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PLANT TISSUE CULTURE AND ITS APPLICATION

Kanchan Lakhera[1,*], Amit Kumar[1], Anju Rani[2], Rekha Dixit[1] and Sonali Rana[1]

Author's Affiliation
[1]Department of Biotechnology, K.V. Subharti College of Science, Swami Vivekananda Subharti University, Meerut, Uttar Pradesh 250005, India
[2]Department of Botany, K.V. Subharti College of Science, Swami Vivekananda Subharti University, Meerut, Uttar Pradesh 250005, India

***Corresponding Author:**
Kanchan Lakhera
Department of Biotechnology, K.V. Subharti College of Science, Swami Vivekananda Subharti University, Meerut, Uttar Pradesh 250005, India.

Email:
lakhera.kanchan@gmail.com

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Abstract

Plant tissue culture has been successfully integrated into plant transformation strategies. Meristem culture has become immensely popular as micro propagation technique for the rapid production of commercially viable plants at industrial level. Meristem culture is also significant for obtaining virus free plant and additionally, meristem culture in combination with other techniques is applied in plant improvement as in production of transgenic plants, plant germplasm storage, regeneration of quality planting material or seeds in association with aeroponic or hydroponic systems.

Keywords: Clonal propagation, callus culture, multiplication, Transgenic plant.

INTRODUCTION

Plant Tissue Culture is a technique of growing and multiplication of the cells, tissues and organs of the plant in a controlled environment of light, temperature, pH etc. under aseptic conditions. German botanist Haberlandt is recognized as the father of the plant tissue culture since Haberlandt in 1902 for the first time attempted to culture the isolated plant cells on an artificial medium. Although he failed to achieve the multiplication of the cells but he observed the growth of the cells for about a month. Based on his findings he put forward the idea of totipotency and established the principles of plant tissue culture.

Thereafter several studies taken up by the eminent scientists led to root/shoot tip cultures, embryo culture, callus culture, discovery of auxins, indole acetic acid etc.

One of the pioneering works done in the plant tissue culture came from the Philip R. White. In 1934 White for the first time reported the successful growth and multiplication of the root tip meristem culture of tomato plant for potentially unlimited periods of time in a liquid media. An important

breakthrough in plant tissue culture came from the work of P. Nobecourt in 1939. He along with White and Gautheret achieved *in vitro* cultivation of plant tissues for an indefinite time period. Plant tissue culture comprises of several types of culture techniques based on the type of organ/tissue used as explant, regenerative pathway, media used for the culture etc. Some of the important tissue culture techniques are meristem culture, callus culture, protoplast culture, organ culture, embryo culture, root culture, anther/pollen culture, cell suspension culture etc. One of the important technique of plant tissue culture that has gained the significance and is most popular for regeneration of plant or its tissue/organ is the meristem culture. Meristem culture is the culture of the apical meristem on nutrient media under aseptic conditions to regenerate the tissue, organ or whole plants. Regeneration of plant from meristem culture technique comprises of 4 steps:

1. **Establishment of explants:** Explant from the mother plant is cultured on the nutrient media under aseptic conditions in a controlled environment. Although many of the plant tissues can be used for the regeneration of the plant or its organs but physiological state of the mother plant e.g. age of the plant, type of tissue from which the explant is derived influence the regeneration capacity of the plant. From all the different types of cells, meristematic cells are the most active cells hence their regeneration capacity is better than the other types of tissues. For plant meristem culture, the nutrient medium that is commonly used is MS media modified suitably according to the specific requirement of the given plant specy.
2. **Subculture of shoot propagules for proliferation:** As the explant is cultured on the sterile nutrient media, after few days shoot starts to emerge and within 3-6 weeks shoot propagule is regenerated. Later the shoot propagules are aseptically cut into small pieces containing atleast one auxillary bud and sub-cultured on the sterile media for proliferation. Sub-culturing is done to increase the number of cultures.
3. **Rooting of Shoot Propagules:** Once the sufficient number of sub-culturing is done, the shoot propagule thus regenerated are aseptically cut into small pieces and transferred to the rooting media containing higher auxin to cytokinin ratio which supports the root formation. Within 3-6 weeks, plantlets are obtained
4. **Hardening of Plantlets:** Plantlets thus regenerated are transferred to the pots carrying sand or peat in the greenhouse for hardening since the plantlets are regenerated under controlled environment hence are unable to stand the harsh field conditions and may not survive if transferred directly therefore hardening in greenhouse is necessary.

