quarantine checks
8. Easy transport of propagation material

3.6.1.2 Ways of Micropropagation

The ways for the regeneration of whole plant from small excised plant parts include the following.

3.6.1.2.1 Regeneration from Existing Meristems

This is also known as axillary shoot proliferation. The existing meristems such as shoot tip or nodal bud are cultured on the different types of media fortified with various plant growth regulators, alone or in combination. The shoot proliferation depends on the type of hormones/regulator used. For axillary shoot proliferation, the commonly used growth regulators are benzylaminopurine (BAP), kinetin, and 2-isopentenyl adenine (2-ip). The regenerants are considered to be genetically stable. The process of shoot tip culture is successful in banana tissue culture.

3.6.1.2.2 Regeneration from Adventitious Meristems

Shoot multiplication either directly or by callus formation can be obtained by inducing adventitious shoot production on mature plant organs such as leaves, internodes/stems, and roots. For initiation of adventitious meristems, a proper combination of growth regulators is needed in culture medium. In general, shoots are formed

when a high ratio of cytokinin to auxin is present, and reverse is true for root formation. The plants regenerated via this method are not always genetically stable, due to formation of mixoploids. The repeated subculture of callus also reduces its potential and regenerative capacity.

3.6.1.2.3 Regeneration by Somatic Embryogenesis

The somatic embryos are bipolar structures, possess both shoot and root meristem, and originate from somatic or vegetative cells. It requires a high level of auxin in culture medium for induction, followed by low auxin and cytokinin concentration in medium. Somatic embryos may arise directly on explants or via callus formation or liquid suspension cultures. The somatic embryos may act as synthetic seeds after encapsulation, which is an attractive alternative for propagation of plants. Among two methods, viz., hydrated or desiccated, for artificial seed production, the production of hydrated seeds is more popular. In this method, the individual somatic embryo is encapsulated in a water-based gel (hydragel such as calcium alginate). Embryos developed through tissue culture technique are mixed with sodium alginate and dropped with pipette into a calcium salt (calcium chloride) solution to form calcium alginate capsules. The capsules are washed in water and then placed on culture medium for germination. Artificial seeds have been produced in banana, citrus, mango, apple, olive, and kiwi fruit.

3.6.1.3 Stages/Steps Involved in Micropropagation

There are four main stages/steps involved in micropropagation of plants, such as explant establishment, shoot multiplication, rooting, and hardening and transfer to soil/field.

3.6.1.3.1 Explant Establishment

The establishment of explants depends on several factors such as the source of explants/genotype; size of explants; type of explants such as leaf, root, and stem from mature or immature plants/seedlings; explant sterilization process; types of sterilizing agents used; and the in vitro culture conditions such as culture media, composition, temperature, humidity, and light. The explants showing growth are considered established.

3.6.1.3.2 Shoot Multiplication

The established explants are subculture on shoot multiplication medium. The medium is fortified with such growth regulators/hormones which avoid callus formation and cause multiplication of established cultures. Thus the proper hormonal combinations result in multiplication. Hence careful use of auxins like IAA, NAA, and 2,4-D and cytokinins like BAP and kinetin is done in culture medium. It is a known fact that cytokinins enhance shoot multiplication.

3.6.1.3.3 Rooting of Shoots

The in vitro regenerated shoots are rooted in the medium containing auxins like IAA, NAA, and IBA. The rooting can also be induced on medium devoid of hormones due to stress conditions. The rooting should also be preferably without formation of callus, thus avoiding somaclonal variants.

3.6.1.3.4 Hardening and Transfer to Soil/Field

The in vitro rooted plantlets thus obtained are hardened/acclimatized before transferring to the field. The plants are hardened in greenhouse under high humidity. The various types of potting mixtures are used to enhance their survival rate in greenhouse.

