



# Plant Archives

Journal homepage: <http://www.plantarchives.org>

DOI Url : <https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.1.067>

## IMPACT AND SCOPE OF TISSUE CULTURE TECHNOLOGY IN FRUIT CULTURE : A REVIEW

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(Date of Receiving-27-11-2023; Date of Acceptance-04-02-2024)

### ABSTRACT

Biotechnology has been globally accepted as one of the important tools for direct application in fruit production. It has a strong and positive influence on the horticulture sector worldwide. Biotechnology includes plant tissue culture (PTC), applied microbiology and applied molecular biology contributing to the production of crops with improved food, feed, fiber, vitamin, mineral and fuel. The technique of PTC is well translated from 'concept' to 'commercialization'. As an industry, PTC is no more a nascent industry in India. It is flourishing with multidirectional growth and multimillion dollar turn over. Several crop plants are routinely propagated (bananas, strawberries, pomegranate, pear, mulberry and pepino, etc.) by tissue culture technique and are being traded domestically and internationally for nearly three decades. Since, PTC is a powerful technique for mass production in many crops, it has become an important tool in the nursery and farming industry. PTC technique has been responsible for bringing about the second green revolution in our country. The growth of PTC industry in India, its impact on the growing needs of the market, its business potential and the challenges this industry is facing are discussed in the review article.

**Key words** : Plant tissue culture, *In vitro* propagation, New varieties, Industry.

### Introduction

Plant tissue culture, or the aseptic culture of cells, tissues, organs and their components under defined physical and chemical conditions *in vitro*, is an important tool in both basic and applied studies as well as in commercial application. It owes its origin to the ideas of the German scientist, Haberlandt, at the beginning of the 20<sup>th</sup> century (John, 2022). The culture of all types of plant cells, tissues and organs under aseptic conditions. This definition also extends to the culture of excised embryos and protoplast culture. Plant tissue culture has an important role to play in the manipulation of plants. Plant tissue culture is an integral part of molecular approaches to plant improvement and acts as an intermediary whereby advances made by the molecular biologists in gene isolation and modification are transferred to plant cells (Haberlandt, 1902).

Tissue culture technology is used for the production

of doubled haploids, cryopreservation and propagating new plant varieties, conserving rare and endangered plants, difficult-to-propagate plants and to produce secondary metabolites and transgenic plants (Hussain *et al.*, 2012). The production of high quality planting material of crop plants and fruit trees, propagated from vegetative parts, has created new opportunities in global trading, benefited growers, farmers and nursery owners and improved rural employment. However, there are still major opportunities to produce and distribute high quality planting material, *e.g.* crops like bananas, strawberries, pomegranate, pear, mulberry and pepino *etc.* (Mahendra *et al.*, 2020).

The main advantage of tissue culture technology lies in the production of high quality and uniform planting material that be multiplied on a year-round basis under disease-free conditions anywhere irrespective of the season and weather (Lalitha *et al.*, 2014). However, the

technology is capital, labour and energy intensive. The energy requirements for tissue culture technology depend on day temperature, day-length and relative humidity and they have to be controlled during the process of propagation (Loberant and Altman, 2010). Individual plant species also differ in their growth requirements. It is necessary to have low cost options for weaning, hardening of micro-propagated plants and finally growing them in the field. Some of the simpler techniques that are more approachable and have been found to be applied directly in plant propagation and genetic improvement of plants are (i) micro-propagation, (ii) meristem culture, (iii) somatic embryogenesis, (iv) somaclonal variation, (v) embryo culture, (vi) *in vitro* selection, (vii) anther culture, and (viii) protoplast culture (Smith *et al.*, 1990).

