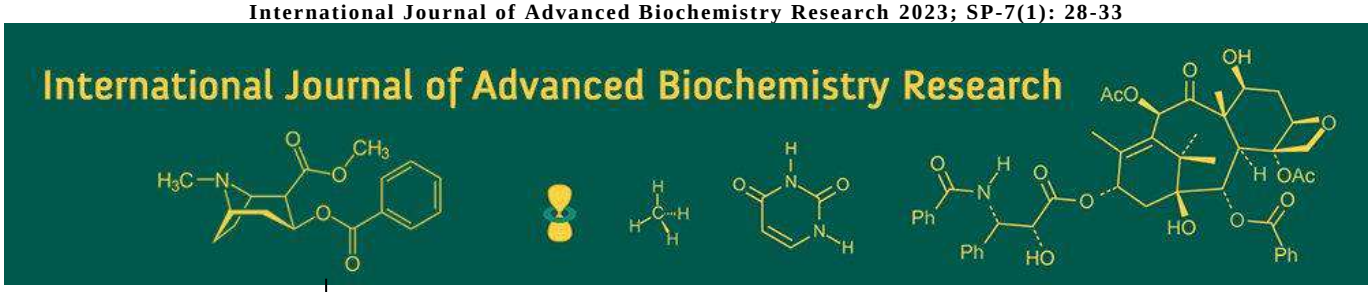

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Plant tissue culture industry in India: Trends and scope

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Abstract
Biotechnology has been globally accepted as one of the important tools for direct application in agriculture. Agricultural biotechnology includes plant tissue culture (PTC), applied microbiology, and applied molecular biology contributing to the production of crops with improved food, feed, fiber and fuel. The technique of PTC is well translated from 'concept' to 'commercialization'. It is flourishing with multidirectional growth and multimillion dollar turn over. Several crop plants are routinely propagated 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.

Keywords: Plant tissue culture techniques, micropropagation, ornamental plants

Introduction
Plant tissue culture technology defined as the culture of all types of plant cells, tissues and organs of the desired plant in a suitable nutrient medium under controlled aseptic conditions. It is based on the theory of totipotency i.e. the ability of a single cell to develop into whole plant. It enables propagation of true-to-type plantlets *en masse* in a relatively short time and does not involve transfer of genes within and across species. This technology considers an integral plant of the plant biotechnology and is an alternative to conventional methods of propagation, has made invaluable contribution to agriculture by enabling the production of disease free, high quality planting material of commercial plants and fruit trees, throughout the year irrespective of the season and weather. The importance of plant tissue culture is in the manipulation of aromatic and medicinal plants for improved agronomic performance and also the ways in which it is beneficial to mankind. Generally, the multiplication of crop through sexual propagation like seed or asexual or vegetative propagation like stems, roots or modified underground structures depend upon their nature of reproduction system, suffered certain level of disadvantages. Plants raised from seeds may not repeat good performance of mother plants and many horticultural plants take a long time to produce seeds/fruits or many of them do not produce viable seeds or desired quality of seeds. However, vegetative propagation suffers with slow, time and space consuming process and usually infected with latent diseases. The technique brings big relief for conventional plant breeding methods of many species, particularly horticultural plants. Traditionally, the production of horticultural plants by farmers and commercial breeders for their own use or for the domestic market has been carried out using conventional breeding practices such as budding, grafting or layering which are time consuming and require a large number of parent propagule materials, necessitating a big production space in the nursery. Therefore, scientists looking alternative effective approach for plant propagation that could helpful to overcome the disadvantages encountered with above described methods. The advent of PTC is giving the answerable solutions against the limitations of conventional plant breeding practices as it full exploited potential enables *in vitro* production of plants on a large scale throughout round year and the transportation of developed microplants is easily way to far-flung places.