3.6.1.4 Factors Affecting In Vitro Multiplication

3.6.1.4.1 Selection of Explants

Selection of healthy explant is important in micropropagation (William and Maheshwaran 1986). The suitability of which depend upon:

3.6.1.4.2 The Organ That Is to Serve as a Tissue Source

Different types of explants have been used by various scientists as the tissue source such as in vitro propagation of banana cultivars through the culture of excised shoot apices is well established as described by a number of researchers

(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). Plant regeneration studies using male inflorescence (Krikorian et al. 1993; Escalant and Teisson 1994) have been reported in important banana cultivars. The shoot tip is considered to be most responsive and better explant as compared to other parts of the plant.

3.6.1.4.3 Size of Explant and Overall Quality of Parent Plant

Many workers have confirmed that the quality of explants primarily determines the establishment of in vitro cultures (John and Murray 1981; Kim et al. 1981; Keathley 1983). Madhulata et al. (2004) observed that larger explants regenerated earlier while too small shoot explants of banana failed to regenerate.

3.6.1.4.4 Physiological State

Physiological state of the plant from which explant is taken is an important factor in the process of micropropagation. Younger trees provide more suitable explants for regeneration than the aged trees (Bonga 1982; Sommer and Wetzstein 1984). The success in micropropagation from mature tree is very much dependent upon careful selection of explant (Murashige 1974; Sommer and Caldas 1981).

3.6.1.5 Methods of Disinfection of the Explants

Successful disinfection of explants is a prerequisite for in vitro culture and often involves a standard set of treatments, which vary with the type and species of explant in question (Thorpe and Patel 1984). Contamination in tissue culture can originate from two sources: either through carryover of microorganism on the surface of explant or in the tissue itself (endophytic microbes). For in vitro propagation of banana, bacterial contamination is a great problem. Huge numbers of explants are destroyed during micropropagation of banana due to endogenous bacteria (Hadiuzzaman et al. 2000).

The various types of disinfectants can be used to overcome the problem of contamination. The surface sterilizing agents like sodium hypochlorite, calcium hypochlorite, and mercury chloride can be used for sterilization of explants. The systemic sterilizing agents like Bavistin (fungicide) and streptocycline (bactericide) can also be useful for prevention of contamination. The above chemicals may be used alone or in combination to enhance the survival rate of explants during sterilization.

3.6.1.6 Problems Encountered During Micropropagation

The success of micropropagation is hampered by these problems. The main problems encountered during micropropagation are as follow.

3.6.1.6.1 Contamination

Bacterial/fungal contaminations in the cultures do not allow the explant/multiplied culture to grow. The problem can be overcome by growing donor plants in growth chambers, systemic fungicide spray prior to explant removal, effective explant sterilization with effective sterilizing agents, performing inoculations in sterilized laminar airflow cabinets, and using sterilized surgical instruments. Fumigation of inoculation room using dilute formaldehyde solution also helps to minimize the problem.

3.6.1.6.2 Release of Phenolic Compounds

The cultured explants of certain plant species secrete phenols into the medium, which cause browning due to oxidation of phenols and formation of quinones, the toxins which affect the growth of cultured explants. The use of antioxidants such as activated charcoal, citric acid/ascorbic acid, polyvinylpyrrolidone (PVP), and polyvinylpolypyrrolidone (PVPP) during sterilization and in the culture medium helps to prevent the browning. Date palm, banana, and guava are the fruit plants having the same problem.

3.6.1.6.3 Variations in Tissue Culture-Raised Plants

Variability occurs due to callusing and regeneration of plants from callus instead of direct shoot induction and proliferation. The variability is highly undesirable in the tissue culture-raised plants.

The plants regenerated via adventitious meristems as compared to axillary meristem are susceptible to mutations, as it is derived from either a single cell or a small group of cells. This may cause variation in regenerated plants. The variation due to callusing can be stopped by addition of growth regulators which inhibit callusing such as triiodobenzoic acid (TIBA), phloroglucinol, and phloridzin and also by reduction of inorganic salt concentration in the culture medium.

