ISSN 2226-3063 e-ISSN 2227-9555
Modern Phytomorphology 16: 4–8, 2022

REVIEW ARTICLE

Micro-propagation techniques in horticultural crops and various factors affecting it: A review

Souvik Kundu*, **Pedada Sindhusha, Anil Kumar**

Department of Horticulture (Vegetable Science), Lovely Professional University, Punjab, India; * souvikkundu787@gmail.com

Received: 14-Dec-2021, Manuscript No.: mp-21-49749 | **Editor Assigned:** 15-Dec-2021, Pre-QC No. mp-21-49749 (PQ) | **Reviewed:** 23-Dec-2021, QC No.: mp-21-49749 (Q) | **Revised:** 28-Dec-2021, Manuscript No.: mp-21-49749 (R) | **Accepted:** 30-Dec-2021 | Published: 07-Jan-2022

Abstract

A German Botanist, Gottlieb Haberlandt, made the first attempt to use the in vitro method to grow plant tissues and gave the basic concept for the cultivation of plant cells, tissues and organs in vitro culture over 100 years ago. At the initial period plant tissue cultures treated as a research tool and only focused on study the development of small, isolated cells and segments of plant tissues. At the pinnacle of the plant tissue culture period during the 1980s, in a moderately brief timeframe, many commercial laboratories were set up to capitalize by the capability of micropropagation for large scale manufacturing of clonal plants for the horticulture industry. Today plant tissue culture applications incorporate significantly more than clonal propagation. The scope of routine advancements has extended to incorporate somatic embryogenesis, somatic hybridization, and elimination of virus as well as the application of bioreactors for mass propagation. Maybe the best estimation of these tissue culture technologies lies less in their application to mass clonal propagation rather than their role in a plant improvement/modification, molecular biology, bioprocessing and germplasm storage, as well as being a basic research tool. Beside this plant tissue culture technique helps in horticulture to increase crop production. This paper highlights the applications, achievements and the limitation of plant tissue culture on horticultural crops.

Keywords: Bioprocessing, bud multiplication, micropropagation, somatic embryogenesis, tissue culture

Introduction

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under aseptic conditions on a nutrient culture medium of known composition. It is widely used to produce clones of a plant in a method known as micropropagation.

Now it is a well-established technology. Like others technologies, it also has different stages of evolution, scientific research, research tool, unique applications and exploitation. At the initial period plant tissue cultures treated as a research tool and only focused on study the development of small, isolated cells and segments of plant tissues. Around the mid twentieth century, it has come in existence that plants could be regenerated or multiplied from callus or as an organ culture through practical applications. After that many commercial laboratories were established throughout the world for mass clonal propagation of horticultural plants. The term 'micropropagation' was introduced to describe accurately the process of the universal mass clonal plant propagation system. Today plant tissue culture applications incorporate significantly more than clonal propagation. The scope of routine advancements has extended to incorporate somatic embryogenesis, somatic hybridization, and elimination of virus as well as the application of bioreactors for mass propagation.

The applications of plant tissue culture include: Clonal propagation, Direct organogenesis, Indirect organogenesis, Axillary shoot multiplication, Somatic embryogenesis, Embryo rescue, Pathogen free plant, Somatic variation, Induced mutation, *in vitro* grafting, *in vitro* screening and selection, *in vitro* gene banks, stock plant banks, Anther or pollen culture for haploid production, Protoplast culture-somatic fusion, Cell culture, DNA transformation system, Biosynthesis in bioreactors (production of secondary metabolites)

Probably the best estimation of these tissue culture technologies lies less in their application to mass clonal propagation rather than their role in a plant improvement/ modification, molecular biology, bioprocessing and germplasm storage, as well as being a basic research tool.

Review of Literature

Fujimura T. and Komamine A. 1975 conducted an experiment to see the effect of different growth regulators on the embryogenesis in a cell suspension culture isolated from the petiole of a domestic carrot, Daucus carota cv. Kurodagosun. Applying of more concentration of 2,4-D or IAA inhibited the embryogenesis whereas zeatin promoted the embryogenesis.

Bennici A. and Cionini P.G. 1979 studied the effect of cytokinins on *in vitro* development of Phaseolus coccineus embryos on media with different concentrations of zeatin and zeatin riboside at the heartshaped and the middle cotyledonary stages. A higher concentration of zeatin responsible for growth of early embryos whereas a higher concentration of zeatin riboside inhibit the growth of early embryos.