Meristem culture has remarkable advantages to other culture techniques like in viral/pathogen elimination. Plants are often infected with the virus/pathogen which passed on from one generation to the next, with the help of meristem culture virus/pathogen free plants can be raised. Other most popular application is the micropropagation of plants using meristem culture. Micropropagation is rather most exploited plant tissue culture technique which has been used for the rapid multiplication of variety of plant species. Another significant application of meristem culture is the propagation of haploid plants. Haploid plants derived from anther or pollen culture always remain sterile unless and until they are made homozygous diploid. Meristem or shoot-tip culture of haploid plants can be used for their propagation.

APPLICATIONS OF PLANT TISSUE CULTURE

1. To obtain disease free plant: Meristem culture is the most common method of obtaining the pathogen free plants from a systemically infected plant. Some of the earlier information on the meristem culture came from Ball in 1946 who grew shoot tips and subjascent regions of tropaeolum majus L. and of Lupinus albus L. in sterile culture and observed that shoot apex has the highest capacity for development into entire plant while tissue subjascent to the shoot apex have limited capacity Morel and Martin in 1952 first applied meristem culture to obtain the virus free plants of Dahlia from infected individuals by culturing shoot tip in vitro. Since 1952 numerous research studies have demonstrated the successful production of virus free plants of different species through the meristem culture technique. Meristem culture since then has been applied to obtain the clean stock of plants free from pathogens. Brants Hendrine D. and Vermeuleen H. in 1965 conducted an experiment entiltled “Production of Virus free fresia’s by means of meristem culture”. In their experiment they

were able to demonstrate the production of virus free freesia plants, using explant derived from the freesia corms, as confirmed by the serological tests through the meristem culture technique. In 1966 Baruch and Quak conducted a study entitled "Virus free plants of Iris Wedgwood obtained by meristem culture" in which it was confirmed that meristem culture produced virus free plants from virus infected mother plant.

Sometimes meristem culture alone is not sufficient to eliminate the virus or other pathogens from the plant. Therefore meristem culture in conjunction with therotherapy or chemotherapy is used to obtain the clones free from plant pathogens. In 2002, M. Balamuralikrishnan *et al.* conducted a research study to assess the effectiveness of the chemotherapy along with meristem culture the eliminate the sugarcane mosaic virus (SCMV) from sugarcane. The obseration clearly showed that antiviral chemotherapy with ribovarin in combination with meristem culture resulted in the successful elimination of the virus.

Morel in 1960 successfully mass propagated Orchid *Cymbidium* using meristem culture technique. In 1974, P.H. Boxus described the in vitro micropropagation method for rapid production of Strawberry plants using axillary bud culture. In 1985 Banerjee and Langhe developed the technique for the rapid clonal propagation of Musa cultivars (banana and plantain) by shot tip cultures grown on a modified semi-solid MS media. In 2000 Anand & Rao developed the protocol for the clonal propagation of Piper berberi Gamble, a critically endangered plant through meristem culture. In 2010, Ramgareeb *et al*. established the apical meristem culture protocol for virus elimination and shoot multiplication in sugarcane. In 2013, Hatira Taskin *et al.* compared the meristem culture technique with the shoot tip culture technique for the production of virus free plants in two different Garlic species and tested the regenerated plants with the RT-PCR assay. It was found that the plantlets produced from meristem culture were free from virus whereas those obtained from shoot tip culture were infected. In 2017, Tania San Pedro *et al*. developed the protocol for the production of grape (Vitis vinifera L.) cultivar Monastrell using single node culture method from virus free mother plant. Aromatic and medicinally important plants rich in secondary metabolites are also being regenerated through meristem culture. In 2014, Mozhgan Molsaghi *et al.* developed an efficient protocol for the micropropagation of Aloe vera through meristem culture. Gibson and Rebicca reported the first protocol on micropropgation of *E. Zwageri* through induction of shoots multiplication by using nodal explant. Fidan et al. (2009) detected, new virus diseases for Turkey in onion and garlic: onion yellow dwarf virus and shallot latent virus

