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A Review on Implementation of Micropropagation Technology at Farmer's Field

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ABSTRACT : A whole plant can be grown from cells, tissues, or organs using the tissue culture technique in a controlled environment. This technology is based on the 'totipotency' of plant cells, or a cell's inherent capacity to regenerate an entire plant given the right media and growing conditions. Plant tissue culture technology, which was first developed almost a century ago, has advanced quickly in the last three decades. Today, horticulture, forestry, and medicinal plantations all make extensive use of it. Tissue culture technology was restricted to laboratories for a while.

KEYWORDS: micropropagation, technology, farmer, implementation, agriculture, totipotency, horticulture

I. INTRODUCTION

Regeneration through tissue cultures of late, it is spreading to the fields of the farmers thanks to the commercial expansion of horticultural and forestry plants in both the public and private sectors, which are then made available to them. In addition, a variety of basic and applied research fields also use the tissue culture technique. This paper provides an overview of tissue culture's significance and range, with a focus on micropropagation technology, its uses, and its potential for commercial success. It also highlights specific instances from the AP-Netherlands Biotechnology Programme involving the transfer of micropropagation technology from research laboratories to production centers and its evaluation with farmers' participation.[1,2]

II. TYPES OF TISSUE CULTURE

Tissue culture literally translates to "growing a plant from a small piece of plant tissue". But in actual usage, this phrase has a broader connotation. To create a complete plant, it makes use of individual cells, protoplasts (cells without cell walls), tissues, meristems, and entire organs. These are the primary categories:

Cell and protoplast culture: This is the process of regenerating entire plants by growing individual cells or protoplasts in predetermined media. Transgenic plants and cytoplasmic hybrids can be produced using this technique.

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Vol. 2, Issue 11, November 2013

Anther culture: This is the process of growing haploid cells from whole anther or micropores. In experiments involving hybridization, haploids are helpful. To create homozygous diploids, they are also applied. In China, hybrid rice is produced extensively using a culture.

Ovary culture: Healthy fruits can be produced by cultivating ovaries from pollinated flowers in a medium. However, its primary use is in experiments.

Embryo culture: By cultivating them in a lab, wide cross hybrid embryos can be saved (recovered). This is widely used in the family of grains and grasses.

Meristem culture: Viral-free plants are created in the lab by cultivating actively growing shoot epithelium. In horticulture, this is frequently used.

Tissue and organ culture: Organ culture refers to the practise of regenerating plants from whole organs such as seeds, leaves, or nodal segments. It is occasionally possible to regenerate entire plants using components of the organs, such as leaf discs. It is most frequently used in the creation of transgenic plants or in genetic transformation. [3,4]

Micropropagation: Micropropagation refers to the process of producing whole plants in vitro using a portion of the plant, such as a leaf, somatic embryo, or node, and then repeatedly multiplying those plants. It is a type of vegetative propagation carried out in controlled lab/greenhouse environments. The plants produced by micropropagation are typically true to type. It is frequently used in horticulture and forestry to produce millions of plants from chosen mother plants. Micropropagation and commercial tissue culture are nearly identical.

III. APPLICATIONS OF TISSUE CULTURE TECHNOLOGY

The use of tissue culture technology benefits both academic research and industrial plant material propagation. To put it another way, the tissue culture technique can be helpful either directly for varietal development or indirectly for mass propagation. Here are some significant applications in horticulture and agriculture:

- Micropropagation of fruit, forest, and ornamental tree species.
- The propagation of high-value plants and vegetable F₁ hybrids.
- The creation of novel varieties via somaclonal variation.
- Plant production free of viruses using meristem culture.
- Production of wide crossed hybrid through embryo rescue.
- Artificial seeds.
- Germplasm preservation through cryo.
- Plant regeneration following genetic modification (transgenics or GMOs)
- Cell culture-based production of secondary metabolites

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Vol. 2, Issue 11, November 2013

IV. MICROPROPAGATION

Micropropagation is the most frequently employed tissue culture application. Traditionally, cuttings, grafting, and air layering have been used to improve and multiply horticulture and forest plants. However, there are some restrictions on how many propagules can be produced from a single mother plant for each of these techniques. Micropropagation, in contrast, permits the year-round production of planting material from a single identified mother tree, albeit with some restrictions. The most crucial requirement is that skilled labour must be used in controlled environments.