The idea of cell and tissue culture were come from German Scientist Haberlandt in 1902. After many trials and errors in the sixties, plant micro propagation by tissue culture method was found commercially successful in the case of orchids. Micropropagation 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 [1]. It is one of the key tool of plant biotechnology that has been extensively exploited to meet the growing demands for diseases free elite planting material in the current century. The remarkable features of plantlets regenerated through microprogation like uniformity in productivity, diseases free clone, vigorous growth and high yield make it widely acceptance in the farmer community of our country. The plantlets of many valuable crops like banana, sugarcane, papaya etc are raising commercially through plant tissue culture that lead to become one of the best profitable industry enterprise in our country. The globally threat of explosive population growth and reckless exploitation of natural plants for self interest badly affect to the existing biodiversity in the world. Many universities and top leading institutes like BARC, Bombay; NCL, Pune; ISC Bangalore etc has been engaged to conserve the level of biodiversity and their research programme study through plant tissue culture technique.

The major components of the technology include choice of explant, growing of explant on a defined medium in glass vessel (*in vitro*), elimination and prevention of diseases, providing appropriate cultural environment and transfer of plantlets from glass vessel to natural environment (hardening). All these constitute protocol for tissue culture that varies from species to species and variety to variety within the same species. Some of the simpler techniques that are more approachable and applied directly in plant propagation and genetic improvement of plants are (i) micropropagation, (ii) meristem culture, (iii) somatic embryogenesis, (iv) somaclonal variation, (v) embryo culture, (vi) *in vitro* selection, (vii) anther culture, and (viii) protoplast culture.

Advantages of Plant Tissue culture technology

The commercial advantages of tissue culture technology over its conventional counterpart are summarized below:

a) Tissue culture could be a useful way for circumventing or eliminating disease, which can accrue in stock plants.
b) Tissue Culture Plants (TCPs) may have increased branching and flowering, greater vigour and higher yield, mainly due to the possibility of elimination of diseases.
c) The method may succeed to propagate plants where seeds or vegetative propagation is not possible or difficult or undesirable. As the capital investment on mother plants is reduced to almost zero, it may be easier to adapt to changing conditions. Additionally, a better programming of the production is possible, because of the greater plant uniformity and the availability in the mass at any time.
d) Enables storage and maintenance of stock plants/germplasm.

Present scenario of Plant Tissue Culture Industry in India

The demand for disease free clones of superior quality plants in ornamental, horticultural, floricultural and agro-forestry sectors, which form the core sectors of agriculture needs always exist. The first Commercial tissue culture born in India in 1987 when NV. Thomas & Go. ltd in kerala established their commercial unit for large scale production of cardarnurn. As a result, several Indian tissue culture industries have expanded rapidly, both in terms of the number of units as well as production capacity. The Biotech Consortium India Limited estimates that there are about 125 commercial plant tissue culture units with a minimum production capacity of about 300 million plants per year from each of the units [2]. Among these, at least 20 of the units have larger production capacities, with 5 to 10 million plants/year. In addition, there are more than a dozen smaller units with 0.2 to 0.5 million plant production capacities where single crops are being produced. Most of these tissue culture units are located in Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu and Kerala. Major companies in the field of plant tissue culture industry such as Hindustan Lever, Tata Tea, Unicorn Biotech, Nath seeds, RPG Enterprises, Indian tobacco, Indo-American Hybrid Seed Company and Hindustan Agri Genetics limited etc. which has doing multi-million dollar business from the past 30 years. Initially PTC units established in India export exotic and ornamental plantlets to the Europe market. Presently, only limited number of PTC units are generated the exporting plantlets material while majority are generating various fruit, ornamental and plantation crops for the domestic local market consumption. Propagation by tissue culture offers good commercial prospect in ornamental plants, vegetables and also fruit plants, where value of the products is high. The Ministry of Science and Technology, Govt of India has already approved more than 150 projects related to plant tissue culture in 80 different universities/Research Institutes. for the R&D and field demonstration purpose. The encouraging and supportive various policies by the Ministry of Science and Technology and Ministries of Commerce, Industries and Agriculture, Government of India, encouraged budding entrepreneurs and industrialists had been setup more than 50 commercial laboratories from 1987 to 1995 year with total capacity of 210 million plants genearted per annum. The farmers are attracted immensely towards the production of quality planting material by micro propagation of various cash crops flowering shrub and trees. 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 centres catering the needs of the tissue culture industries in various parts of the country. In India, a number of plant species ranging from fruit, cash crops to spices and medicinal plants are being produced using PTC technology (Table 1).