### **Micropropagation industry in India**

Micro-propagation is the application of tissue culture technique to the propagation of plants starting with very small parts grown aseptically in a test tube or other suitable containers (Lone *et al.*, 2020). Micro-propagation is one of the key tools of plant biotechnology that has been extensively exploited to meet the growing demands for elite planting material in the current century. There exists a large demand for disease free clones of superior quality plants in pomology (Sanghera, 2021). This need has been successfully tapped through micro-propagation by the application of techniques of plant tissue culture thereby effectively translating the concept of technology for the commercial needs. As a result, several hundred plant tissue culture laboratories have come up worldwide, and more so in India (Singh and Shetty, 2011). The commercial tissue culture was born in India in 1987, when A.V. Thomas and Company Kerala (AVT) established their first production unit in Cochin for clonal propagation of superior genotypes of selected cardamom plants (Bijalwan, 2021). Currently 78 commercial tissue culture production units have been recognized by the Department of Biotechnology (DBT), Govt. of India under the National Certification System for Tissue Culture Raised Plants (NCS-TCP) (Shingote *et al.*, 2019).

### **Units Operating across India**

Apart from central research laboratories, research centers, and universities, there are some PTC units involved in commercial micro-propagation. Some of these laboratories work on regular basis, while some of them depend on the government. Financial and technological help is been given to them from the government to promote tissue culture industry in the country. Most of them are in Maharashtra and Karnataka states. It is interesting to note that being a country of variety of

climatic differences; India possesses these units across its land. For example, Rajasthan and Darjeeling have their units fully operative, despite extreme climate limitations in both the regions (Abebaw *et al.*, 2021). They also vary in their management and production. Maximum yield is obtained by Kalindi Biotech, located in Rishikesh, Uttarakhand (20 million plants per year). The lowest amount of plants is produced in Cost ford Promoted Unit, Thiruvananthapuram, Kerala and Rallis India Ltd, Bengaluru, Karnataka, with only 0.1 million plants per year. Most of them lie in the range between 5 and 10 million plants per year. Considering the production and number of units present, Maharashtra tops the list of annual production with 31 million plants per year with 25 units. Karnataka stands second in line with 31 million plants with 9 units. Odisha and Rajasthan are reported to produce the lowest amount of plants 1 million per year with 1 units each (Patil *et al.*, 2021).

### **Objectives of PTC**

The commercial production of fruit plants needs rapid propagation of superior varieties with increased yield. Although the rate of propagation varies in different species based on their genetic composition and environmental conditions, herbaceous species are preferred over woody perennials for the enhanced production (Abebaw *et al.*, 2021). Furthermore, herbaceous plants used for germplasm storage and can help in the maintenance of stock under controlled conditions (Mavituna, 1988). Meanwhile, it becomes essential to eliminate plant propagative material to recover good yield at the end. Thus, protective measures like virus indexing can be performed with the explant before the culturing. It makes the progeny virus-free; hence, a better yield can be obtained (Reinert and Bajaj, 2013). In course of plant production, one must adhere to the maintenance of desirable genes in the produced plants. Regeneration from cell and callus suspension is more difficult than micro-propagation. More research needs to be done in this aspect to develop genetically similar plants without the occurrence of any undesirable gene (Jain, 2012). With the advancements in plant biotechnology, it is possible to induce genes within the plant genome (Limera *et al.*, 2017). This can be exploited to induce desirable and heritable genes in regenerated plants. Along with this, techniques such as protoplast fusion and generation of plants from fused protoplasts also prove to be efficient for exchanging genetic material (Bhojwani *et al.*, 2018). In PTC, embryos could use a hand in micro-propagation, in case of failed cultures. Therefore, embryo rescue needs to be practiced with suitable modifications to endeavor a good yield of healthy plants (Patil *et al.*, 2021).