The chief objective of clonal or vegetative propagation is to produce progeny plants which are identical in genotype with their parent plant. This is achieved by the following processes which are employed for micropropagation of different plant species: (i) proliferation of axillary buds, (ii) induction of adventitious buds, bulbs, protocorms etc., and (iii) somatic embryogenesis.

Need for Micropropagation: Vitroplants present a number of characteristics that have led to their use for strictly agronomic purposes, and encouraged their use for international germplasm exchange and the conservation of genetic resources. [5,6]

Micropropagation Technique:

Procedure of the invitro propagation of shoot tip are :

- Culture initiation, which includes harvest of plant material, disinfections, and culture initiation.
- Multiplication stage with several subcultures on multiplication medium, growth stage

All plant parts with a shoot meristem can be used as planting material. Terminal inflorescence apices with young flower buds can also be used (DoreSwamy and Sahijram 1989).

To guarantee quality and trueness to type of the mother plant, it is important to pick explant from selected shoots that have flowered. The material must be carefully checked for different viruses (BBTV, CMV, BBMV and BSV) ELISA detection tests can be used.

Before transfer to the laboratory, explant should be washed and reduced to small size. Double disinfection firstly of large blocks of tissue 3 cm in diameter and subsequently of an 1-2 cm cube is carried out. The most commonly used disinfecting agent is sodium hypochlorite at a concentration of between 0.25 and 0.75%, together with a wetting agent.

The shoot tip is excised in aseptic conditions. The most widely used explant consists of a 2.5 mm cube including meristem. Before culture initiation a preliminary treatment with antioxidants is sometimes undertaken.

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Vol. 2, Issue 11, November 2013

The most widely used medium is that of Murashige and Skoog (1962) with 3% sucrose, enriched with a cytokinin to induce bud proliferation. BA at a concentration of 2.5 mg/l is generally used alone but some authors add auxins or adenine sulfate, which may show a synergetic effect with cytokines and media required to specific specie, needs to be standardized.

Culture generally takes place on a semisolid medium with low light intensity or even in the dark and at a temperature of 26-30°C.

Multiplication Stage:

Multiplication is performed by splitting and sub-culturing newly formed shoot or bud clusters on fresh media under conditions the same as, or very similar to those used in the initiation stage. In particular, the composition of the media is often the same, the most common being a concentration of between 2 and 5 ml/l of 6-benzyl aminopurine. The multiplication rate is a direct function of the concentration. Concentrations of BA above 10 mg do not appear to increase efficiency, and may even disturb shoot development.

As in the initiation phase, the effect of phenotype is very strong, and appears to be genome dependent.

Poor proliferation rates may be improved by wounding or decapitation of the meristem. Some workers prefer to split the shoots and subculture the resulting halves as individuals. Much discussion has taken place to determine whether the newly formed shoots originate adventitiously or from axillary buds. This may depend on the origin of the explant cultured, but the hypothesis of adventitious origin appears to be gaining ground, except in the case of shoots that arise directly from the whole or portions of the terminal meristem.[7,8]

The multiplication phase can take place in a liquid medium, but this may lead to problems of asphyxia. However, these problems can be avoided by using an original procedure of automatic temporary immersion which also has the advantage of increasing both the multiplication rate and the accumulation of dry matter (Alvardet *al* 1993). The aim of the growth stage is to obtain plantlets that will produce roots rapidly and be large enough to survive in the outside environment. On the multiplication medium the growth of roots is strongly inhibited by the presence of cytokinins. For this reason, plantlets need to be subcultured as individuals or in clusters of very few shoots on a medium devoid of growth regulators or containing only auxins, NAA being preferred.

To obtain plants that are as vigorous as possible, the ratio of the volume of the vessel to the number of plantlets must be higher than in previous stages. Studies on *in vitro* respiration and photosynthesis have shown that photosynthesis increased by additional lighting and a supply of CO₂ resulted in better *in vitro* development, which in turn led to an advance in development during hardening.

The vigor of the plantlets can be exploited more pragmatically by carrying out this last phase in tropical conditions similar to those used in hardening or even by hardening plantlets shortly after the end of the proliferation phase.