Table 1: Large-Scale Micropropagation Plants by Tissue Culture Technology

Plant Category	Plants
Fruits	Banana, grapes, cashew, pineapple, strawberry, sapota, watermelon, apple and citrus.
Cash crops	Sugarcane, potato and tapioca.
Spices	Turmeric, ginger, vanilla, large cardamom, small cardamom, vanilla and clove.
Medicinal plants	*Aloe vera*, geranium, stevia, patchouli, rosemary, gloriosa, tulsi
Ornamentals	Gerbera, carnation, anthurium, lily, syngonium, cymbidium, limonium, dracena, philodendron, rose-miniature, caladium, gentiana and cactus.
Trees	Teak, white teak, bamboo, eucalyptus, populus, pine and red sanders.
Bio fuel	Jatropha, Pongamia

Outsourcing of the Plant Tissue Culture Industry in India

The Applications of plant tissue culture (PTC) technology, one of the most commercially successful biotechnologies, in horticultural plant propagation has offered new opportunities to many PTC companies in Europe and US to outsource production activities to developing countries such as India, where there is an abundant source of cheap labor available [3]. Outsourcing is an attractive option because PTC is a labor-intensive technology, and labor inputs form a significant proportion of the total production cost. Given the quality of available technical support, inexpensive labor and low transportation costs, global PTC companies started outsourcing production orders to Indian PTC units. The present status of existed tissue cultured plants and products hold global market estimation of US$ 15 billion. According to one estimate, there are more than 248 commercial micropropagation companies in Western Europe and 250 laboratories in the United States of America [4], with an increasing number in Asia and Eastern Europe. The easily available of lower cost manpower resource offering the opportunities to countries like Israel, the USA and UK for setup PTC industry in India.

Evolution of New Varieties by Plant Tissue Culture

The use of plant tissue culture platform to generate somaclonal variation is one means of generating variation that may be needed in breeding program. Deliberate attempts to induce variations in tissue culture have been in progress for the last 60 years and a large number of variants in ornamentals and horticultural crops have been reported [5]. These include sugarcane - increase in cane and sugar yield, and resistance to eye-spot disease [6]; potato - improvement of tuber shape, colour and uniformity, and late blight resistance [7]; tomato - increased solids, resistance to *Fusarium* race 2 [8]. In the ornamental sector, *Syngonium* provides an excellent example of somaclonal variant where 22 new cultivars, all somaclonal variants, were selected from large populations of tissue-cultured material grown in commercial greenhouses. Among the other agricultural crops, CIEN BTA-03, a variant of Williams variety of Banana resistant to yellow Sigatoka disease; AT626 & BT 627 of sugarcane variant resistant to sugarcane mosaic virus A and B are released for commercial usage. In addition, 'Ono', a sugarcane variant from variety, Pindar resistant to Fiji disease; ATCC 40463, a tobacco variety with enhanced flavour; DK 671, corn variety with higher yield, with lasting green colour and higher seedling vigour are the varieties from overseas inventions. Bio-13 a variety of Citronella released by CIMAP, India; Bio- 902, Bio-YSR variants of *Brassica* parent 'Varuna' with enhanced seed yields are from India that are being multiplied and are cultivated successfully on commercial scale.

Integration of Technology from Lab to Land

Tissue culture technology contributes significantly to the improvement of agricultural productivity and food security. However, it must be integrated into production systems in a cost effective manner. Wherever practical, direct use of the tissue-cultured plants is the best strategy to obtain the full benefits of this technology. The price of the products, i.e. plants, tubers, bulbs and cuttings, must be competitive with those obtained from conventional propagation. While many plants that are propagated from vegetative parts can be micropropagated, the cost of tissue-cultured plants is still too high for direct growing by the farmers. For example, micro- and mini-potato tubers and micropropagated plants of chrysanthemum, strawberry, sugarcane and cassava are too expensive for direct field planting. In such cases, tissue-cultured plants are grown as super-elite or elite material and the costs are brought down by conventional propagation one or more times. Thus, the costly tissue-cultured plants are used as nuclear material, and their further multiplication is by conventional vegetative propagation in the field to produce large quantities of pathogen-free planting material. In addition, the handling of bulky conventional planting material, such as potato tubers, banana corms and sugarcane sets adds to the transportation costs. Hence, the cost of tissue-cultured plants has to be brought down substantially, so that farmers benefit through increased productivity.