## **Role of Government in Plant Tissue Culture Industry**

Micro-propagation industry was identified as a priority area by the Government of India. Over the last 15 years the Ministry of Science and Technology has supported 150 projects for R&D and field demonstration in nearly 80 different universities/Research Institutes. The strong R&D support from the Ministry of Science and Technology and favorable policies of the Ministries of Commerce, Industries and Agriculture, Government of India, encouraged entrepreneurs and technocrats to set up more than 50 commercial laboratories between 1987 and 1995, with a total installed capacity of 210 million plants per annum (Purohit *et al.*, 2011). It was seen that from 1986-1989 the targets achieved were 50% of the installed capacity. In 1991, there was a decline and only 20% of target was achieved. In 1996, there was a drastic reduction in the number of plants with regard to an increase in the number of units. The history of commercialization in India is a story of 'Rise and Fall and Rise Again'. The percentage increase in production decreased by 50 percent from 1991 to 1994 and in 1998 there was a negative percentage showing rapid decline. However, between 1999 till date there has been an average of 35% rise in tissue culture production per year. This trend resulted into better capacity utilization of the existing facilities by 2002 and additional facilities are now being set up to increase the total installed capacity in the country to 300 million plants per annum (Prakash, 2004). To facilitate the commercialization further, the Government of India has set up a National Facility for Virus Diagnosis and Quality Control of Tissue Culture plants, at New Delhi with 5 satellite centers catering the needs of the tissue culture industries in various parts of the country. A concerted effort is being made by the Government and the industries to ensure that Plant Tissue Culture, a technology with enormous commercial potential, would be an important industrial activity during the 21<sup>st</sup> century (Dogra, 2023).

### **Indian Scenario vs Rest of the World**

The Indian scenario of tissue culture industry clearly indicates that it is a flourishing industry. The analysis of the product range indicates that it is mainly concentrating on fruit crop, and horticultural crops (Suman, 2017). Fruit plants like (bananas, strawberries, pomegranate, pear, mulberry and pepino etc.) are being produced. The global scenario of tissue culture industry has been well reviewed by Bijalwan (2021). Among the Asian countries that are active in commercial tissue culture fruit plants production, it is in India that about 125 commercial units are

functioning, while Indonesia and Japan have 33 units each, Korea has 20 and Thailand has 18 units (Bhat, 2022). Other global countries involved in tissue culture plants production include European countries, USA, Canada, Australia, New Zealand, Israel, Middle East, South and Central America and Africa. All these countries together produced about 900 million plants (Pierik, 1991).

### **Commercialization: A scenario of India and Contemporary World**

The modern-day PTC involved with the micro-propagation, it is limited to lab scale production, which greatly differs from commercial or large-scale production. Thus, it becomes important to establish PTC industries well-equipped with all the facilities that permit extensive production of plants to produce plant products. Due to the above factors, commercial application of PTC was first established in US with micro-propagation of orchids in 1970s. Since then, the industry has witnessed tremendous support and global expansion. The number of production units as well as number of plants has been increased from 1985 to the present (Govil and Gupta, 1997). The current scenario depicts the presence of enough scope for the expansion of commercial PTC as the demand far exceeds production, with an estimated 15 billion USD market per annum. The global biotechnology business is estimated to be approximately 150 billion USD, of which 50–60% is attributed to agri-business, which raises annual demand of tissue culture products by 10%. During 1990, production increased from 130 to 500 million plants, where five major commercial units produce 15–20 million plants per annum. Although it appears like PTC industry is nearly halted in developed countries due to increased production lower wage scales. This leads to the production of plants at cheaper costs. The Western Europe has a capacity to produce 212 million plants, with 37 units producing more than 1 million plants per annum. Netherlands produces about 62 million reengineered plants every year with 67 commercial units. In Germany, the production raises up to 8 million with 21 functional units (Bhatia *et al.*, 2015).

### **The various PTC Technologies used to propagation of fruit crop**

In the past 20 years, tissue culture has become a useful research tool for fruit culture. The initial impact of tissue culture on the nursery industry (Morel, 1964) clonally propagated orchids from shoot tips. Subsequent studies be traced back to this research, and great progress has been achieved with other horticultural species. Research on fruit trees developed later, but so much work has been accomplished that, within a few years, it has

been possible to move from laboratory studies to commercial nursery production. The scientists and nursery workers have played a role in this technology and their contribution be stressed in this review. There are at present three techniques of *in vitro* propagation adventitious meristem formation, somatic embryogenesis, and axillary shoot multiplication.