Behki R.M. and Lesley S.M. 1980 conducted an experiment to see the response of leaf callus supplemented with different combinations of growth regulators and subcultured on Murashige and Skoog medium. They concluded that the composition of induction and differentiation media and the duration of the induction period affect the shoot regeneration. The maximum shoot regeneration from callus and leaf explants was also affected by a critical ratio of ammonia to nitrete.

Barden K.A. *et. al.* 1986 conducted an experiment on leaf discs of tomato (*Lycopersicon esculentum*, Mill.) to regenerate somaclones from tobacco mosaic virus fully susceptible isogenic line at Glasshouse Crops Research Institute, Littlehampton, U.K. Out of 370 somaclones which were inoculated with TMV-Flavum, six somaclones were selected as virus free and partially resistant to TMV.

Bentz S.E. *et. al.* 1998 developed a micropropagation system to study the factors affecting *in vitro* propagation of Yucca glauca. Murashige and Skoog medium was prepared with different combination of NAA and BA for culturing shoot tip explants from mature plants. With the increasing concentration of BA shoots were expand more than the media (presence of NAA and absence of BA).

Micropropagation

Plant tissue culture also known as micropropagation which is used to produce clones of plant under aseptic condition or in a controlled environment. For this process a liquid or a solid medium is required for proper growth and multiplication of an explant under a controlled environment and these conditions include proper temperature, proper nutrient solution with growth regulators and proper gaseous and liquid environment with proper relative humidity. Plant tissue culture relies on the fact of totipotency which emphasize that a plant cell have the ability to regenerate a whole plant. For micropropagation a single cell, protoplasts (plant cells without cell wall), pieces of leaves or root can be used to regenerate a whole plant on a culture media with proper concentration of nutrient and plant growth hormones. The different stages of micropropagation are:

- selection of mother plant
- establishment of aseptic culture
- shoot multiplication
- root multiplication
- transplanting

It is a very useful method for making a clonal forest and field as it helps in the propagation of a large number of plants in a short time from a small piece (explant) of a stock plant. Depending on the species the explant may be taken from leaf, shoot tip, lateral bud, stem of root tissue.

Advantages of micropropagation

The unique advantages of micropropagation which are not possible with conventional propagation are:

- Rapid multiplication of genetically similar plants (clones) with desirable traits. A single plant cell can be multiplied into thousand plants in a very short time and those can be grown under greenhouse conditions without seasonal interruption.
- Multiplication of plants can be possible without seeds or necessary pollinators to produce seeds.
- Regeneration of genetically modified plants from plant cells helping a nurseryman to choose superior clones of ornamental plants in sufficient quantities having an impact on the landscape plant market.
- The production of plants in a aseptic condition promotes the plants with greatly reduced chances of transmitting diseases, pests and pathogens.
- Micropropagation is better than those plants which are germinate from seeds as there are very low chances of germinating and growing healthy, e.g. orchards and nepenthes.

Applications of micropropagation

Plant tissue culture is now widely used for many purposes;

the commercial applications of plant tissue culture are:

- It is very useful for screening cells rather than a plant for advantageous characters, e.g. herbicide resistance/tolerance.
- This is an alternative method of vegetative propagation for mass production with less time. Desirable variety can be produced in large quantities with desirable traits and the time to produce a new variety is reduced by 50%.
- Diseases free varieties can be developed by using meristem tip culture through micropropagation.
- By crossing distantly related species to regenerate the novel hybrid through protoplast fusion can also be possible.
- For production of doubled monoploid plant by colchicine treatment from haploid cultures to achieve homozygous lines more rapidly in breeding programmes.
- It also useful for seed production of certain crops which need a high degree of genetic conservation for seed production.
- This method holds a better scope and future for the production of important plant-based phytopharmaceuticals. It also helps for successful production of valuable, specific and yet undiscovered plant chemicals.
- Micropropagation helps in *in vitro* conservation of germplasm for those plants which do not produce seeds or which have recalcitrant seeds those can't be stored under normal storage conditions in seed gene banks.