The production of disease-free planting material from plant tissue culture that gives improved yield and quality in case of potato, banana, citrus, date palm, pineapple, papaya and many ornamentals. Seed potato production is a highly specialized business, and requires highly skilled growers. The mini-seed tubers are not used for growing the potato crop. Instead, these are further multiplied by conventional propagation in the field, at least twice, and sometimes even 3 to 4 times for the production of certified seed tubers. Similarly, the sugarcane growers in Punjab, India have switched to high quality seed cane that is derived from tissue cultured plants, which are propagated 3 to 4 times from conventional cuttings. The lead in commercial sugarcane tissue culture technology has been taken by the sugar cane mills, which have established their own laboratories for large-scale seed cane production. The state agricultural university provides the disease-free indexed cultures to the sugar mills. Such a model that includes the university for research and trouble-shooting problems, private industry (sugar mills) that multiplies the sugarcane seed material through tissue culture, and cane growers who produce the crop, has been very successful in integrating tissue culture technology for the benefit of the farmers and processors. This tripartite cooperation has rapidly replaced conventional sugarcane seed production in the state. Recently, direct planting of micropropagated sugarcane plants has been profitably undertaken in Maharashtra, India.

Government Schemes and Incentives in plant tissue culture industry

The Government of India has identified micropropagation industry as a priority area for further research, development and commercialization. The statistical survey reported by Prakash [9] reveals very interesting data on this aspect. The formation of the Department of Biotechnology and subsequent hi-tech industry status given to PTC production has encouraged several corporate houses to establish production centers [10]. In 1991, the Government of India identified micropropagation of plants as an industrial activity under the Industries (Development & Regulation) Act of 1951, and several incentives are now offered [11]. Over the last 20 years, the Ministry of Science and Technology has supported 150 projects for research and development in this field. A concerted effort in terms of favorable policies from the Ministries of Science and Technology, Commerce, Industries and Agriculture, Government of India have encouraged entrepreneurs and technocrats to set up more commercial units of plant tissue culture industry with enormous commercial potential, would be an important industrial activity during the 21st century. To encourage the tissue culture industry, various central and state government departments have framed several schemes which are as given below:

(a) Ministry of Agriculture: The Department of Agriculture and Cooperation under the Ministry of Agriculture, Government of India has the following programmes and schemes for promotion of horticulture.

1. There is a provision for assistance of upto Rs. 21 lakhs and Rs. 10 lakh for setting up tissue culture units in public and private sector respectively subject to a maximum of 20% of the project cost.
2. Under the Integrated Development of Fruits scheme assistance is given for purchase of planting material under the area expansion programme for the following crops: -

a) Rs. 7,000/hectare for plants of Guava, Amla, Date Palm, Plum Peach, Bes, Fig and citrus.
b) Rs.10,000/hectare for plants of mango, almond, pomegrante, apple, nuts, apricot, olive, papaya, litchi and sapota.
c) Rs. 30,000/hectare for plants for Bananas and pineapples.
d) Rs. 70,000/hectare for plants of grapes and strawberry.

In addition, 50% subsidy is given to the farmers for purchase of tissue culture banana by the Andhra Pradesh State Agriculture Department under the Macro Management Scheme.

(b) Agriculture and Processed Food Products Export Development Authority (APEDA): Under the Ministry of Commerce and Industry, state-of-the-art airfreight trans-shipment centre has been set up for tissue culture plants (perishables) at New Delhi, Bombay and Bangalore airports. Airfreight subsidy up to 25% of the freight cost is provided to tissue culture plants. 50% subsidy is given for the development of infrastructure like refrigerated van, packing, export promotion, market development, consultancy services, feasibility studies, organization building and human resource development. Financial assistance is also given for strengthening quality control facilities implementation of ISO 9000.