### **Adventitious Meristem formation (Organogenesis)**

The initiation of meristematic activity for shoot and/or root production has been described and the steps are well known. These meristems probably originate from a small group of cells that either superficial or situated inside the callus. The induction of morphogenesis is often controlled by the cytokinin: auxin ratio of the basal medium (Skoog and Miller, 1957). To obtain shoot formation, a high cytokinin concentration is required, while a high auxin level promotes the induction of root primordia. Generally, when using this technique for propagation, shoot formation is induced first and then the shoots (micro-cuttings) are detached and rooted in a different medium. This method for example, is used for kiwifruit, grapes, *Prunus* spp., and *Malus* spp (Young *et al.*, 1984). Although, growth regulators are responsible for the induction of organogenesis, this process is also under the control of physical, nutritional and physiological origin of callus factors (Vasil and Vasil, 1980). It is possible to propagate an enormous number of plants from a single meristem. Application is limited by difficulties in the control of adventitious meristem formation in several woody species or specific clones within the species, and because of the risk of obtaining aneuploid, polyploid, or aberrant plants.

### **Somatic embryogenesis**

Somatic embryogenesis is a natural phenomenon in several fruit tree species (*e.g.*, Citrus, Mango, *Malus* and *Juglans*) and occurs as an alternative (apomixis) to or together with sexual reproduction (polyembryony). It is often difficult to differentiate zygotic and somatic embryos (Reinert, 1958; Steward and Mapes, 1958) were the first to obtain somatic embryos *in vitro* from culture of secondary phloem. This finding has been observed subsequently in many angiosperms. Somatic embryos also have been produced from tissues of mature and immature embryos as well as petioles, leaves, roots, flower axils, anthers, mesophyll cells and protoplasts (Vasil and Vasil, 1980). These embryos originate by several mitotic divisions, generally starting from a single cell on the callus surface or from the periphery of cell clumps in liquid culture. Growth regulators are involved in somatic embryogenesis but their action is still unclear. It seems that auxins (2,4-D in particular) are required to induce

embryogenesis, but this substance is not necessary and may even have an inhibitory effect on embryo development. Embryo induction usually requires an auxin-rich medium and embryo development requires an auxin-poor medium. *In vitro*-induced embryogenesis has a great potential for the species that respond to this technique, because it is possible to obtain clonal production of plants rapidly. This clonal production has been achieved for an increasing number of species. *In vitro* somatic embryogenesis has been reported in many fruit crop species including citrus, apple, strawberry and papaya (Litz, 1985).

### **Axillary shoot multiplication**

This technique is based on the *in vitro* formation of new vegetative axes and buds produced in the axils of young leaves or leaf primordia, starting from a single bud or already differentiated meristem. At the beginning of the culture, this method is less efficient than organogenesis or embryogenesis but, after frequent subcultures, the rate of multiplication may increase greatly. This increase in the proliferation rate seems to depend on the process of rejuvenation that occurs during culture. Also, positive results obtained with the use of 1,3,5-trihydroxybenzene phloroglucinol (PG) in the nutrient medium seem to be related to the rejuvenation process; in fact, the positive effect of PG is greater in cultures that are initiated from older stock plants. The rejuvenation process also has a positive influence on the rooting phase, increasing the percentage of rooted shoots. Most information on this process relates to apple trees (Loreti and Morini, 1982). It has not been possible in commercial nursery production to achieve these results, but, considering the species, the number of plants produced.