Micropropagation techniques

It is a simple technique. The basic protocol based on the MS medium (Murashige T., and Skoog F. 1962) which established a whole research field and industry with a numerous modifications by 1960s. However, some species and cultivars have not responded to this protocol. Too often the scientists published this protocol for those species which are not reproduced by other laboratories nor do not hold up under sustained production. This situation has led the scientists to revisit the basic principles.

Modern plant tissue culture is conducted under aseptic conditions with filtered air (free from microorganisms). Living plant materials are almost contaminated with microorganisms on their surface or even interiors, so surface sterilization of explant with 70% ethanol or Mercuric chloride along with fungicides is required. Then explant is placed on a solid or liquid (for suspension culture) based culture medium. These Medias are generally constructed of inorganic salts, organic nutrients, vitamins, growth hormones and gelling agent (for solid media). The concentrations of growth hormone are highly effective on the morphology of the initial tissue of explant. For example high auxin and low cytokinin concentration results in root proliferation, while low auxin and high cytokinin concentration results in shoot proliferation.

A proper concentration of both plant hormones, auxin and cytokinin will often produce an unorganized growth of cells in a media, which is known as a callus, but the characteristics of outgrowth will depend on the plant species as well as composition of the medium. With the growing of cultures, the callus cells need to transfer to a new media (subcultured) for better growth and for alter the morphology of the culture. A skilled and experienced tissue culturist can determine that which pieces to culture and which to discard. When shoots emerge from a culture then it have to transfer to a rooted media (high auxin concentration) for development of root and produce a plantlets. When these plantlets are mature enough it can be transferred to potting soil for further growth in greenhouse. The procedure of micropropagation techniques consist of 5 stages:

- Stage 0-Initial stage
- Stage I–Culture initiation
- Stage II –Bud multiplication
- Stage III–Plantlet regeneration and
- Stage IV–Acclimatization (Hardening)

Factors Affecting *in vitro* Growth

Choice of explant

The portion of the plant which is obtained for culture is known as explant. Based on certain model system it has been seen that a totipotent explant can be cultured from any part of the plant. The plantlets which are developed by tissue culture, is it haploid or diploid will also be determined by the choice of explant material. With the inappropriate explants the risk of microbial contamination also increased. So, the choice of proper explant is very important for conducting tissue culture. The meristimatic ends of the plants like shoot tip, auxiliary bud tip and root tip are commonly used as they have potential of high rates of cell division and produce required growth regulating hormone including auxins and cytokinins.

Some explants collected from root tip, are difficult to

isolate and are contaminated with soil micro-flora which form a tight bonding with the root systems, or even born within the root. Soil particles also bound to attaches are very hard to eliminate without injury to the roots that's lead to microbial attack.

Explant size and thin section culture system

To induce a desirable morphogenic character in vegetative tissues would be the most significant advancement in plant tissue culture by proper *in vitro* manipulations. The success of this morphogenic characters are determined by tissue culture. To influence the organogenic potential of the cultured tissue several explant related factors are responsible (Benson E.E. 2000). These include growth factors, genotype and whole plant physiology of the mother plant. Along with, there is a negative correlation between explant size and number of cells potentially has also been seen for oraganogenesis.

However, before the late 1990s this observation was not so much popular to the researcher (Lakshmanan P. *et al.* 1995 and 1996). In 1995, (Lakshmanan P. *et al.*) had shown that the in vitro orchid protocorms production can be improved enough by only manipulating the size of the explant. For example, a 0.6 mm thick section produced the number of protocorms 5 times greater than produced by a single shoot tip (6 mm-7 mm long) cultured under same conditions. This thin section culture system is a novel approach in plant regeneration of various orchards (Lakshmanan P. *et al.* 1996), brassicas (Cheng P.K. *et al.* 2001).

The *in vitro* environment

Besides work we have to improve some interesting area of *in vitro* biology such as autotrophy and hormone physiology (auxin regulated axillary growth) (Reinhardt D. *et al.* 2000) for our better understanding. We need to gather more information about the field of *in vitro* dynamics and mineral nutrition (Williams R.R. 1995). Light quality is an important environmental factor, as it affects the direction of *in vitro* plant morphogenesis (Morini S. *et al.* 2000) and also act as a switch between gametophytic and sporophytic pathways. In plant tissue culture the stress by extensive cutting and stress injury of tissues led the programmed physiological changes in plants (Leon J. *et. al.* 2001).