(c) National Horticulture Board (NHB): For setting up tissue culture lab there is a provision for back-ended capital subsidy not exceeding 20% of the project cost with a maximum of Rs. 25 lakh per project. Such subsidies are also extended to build up greenhouse and climate controlled poly house/shade house. The units planning expansion in the domestic market by having a network of nurseries or additional hardening facilities can avail this scheme. The provision also exits for high quality commercial horticulture crops, Indigenous crops/produce, herbs, aromatic & medicinal plants, seed & nursery, bio-pesticide and establishment of Horticulture Health Clinics/Laboratory. In all these cases, the subsidy is routed through the involvement of a financial institution on the completion of the project.

(d) Small Farmers Agri-business Consortium (SFAC): SFAC under the Ministry of Agriculture gives soft loans up to 50 lakhs for setting up small tissue culture labs by co-operative societies formed by small scale farmers.

(e) Department of Biotechnology (DBT): DBT supports research and development projects across the country at various laboratories in the universities and research institutions for development and standardization of tissue culture protocols. The private tissue culture units are entitled for expansion of existing units as a Phase II activity under a scheme called Small Business Innovation Research Initiative (SBIRI). To promote the adoption of tissue culture technology by the industry and the end user, the department has established two micropropagation technology parks (MTPs) which provide a large number of service packages and have an important mandate of training and generating skilled manpower. The MTPs have transferred about 10 technologies to the industry and have also provided consultancy and taken up turn-key projects for various end users and state departments. The department has also set up a national facility for virus diagnosis and quality control of tissue culture raised plants, which are located at 6 different centers in India to ensure supply of disease free plants to the end users.

(f) State level incentives: The states of Karnataka, Gujarat, Maharashtra, and Andhra Pradesh are giving financial assistance for setting up tissue culture units under the new agro-industrial policy. Karnataka gives capital subsidy of 20% on investments whereas the subsidy is 6% in Gujarat. Maharashtra gives a subsidy on power consumption. In addition, state government provides subsidy to the farmers for purchase of tissue culture plants under various schemes.

(g) Financial Assistance by Banks: Apart from the fiscal incentives given by the central and state governments, the financial institutions have also been financing tissue culture projects as a priority sector. Some nationalized banks like Canara Bank has opened a special cell for financing high tech agriculture projects. National Bank for Agriculture and Rural Development (NABARD) under its refinancing scheme has supported some 30 projects.

National Certification System for Tissue Culture Raised Plants (NCS-TCP)

Propagation of disease free and high quality planting material of ornamentals, horticultural, agroforestry and various economical plant species by Tissue culture technology through micro propagation has revolutionized the agriculture scenario worldwide and witnessed significant growth in the market. Lack of effective quality parameters in micro-propagation may result into spread of virus infected and/or somaclonal variants which may hamper its growth resulting in lower levels of acceptance of this promising technology. Therefore, it needs a quality management system which is crucial for the healthy growth of the micro propagation industry. Accordingly, NCS-TCP has been established by the Department of Biotechnology (DBT), Government of India which is first of its kind in the world. It is a dynamic and comprehensive system intended for facilitating production of quality tissue culture plants and providing mechanisms for certification of quality tissue culture plants. NCS-TCP has received a very enthusiastic response from the industry. More than 90% of the leading tissue culture companies have got themselves recognized under the system and many other small units are in the process of assessment. Significant impact of NCS-TCP includes strengthening the capacities of tissue culture companies, enhancing their visibility resulting into market reach, increase in the production capacity of recognized companies, introduction of new crops and increase in the number of samples referred to testing. It may be concluded that this system has provided conducive environment for the growth of plant tissue culture industry in India and has potential to be replicated worldwide.