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Vol. 2, Issue 11, November 2013

Induction of Adventitious Buds, Bulbs, Protocorms

In Many plant species, rapid clonal propagation is achieved by the inducing adventitious buds, bulbs, protocorms etc. For example excised shoot tips of most orchids, e.g., Cymbidium, Vanda etc., Micropropagation of lily (*Lilium longiflorum*) is done by inducing bulb formation on cultured pieces of bulb scales. Adventitious shoot bud formation in vitro is exhibited by many plant species, e.g., *Chrysanthemum*, *Hyacinthus*.

Somatic Embryogenesis

Somatic embryogenesis, especially through cell suspension, is considered to be the most promising technique for the future, in particular for its high potential multiplication rate. Somatic embryogenesis enables the production of encapsulated and artificial seeds, facilities cryopreservation or desiccation and, as a result, simplifies the the management of in vitro populations.

The future of Somatic embryogenesis techniques for mass micropropagation in crop like banana will depend on trueness-to-type of regenerated plantlets under field condition [6,7]

Advantages of micropropagation :

- Limited space needed
- Plants can be produced round the year
- True to type plants can be produced
- Disease (virus) free plants can be obtained
- Early flowering possible

Disadvantages of micropropagation :

- High capital, recurring costs and skill intensive
- Contamination control major challenge
- Variability possible sometimes (Eg. multiple shoots, somatic embryogenesis)

When compared to planting material from seed raised plants, micropropagation or clonal propagation has some distinct advantages. The following table lists the key distinctions.

International Journal of Innovative Research in Science, Engineering and Technology

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Vol. 2, Issue 11, November 2013

	Seed Raised Plants	Tissue Cultured/Clonal Plants
1	Genetically variable - Gene complexes both for production (short term economic yield) and future adaptability -Immediate fitness and long term genetic flexibility	Uniform -Stress on short term economic yield at the expense of future adaptability -Immediate fitness only
2	Low maintenance costs -Low inputs and moderate yield	High maintenance costs -High inputs and high yield
3	Advisable under poly cultural forestry -Species-rich, various species serve different purpose/need	Advisable under monocultural conditions -Low genetic diversity
4	Productivity Low (per species basis) High (on cumulative basis)	High
5	Advisable for conservation/ revegetation/ environmental forestry and wasteland development	Advisable for industrial forestry but selectively for other types of forestry

V. DEVELOPMENT OF MICROPROPAGATION TECHNOLOGY IN INDIA

One of the first nations to conduct tissue culture research, including micropropagation, was India. Many horticulture and forestry species now have standardised and expanded protocols. University of Delhi is one of the top research institutions that created the T.C. protocols. In the public sector, there are institutions like the National Chemical Laboratory, M.S. University of Baroda, National Botanical Research Institute (NBRI), ICAR Research Institutes, and Bhabha Atomic Research Centre. In the private sector, there are organisations like Tata Energy Research Institute (TERI), Indo American Hybrid Seeds (IAHS), and A.V. Thomas and Co. Numerous short- and long-term projects for the protocol development of horticultural and forestry species have been funded by the department of biotechnology. Two pilot projects that later developed into technology parks were set up at TERI in New Delhi and NCL in Pune. DBT has also successfully carried out pilot projects. Several species, including banana, sugarcane, teak, poplar, and a large number of ornamentals, including rose, gerbera, ficus, and anthuriums, are now produced commercially and sold both in India and abroad thanks to the joint efforts of the public and private sectors. Earlier this year, the Department of Biotechnology published a compilation of tissue culture protocols that are available for commercialization in India.[4,5]

International Journal of Innovative Research in Science, Engineering and Technology