3.6.1.6.4 Mortality in Greenhouse

Tissue culture-raised plants have different leaf morphology, poor photosynthetic efficiency, malfunctioning of stomata (open), and reduced epicuticular waxes and thus are amenable to transplantation shock. Hardening of such plants is thus must before transplantation under field conditions. Conservation of moisture by creating high humidity around the plants, partial defoliation, and application of antitranspirants are useful for hardening of in vitro-raised plants.

3.6.1.7 Limitations

Limitations of micropropagation are as follows:

1. The facilities are costly.
2. Highly technical skill is required to carry out different procedures.
3. Pathogens, once appear in the system, multiply at a very fast rate and deteriorate the whole culture.
4. Plants having high levels of phenols (mango, date palm, coconut, etc.) usually do not respond to micropropagation techniques.
5. Establishment of laboratory-raised plants in the field is a very difficult task.

3.6.2 Meristem Tip Culture

This technique is widely used in fruit plants. In this method, the meristem tip consisting of one or two pairs of leaf primordia is cultured in a medium. After a few weeks, the plantlets are regenerated, and after hardening of the plantlets, these are transplanted in the soil under natural environmental conditions. Meristem tip-cultured plants give rise to polyploid plants instead of diploid plants. Moreover, meristem tip culture is very useful for the elimination of viruses from infected plant material. Rapid multiplication of the plants, which are otherwise not easily propagated by vegetative means, is also possible through meristem culture. Plants produced are free from pathogens and can be stored for longer period and in smaller space.

3.6.3 Micrografting

It is difficult to regenerate complete plants from meristem in woody species like most of fruit and forest plants; thus as an alternative micrografting is done to produce virus-free plants. The various steps in micrografting include scion preparation, rootstock preparation, in vitro grafting, and acclimatization/hardening of the plants. The in vitro-raised nucellar seedlings are used as rootstocks. The scion (meristem 0.1–0.4 mm) is obtained from either young growth of field-grown trees, defoliated glasshouse-grown plants, or in vitro proliferated nodal segments obtained from mature trees. The grafting is done with the help of stereomicroscope, under aseptic conditions. Several viruses have been eliminated via micrografting in fruit plants such as Citrus tristeza virus, peach latent mosaic viroid, and pear vein yellow virus.

3.6.4 In Vitro Mycorrhization

Recently, attention has turned to the possible beneficial effects of microorganisms in in vitro plant cultures. For example, the root endophyte *Piriformospora indica* promotes explant hardening (Sahay and Varma 1999); *Pseudomonas* spp. can reduce hyperhydricity (Bela et al. 1998); and *Bacillus pumilus, Alcaligenes faecalis*, and *Pseudomonas* spp. improve shoot multiplication (Monier et al. 1998). Mycorrhization in micropropagation, particularly the use of arbuscular mycorrhizal fungi (AMF), is now gaining momentum due to a demonstrated positive impact on posttransplant performance of in vitro-grown plants (Lovato et al. 1996; Rai 2001). Improved nutrient uptake, water relations, aeration, soil pH

balance (Sylvia 1998), and their potential use as bioregulators (Lovato et al. 1996) have recently heightened research interest in AMF, contributing to the development of effective AMF production methods, mycorrhization of in vitro plants, and screening for efficient AMF strains. The potential of different AMFs for application in commercial micropropagation industries can now be tested using an array of tools. CPB has also completed the project on mycorrhiza. The mycorrhiza has been used as biohardening agent. It has been observed that mycorrhiza enhances the survival percentage of in vitro-raised plant. The study has been done in banana, sugarcane bamboo, etc.

3.7 Research Work on Fruit Propagation at the Centre for Plant Biotechnology (CPB), Hisar

CPB, Hisar, is engaged in the in vitro multiplication and production of elite germplasm of various medicinal, horticultural, and other crop plants. CPB has standardized the in vitro multiplication protocol for fruit crops like banana, guava, citrus, and strawberry. The center is also engaged in commercial production of two cultivars of banana, i.e., Robusta and Grand Naine (G-9), and supplying the planting material to the users.

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