Demand VS Supply

The demand for micropropagated plants in agriculture, horticulture and in social forestry is growing by the day, since the traditional methods of propagation do not yield sufficient quantity and in some crops they are cumbersome. The emerging scenario on the growing use of tissue culture plantlets predicts that each state in our country should, at least, have ten tissue culture laboratories. The major consumers of tissue culture plants are the State Agriculture and Horticulture Departments, Agri Export Zones (AEZs), sugar and paper industries, private farmers and floriculturists. State-wise, the requirement of the crop type is different for the domestic consumption. It is important to note that the demand for some crops like banana, grapes, pineapple, strawberry, sugarcane, potato, turmeric, ginger, cardamom, vanilla and ornamentals like anthuriums, orchids, chrysanthemums, rose, lily, and gerberas are on the rise in different states in the country. Small quantities of medicinal plants like Aloe, Coleus, Chlorophytum, Digitalis, Melaleuca, Patchouli, Gloriosa and forestry crops like Bamboo, Teak, Eucalyptus, Sandal, Mangium are also produced and consumed in the domestic market [12]. A market survey made on tissue cultured plants by the Biotech Consortium India Limited [12] for the Department of Biotechnology and Small Farmers Agri-business Consortium reveals that a total of about 45 million plant species mentioned above was for the domestic consumption values Rs. 38.5 crores. The survey had also projected that for the year 2007-08 the overall market demand for tissue culture plants would be 145 million plants of the above species valuing Rs. 136 crores, with a growth rate of 20-25%. The consumption of plants for 2002-03 has been approximately 45 million plants with banana constituting 41% share followed by sugarcane at 31% due to the introduction of ethanol blended petrol and ornamentals at 14%, spices at 6% and medicinal plants at 4%.

It can be noted that there is growing awareness of superiority of tissue cultured plants, and demand for crops like banana, grapes, papaya, ginger, turmeric, cardamom, vanilla, potato, Jatropha is increasing. The impact of tissue culture technology in bridging the gap between the demand and supply could be exemplified by quality planting material of banana and Jatropha. Banana is being cultivated in India in an area of about 500,000 hectares with an average productivity of about 15 kg of yield per plant. However, by replacing the conventional methods of use of suckers with tissue cultured plantlets, the productivity can be enhanced to about 50 kg/plant from the same area. At present, India is the largest producer of banana in the world with about 30% of total global production. With increased productivity/unit area, the export capabilities can certainly be improved. This is possible by adopting the cultivation with virus indexed, tissue cultured plants instead of using the conventional suckers. Similar is the case with *Jatropha curcas*. The demand within the country for quality Jatropha plants is about 5 billion (50,000 lakhs). This huge quantum of quality planting material supply is possible either through the adoption of tissue culture technique or by providing hybrid seeds.

When it comes to the international demand, the foliages and ornamentals have a great potential and the products have an unending elongated list. Major pot plants and landscaping ornamentals like Ficus, Spathiphyllums, Syngoniums, Philodendrons, Nerium, Alpenia, Yucca, Cordylines, Pulcherrima, Sansevieria, Gerbera, Anthuriums, Rose, Statis, Lilies, Alstromeria etc. are routinely produced by various plant tissue culture laboratories in India. About 212.5 million plants including 157 million ornamental plants amounting to 78% of the total production are reported [13, 14]. It may be pointed out that tissue culture laboratory can also be used to produce biofertilisers like rhizobium, azotobacter, azospirillum, phosphate solubilising bacteria culture as well as mushroom spawn culture that indirectly contribute to the agricultural sector.

Future Prospect of Plant Tissue Culture Industry

Biotechnology is increasingly acknowledged as a powerful and pervasive force that can significantly alter agricultural production and labor absorption. Plant tissue culture techniques have a vast potential to produce plants of superior quality, but this potential has not been fully exploited in the developing countries. Plant tissue culture has becoming an integral part of plant breeding and involvement of the rapid introduction of improved plants. Bringing new improved varieties to market can take several years if the multiplication rate is slow. *In vitro* propagation can considerably speed up this process. For example, the development of pest- and disease-resistant plants through biotechnology depends on a tissue culture based genetic transformation. The improved resistance to diseases and pests enables growers to reduce or eliminate the application of chemicals. The potential of plant tissue culture in increasing agricultural production and generating rural employment is well recognized by both investors and policy makers in developing countries. The current progress of the

industry is encouraging but the expected rapid growth has not taken place due to less awareness amongst the people about application of this technology. Although within the country the research groups have put lots of effort in standardizing protocols for several plant species, the benefits have not been sufficiently demonstrated to the farmers at the field level. Therefore, the agricultural extension workers of concerned Universities and experts should work together to disseminate the information of plant tissue technology to the farmers for handling and growing tissue cultured plants for further multiplication under high health status. These trends have augmented the firm footage of tissue culture industry as an established input into agriculture and have further opened up avenues for future growth.

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.

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