Current Developments (New Culture Systems)

Bioreactors

It is proven fact that liquid media is the best media than solid based media for growth of most of the plants in culture. Depending on the desired final product and investigation of species, many innovative approaches were adopted to increase the productivity of liquid culture system (Aitken-Christie J. *et al.* 1995). Since the basic micropropagation plan by (Murashige T. 1974), the one of the major development of plant tissue culture industry is the application of bioreactors. The advantages of bioreactors systems compared to traditional tissue culture are: it is time and labour consuming, easy to scale up, increased growth and multiplication by forced aeration and by the use of liquid media it is improved the nutrient availability. On the basis of suitability for various plant species and their specific needs, several new approaches were adapted to develop bioreactors (Aitken-Christie J. *et al.* 1995 and Peak K.Y. *et al.* 2001). The basic systems are:

- Aeration-agitation bioreactor
- Spin filter bioreactor
- Gaseous phase bioreactor
- Rotating drum bioreactor
- Air-driven bioreactor (Lee J.M. 2004)

These systems are already used to mass production of more than 80 crops (Takayama S. 1991) and it is now practice to evaluate several other plant species for production (Peak K.Y. *et al.* 2001). It is proved that bioreactors are more superior to traditional *in vitro* technique as a plant production technique for all the species.

In recent, somatic embryo-based mass propagation system was developed by using a bioreactor for the recalcitrant species *Coffea arabica.* In brief, bioreactors have the potential to improve the quality of the product with less cost, but for commercial benefit it needs further more development.

In vitro mycorrhization

Mainly, for plant tissue culture technique, aseptic conditions are more essential but from the recent study, it has been seen that researcher are focused on the possible beneficial effects of microoraganisms in *in vitro* plant culture. For example, explant hardening is promoted by the root endophyte piriformospora indica (Sahay and Varma 1999); *Psuedomonas* spp. can reduce hyperhydricity (Bela J. *et al.* 1998) and shoot multiplication is improved by *Bacillus pumilus, Alcaligenes faecalis* and *Pseudomonas* spp. (Monier C. *et al.* 1998). The use of Arbuscular Mycorrizal Fungi (AMF), mycorrhization in micropropagation, is now gained popularity because of its positive impact on post-transplant performance of *in vitro* grown plants (Lovato P.E., *et al.* 1996 and Rai M.E. 2001). The recent tending research interests in AMF are: improved nutrient uptake, water relations, aeration, soil pH balance (Sylvia D. 1998) and their utilization as

a bioregulators (Lovato P.E. *et al.* 1996), which is being performed to development of effective AMF production methods and screening for efficient AMF strains.

Conclusion

Plant tissue culture is now a popular and well established technology for its significant contributions to the plant propagation and it also helps to improve of agricultural crops in general. Proper understanding of the biological process that allows the manipulation of *in vitro* morphogenesis and studied on various molecular, physiological and biological aspects of plant hormones that can help through providing information to address the issues of *in vitro* recalcitrance or about *in vitro* plant growth and development.

References

Murashige T., Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* **15:** 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Benson E.E., (2000). *In vitro* plant recalcitrance: An introduction. *In vitro: Cell Dev Biol Plant* **36:** 141-48. http://dx.doi.org/10.5958/0974-0112.2019.00042.2

Lakshmanan P., Loh C.S., Goh C.J. (1995). An *in vitro* method for rapid regeneration of a monopodial orchid hybrid Aranda Deborah using thin section culture. *Plant Cell Rep* **14:** 510-514. https://doi.org/10.1007/BF00232785

Lakshmanan P., Lee C.L., Goh C.J. (1996). *In vitro* propagation of commercial orchids: An assessment of current methodologies and development of a novel approach-thin section culture system. *J Orchid Soc Indian* **10:** 31-41.

Cheng P.K., Lakshmanan P., Swarup S. (2001). High frequency direct shoot regeneration and continuous production of rapid-cycling *Brassica oleracea. in vitro. In vitro: Cell Dev Biol Plant* **37:** 592-98. https://doi.org/10.1007/s11627-001-0104-0100

Reinhardt D., Mandel T., Kuhlemeier C. (2000). Auxin regulates the initiation and radial position of lateral organs. *Plant Cell* **12:** 507-518. https://doi.org/10.1105/tpc.12.4.507

Williams R.R. (1995). The chemical micro-environment. In: AitkenChristie J, Kozai T and Smith MAL (eds), Kluwer Academic, Netherlands. 405-439.