2. To obtain rapid multiplication: Mersitem culture has been initially used for rapid multiplication of the plants and for freeing the plant of viruses, viroids and other plant pathogens. Later, meristem culture technique has been used in association with other techniques and methods to further the advancement in plant biotechnology. Meristem culture has gained industrial significance as a technique for large scale production of quality plating material as microplants, tubers etc. In 2017, Karla A. Quiroz obtained the meristem culture of the Chilean strawberry (Fragaria chiloensis (L.) Duch.) Eliminating virus from the infected plants and produced the plantlets as high quality planting material for propagation for farmers. Meristem culture is used in the propagation of the novel intergeneric hybrids depending on its micropropagation potential. In 2010, A.N. Sutan *et al.* developed a protocol for in-vitro propagation of two intergeneric varieties of Fragaria x Potentilla called Seranata and Pink Panda and assessed the effect of season and culture medium on the micropropagation potential of both the ornamental strawberry varieties. Kumar, A. *et al.,* (2015) observed that Plant growth regulators effect on the callus induction and plant regeneration.

3. To obtain Transgenic plant: Most methods of plant transformation applied to genetically modified crops require that a whole plant is regenerated from isolated plant cells or tissues that have been genetically transformed. This regeneration is conducted *in vitro* so that the environment and growth medium can be manipulated to ensure a high frequency of regeneration. In addition to this, the regenerable cells must be accessible to gene transfer by whatever technique is chosen.

Meristem culture also being used in combination with molecular techniques in the production of transgenic plants. In 1988, E.C.Ulian *et al.* transformed petunia by A. tumefaciens carrying the genes

for kanamycin resistance and beta-glucuronidase using in-vitro shoot tip meristem culture of petunia variety rose flash and regenerated the transformed plants. In 1999, S. Zhan *et al.* produced the transgenic lines of the barley (Hordeum vulgare L.) and oat (Avena sativa L.) using shoot meristem culture derived from germinated seedlings. The similar application is done by Shah *et al.* (2013)

4. Combine with other techniques: Meristem culture in combination with other plant biotechniques like hydroponics and aeroponics system is used to greatly enhance the production of quality palnting material or seeds. In 2012, M Chiipanthenga *et al.* described the procedure for obtaining the quality potato seeds through aeroponics system. Clean potato plant stock was obtained using meristem culture and then tuberization was induced under aeroponic system resulting in the production of potato seed tubers rapidly that were free from pathogens.

Meristem cultures may also be utilized in plant improvement. There is a possibility of utilizing meristem cultures for somaclonal vaiation to obtain plants with not true-to-type characteristics in meristem clones. In 2010, V. Rosenberg *et al.* regenerated plantlets from meristem culture through virus-eradication procedure and observed the variability in meristem clones. The study showed that plant regenerated differed in their yield, number and weight of the tubers and blight resistance. Meristem cultures are also employed in plant germplasm conservation. Meristem culture has been effectively used as in-vitro technique for the long-term plant germplasm storage. Cells of the meristem are less differentiated and are more stable genetically hence the progeny (plantlets) regenerated from the meristem culture have resulted in the maintenance of greater genetic stability as compared to other methods of in vitro plant regeneration. In 2015, Geeta Morwal *et al.* established a protocol to regenerate plants from meristem culture using nodal segments as explants for the long term germplasm storage of Vanilla planifolia.

5. Organogenesis: Organogenesis relies on the production of organs, either directly from an explant or from a callus culture. There are three methods of plant regeration via, organogenesis. The first two methods depends on adventitious organs arising either from a callus culture or directly from an explant. Alternatively, the third method is by axillary but formation and growth, which can also be used to regenerate whole plant from some types of tissue culture. Organogenesis relies on the inherent plasticity of plant tissues, and is regulated by altering the components of the medium. In particular, it is the auxin to cytokinin ratio of the medium that determines which developmental pathway the regenerating tissue will take.

CONCLUSION

Ability of a cell to regenerate into whole organism is called as totipotency. In plants, cellular totipotency is being exploited in various plant tissue culture techniques. Plant tissue culture techniques especially meristem culture has been instrumental in research and advances made in the development of plant biotechnology. Meristem culture was initially used for rapid multiplication of the plants and for freeing the plant of viruses, viroids and other plant pathogens. Later, meristem culture technique has been used in conjunction with other techniques and methods in plant improvement like in combination with molecular technique it is used to obtain transgenic plants, meristem cultures are also used in plant germplasm storage. Meristem culture technique is exploited to obtain the somaclonal variants with novel traits. In this review we have only scratched the surface of potential of plant tissue culture.

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