| A Monthly Peer Reviewed & Referred Journal || Impact Factor: 1.672 |

Vol. 2, Issue 11, November 2013

VI. PROBLEMS IN TECHNOLOGY ADOPTION

Despite having a number of benefits, micropropagation technology has yet to become widely used in the nation for commercial purposes. India is home to many different fruit and forest species, and scientists from agricultural and forestry research institutes have identified and developed a number of high yielding mother plants and varieties that can be propagated through tissue culture and sold to farmers at reasonable prices. The reach is enormous. The country's high energy costs force producers to charge higher prices for their goods than small and marginal farmers can afford, which poses a number of practical challenges to the widespread adoption of this technology. A typical ornamental micropropagation project costs 40% for labour, 20% for energy, 10% for materials, and 30% for sales and marketing. However, energy costs in India can be as high as 40%–50%, making it difficult for most businesses to invest in sales promotion, marketing, or extension services. Other issues include the lack of affordable alternatives to traditional methods of propagation. Due to seasonality, the majority of agricultural crops have peak demand periods that are challenging to plan for in terms of production and stocking. Constraints on storage, transportation, and poor infrastructure all contribute to the difficulties in getting this technology to farmers. These drawbacks have cancelled out the benefits that the nation gets from having skilled labour. Due to the aforementioned limitations, India is unable to take advantage of the expanding global market for tissue cultured ornamentals. India is outpaced by even small nations like Singapore and Thailand. Some of the top companies in the private sector struggled to keep up with the high costs and gradually cut back on their tissue culture activities. A few export-oriented units (EOUs) had promising beginnings, but issues with the quality of the shipments in the recipient nations and, in some cases, IPR violations on the mother plants, soon surfaced. Few people are still alive today. India has a sizable domestic market for hybrid vegetables, forest plants, and fruit seedlings. In order to make these planting stocks affordable for small and marginal farmers, the micropropagation technology needs to be made more widely available. This industry can only be sustained over the long term by an ongoing domestic market. Due to the rising awareness of the cultivation of better planting material, crops like sugarcane, banana, papaya, ornamentals, medicinal plants, and teak, among others, present good, viable opportunities within the nation. Micropropagation industries must be less expensive than other agro-based units. Decentralised, produce planting material for a specific agroclimatic condition, and establish effective extension services in the target area to meet local needs.

VII. EXPERIENCES FROM THE AP-NL BIOTECHNOLOGY PROGRAMMES ON TRANSFERRING MICRO PROPAGATION TECHNOLOGY

In the A.P. districts of Nalgonda and Mahaboobnagar, a pilot project was started to scale up and distribute the micropropagation technique to farmers' fields through selected NGOs. The pre-project activity started in 1994 with a need assessment survey in which five villages in each district were surveyed using PRA, and potential micropropagation technology interventions were identified. According to thorough studies, farmers selected plants including tamarind, custard apple, teak, neem, and soap nut. Farmers in the investigated villages were aware of these species' use and appropriateness for their communities, but they were unaware of the significance of micropropagation. However, some forward-thinking farmers (10%) were aware that tissue culture technology allowed for the quick production of a large quantity of high-quality planting material, which was typically superior

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Vol. 2, Issue 11, November 2013

to plants grown from seeds. Two species, teak (because of its high value and farmers' preference) and neem (because of its drought resistance, suitability for the target villages, presence of neem-based industry in the area, and the growing use of neem products in IPM), were chosen for multiplication under the pilot project based on the prioritisation. CRIDA has been undertaking country wide surveys and assessment of germplasm of these species for desirable traits since 1995. In case of neem, mother trees with high azadirachtin and seed yield, were identified. While straight bole, golden yellow colour wood and relative tolerance to skeleton were the characters considered in the teak mother tree (cl. Teli). Laboratory level protocol for these two species were further refined and scaled up to produce at least 50,000 plants per annum. Three independent pilot laboratories were established at CRIDA, Gaddipally (SAIRD) and Kothakota (YFA) successfully transferred and adopted from CRIDA to these two rural NGOs. Based on our recent interactions with farmers, the use of tissue-cultured teak plants was well received. Many farmers are willing to pay market rates to purchase the plant material. However, the reaction to neem has not been good. Although this species is better suited for the target regions, farmers do not completely understand all of its benefits. Neem trees are also widely distributed in the fields and homesteads in the communities. However, there is a chance that the demand for neem tissue cultured plants would rise as the usage of neem products is extended in IPM and neem seeds become more expensive.