The first phase involves disinfections to establish aseptic cultures; elimination of viruses, fungi, and bacteria requires special techniques (Wong, 1981). Greenhouse-grown stock plants are preferred because almost 100% of field-grown material is infected. Next, the sterile bud is re-cultured on a new medium that contains growth regulators, including a cytokinin to break apical dominance. After a period of time varying from 2 to 8 weeks (depending on the species), miniature shoots form and axillary buds break and immediately develop shoots. The number of new axes that are formed from one explant increases until the third or fourth subculture and then tends to stabilize. This process, in theory, could be repeated indefinitely, but, for reasons still unknown, the material sometimes appears to age and become less responsive. The problem of genetic stability and of production of true-to type plants must be kept in mind; it is better to limit the

number of subcultures to avoid this risk. The control of morphogenesis during the multiplication phase to achieve early bud break is moderated by cytokinins. Typically, A-phenylmethyl-1-purin-6-amine (BA). The next step involves induction of root primordia rooting phase. The single miniature shoots (1-3 cm) of three to 10 internodes are rooted in a medium different from the proliferation medium. Growth regulators involved in this process are auxins. For improved control, a transition phase lasting about 2 weeks is necessary, during which time the shoots elongate to allow easier handling. The material also is conditioned during this time for example; interruption of photoperiod for a few days before rooting seems to have a positive effect (Jacoboni and Standardi, 1982). Conditioning reduces the negative effect of cytokinins on root formation and hardens the plantlets, a process important after transplanting. Auxins applied within the agar medium in which the shoots are planted, or these handled as micro-cuttings by dipping the shoot ends for a short time in a concentrated solution and then planting them in a nutritional medium without growth regulators, or even directly *in vivo*. The direct planting, which significantly lowers production costs, has given positive results with several species (Simmonds, 1983). Transplanting and acclimation is a critical phase in the process. Recent research conducted on several species has pointed out that leaves of tissue cultured plants often show some structural changes compared to plants grown *in vivo*. Changes are involved in mesophyll tissues (Brainerd *et al.*, 1981), the amount of epicuticular waxes and the stomata. The efficiency of stomata seems to be altered to reduce water loss resistance of the plantlets during acclimation. The transfer to *in vivo* conditions usually follows a very simple procedure. At the proper time, the plantlets are treated with fungicides and transferred from the jar or test tube to containers filled with previously sterilized peat and sand (or other materials) and then placed under controlled conditions. It is important to maintain high relative humidity and low light intensity. Callusing of the shoot end during the rooting phase must be avoided.

### **Women in plant tissue culture research and industry**

The Government of India declared 2001 as the year for Women's empowerment. Recent trends have demonstrated the impressive contributions of womenfolk in various fields. Remarkable contributions have been made by women scientists in plant tissue culture area. Important breakthrough results in the field of plant tissue culture such as perfection of ovule culture (Behara, 2013). The eminent women scientists working in the research field of plant tissue culture would consist of a long list.

Most significant fact is that, this industry employs women staff to the extent of about 85% of the total staff required for this industry (Lee, 1999). The kind of working environment available to the staff is more suitable to the womenfolk. Besides, the work demands lot of involvement, patience, dedication, and commitment, which complement each other. The industry is poised to synchronize with the interest of women empowerment that has been greatly contributing to the success of this industry in India.

### **Conclusion**

The plant tissue culture technology has been very successful as an industry and has greatly contributed to successful agriculture. The technology has created several employment opportunities and opened up many entrepreneurial fields. Usage of tissue culture-generated plants has increased productivity per unit area, particularly in horticultural crops. This industry has made available different unique commercial plant species such as ornamentals and foliages in large scale, which were not produced earlier by the conventional methods. Tissue culture has been one of the main technological tools and reasons that have contributed to the 'Second Green Revolution and Gene Revolution'. India is being looked upon by the world as the main technology base for production and supply of economically important plant varieties. With more innovative work and intensive exploitation of our flora, the tissue culture technique will help us in consolidating our leadership at the global level.

### **Acknowledgements**

All contributors, who do not meet the criteria for authorship should be listed in acknowledgements section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provide only general support. Authors must acknowledge the organizations that have provided financial support for their work in the manuscript with grant award number here.

### **Authors contribution**

Conceptualization, SP; investigation and resources, RD, SP; original draft preparation RN; supervision. All authors have read and agreed to publish version of the manuscript.

Compliance with ethical standards.

**Conflict of interest :** Authors do not have conflict of interests to declare.

**Ethical issues :** None.

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