VIII. SOME COMMON PERCEPTION ON TISSUE CULTURED PLANTS AND THE SCIENTIFIC FACTS

Tissue culture technology has moved to the fields during the last decade. Banana, sugarcane, teak and ornamentals are some of the species routinely produced by public and private sector laboratories and marketed to farmers. There are some apprehensions and misconceptions among farmers and extension workers. Cultured plants vis-a-vis see are clarified below. There are some apprehensions, misconceptions and extension workers on the merits and demerits of tissue or vegetative propagated plants. The most important points are clarified below. [8]

Perception	Fact
Tissue cultured plants are much superior to seedlings or vegetatively propagated plant material	The superiority depends on the mother plants used. Since most of the plants produced through tissue culture are already selected based on performance, they might yield more as compared to local germplasm. The main advantage from tissue culture comes from uniformity in growth and yield which gives an overall economic advantage on area basis, but micropropagation per se does not result in superior planting stock

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Vol. 2, Issue 11, November 2013

Tissue cultured plants are disease/pest resistant	Plants do not become resistant merely by tissue culture. However, some species produced through meristem culture will be virus free atleast for few generations. Basically,, tissue culture cleans the plant from inherent pathogene so that the plants will be vigorous for few generations'
Tissue culture plants do not survive in the field conditions	Not true. Properly hardened T.C. plants with good root system will have same survival rates as that of seedlings or vegetative propagated plants
Tissue culture timber plants like teak mature much earlier than the stumps	Not true. The maturity depends on the mother plant, the growth rate and interaction with the climate. There is no data to show that tissue cultured timber plants can be harvested earlier than normal plants
Tissue cultured plants are fast growing as compared to stumps and seedlings	Rate of growth Is the character of the mother plant. It does not increase due to tissue culture, though initial vigorous growth may be possible because of clean and healthy planting stock
The root system in tissue cultured plants is not well developed and suitable for field survival	Tissue culture is a method of vegetative propagates in The roots, are induced by hormone treatment of micro shoots. Therefore, like in all other vegetative propagated plants (except on in situ grafted or budded plants), the root system will be adventitious. This type of root system has both merits and demerits depending on the life cycle of the plant and the harvest cycle. Tap root system, no doubt gives greater anchorage but even the adventitious root system in perennial trees eventually develops in to a set of strong roots which provide good support.
Tissue culture method of propagation causes variations in the plants produced	It is true in some cases. When the propagation is based on nodal explants or meristems. The chances of variation are extremely low. However, when other methods like somatic embryogenesis involving a callus phase is used there are chances for either negative or positive variations

Therefore, unless it is employed to generate virus-free plants, the planting material of any species does not become superior just because it was produced by micropropagation. The mother plant is primarily responsible for the quality of the planting material. One of the many techniques accessible to scientists and nurserymen for vegetatively propagating superior plants is micropropagation. However, it is a strong and distinctive technology that allows for the quick and efficient production of a huge number of propagules. The kind of natural regeneration for a specific species, any recalcitrance, and the necessity for high-quality planting materials at reasonable prices must all be taken into consideration when choosing a propagation strategy.

International Journal of Innovative Research in Science, Engineering and Technology

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Vol. 2, Issue 11, November 2013

REFERENCES

1. Alvard, D.; Cote, F.; Teisson, C. (1993), Comparison of methods of liquid medium culture for banana micropropagation, *Plant Cell, Tissue and Organ Culture*, 32, 55-60.
2. *Biotechnology in Horticulture and Forestry*, by VibhaDhawan, Plenum Press, NewYork,1989.
3. Dore Swamy, R. and Sahijram, L., 1989. Micropropagation of banana from male floral apices cultured in vitro. *ScientiaHortic.*, 40: 181-188 ...
4. Dryland Agriculture, in Andhra Pradesh; Needs Assessment and Options for Biotechnology- A report by Institute of Public Enterprise Hyderabad, 1994.
5. Murashige, T. and Skoog, F. (1962) A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Plant Physiology*, 15, 473-497.
6. *Plant Biotechnology* by S.S. Purohit, published by Agro Botanical Publishers (India), Bikaner,1997-1998.
7. *Plant Tissue Culture: From Research to Commercialisation- A Decade of Support*, Department of Biotechnolog, Ministry of Science and Technology, Government of India, November, 2000.
8. Status of Plant Biotechnology in India- An Assessment from the Small Farmer Perspective (A Study Sponsored by AP-NL Biotechnology Programme) By Naresh Sharma & P. S. Janaki Krishna, Institute of Public Enterprises,O.U.campus,Hyderabad-500 007,India.