Morini S., D Onofrio C., Bellocchi G., Fisichella M. (2000). Effect of 2,4-D and light quality on callus production and differentiation from *in vitro* cultured quince leaves. *Plant Cell Tiss Organ Cult* **63:** 47-55. https://doi.org/10.1023/A:1006456919590

Leon J., Rojo E., Sanchez-Serrano J.J. (2001). Wound signalling in plants. *J Exp Bot* **52:** 1-9. https://doi.org/10.1093/jexbot/52.354.1

Aitken Christie J., Kozai T., Ann Lila Smith M. (1995). Automation and Environmental Control In: Plant Tissue Culture., Dordrecht, *Kluwer Academic Publishers*.

https://books.google.co.in/books?hl=en&lr=&id=NEbrCAAAQBAJ&oi=fnd&pg=PR7&dq=10.%09Aitken+Christie+J,+Kozai+T,+Ann+Lila+Smith+M.+Automation+and+Environmental+Control.+In:+Plant+Tissue+Culture.,+Dordrecht,+1995,+Kluwer+Academic+-Publishers.&ots=Vg6S1EQFYW&sig=QOl8UWo4tGCiw6xQjUWTTYTe26g&redir_esc=y#v=onepage&q&f=false

Murashige T. (1974). Plant propagation through tissue cultures. *Ann Rev Plant Physiol* **25:** 135-66. https://www.annualreviews.org/doi/abs/10.1146/annurev.pp.25.060174.001031?journalCode=arplant.1

Peak K.Y., Hahn E.J., Son S.H. (2001). Application of bioreactors for largescale micropropagation systems of plants. *In vitro: Cell Dev Biol Plant* **37:** 149-157. https://link.springer.com/content/pdf/10.1007/s11627-001-0027-9.pdf

Lee J.M. (2004). Plant cell culture and its applications. In: R. M Goodman (ed), Encyclopaedia of Plant and Crop Sciences, Marcel Dekker, USA. 931-933. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Lee+JM+%282004%29.+Plant+cell+culture+and+its+applications.+In%3A+R.+M+Goodman+%28ed%29%2C+Encyclopaedia+of+Plant+and+Crop+Sciences%2C+Marcel+Dekker%2C+USA%2C++2004%3B931-33.+&btnG=

Takayama S. (1991). Mass production of plants through shake- and bioreactor-culture techniques. In: Bajaj YPS (ed), Biotechnology in Agriculture and Forestry High-Tech and Micropropagation 1. *Springer-Verlag*, Berlin **17:** 495-515. https://doi.org/10.1007/978-3-642-76415-8_27

Behki R.M., and Lesley S.M. (1980). Shoot regeneration from leaf callus of Lycopersicon esculentum. *Zeitschrift für Pflanzenphysiologie* **98:** 83-87.

Sahay N.S., Varma A. (1999). Piriformospora indica: A new biological hardening tool for micropropagated plants. *FEMS Microbiol Lett* **181:** 297-302. https://doi.org/10.1111/j.1574-6968.1999.tb08858.x

Bela J., Ueno K., Shetty K. (1998). Control of hyperhydricity in anise (*Pimpinella anisum*) tissue culture by *Pseudomonas spp*. *J Herbs Spices Medicinal Plants* **6:** 57-67. https://doi.org/10.1300/J044v06n01_08

Monier C., Bossis E., Chabanet C., Samson R. (1998). Different bacteria can enhance the micropropagation response of Cotoneaster lacteus (Rosaceae). *J Appl Microbiol* **5:** 1047-1055. https://doi.org/10.1111/j.1365-2672.1998.tb05270.x

Bennici A., Cionini P.G. (1979). Cytokinins and *in vitro* development of Phaseolus coccineus embryos. *Planta* **147:** 27-29.

Bentz S.E., Parliman B.J., Talbott H.J., and Ackerman W.L. (1988). Factors affecting *in vitro* propagation of Yucca glauca. *Plant Cell Tissue Organ Culture* **14:** 111-120.

Lovato P.E., Gianinazzi Pearson V., Trouvelot A. (1996). The state of mycorrhizas and micropropagation. *Adv Hort Sci* **10:** 46-52. https://www.torrossa.com/gs/resourceProxy?an=2209206&publisher=FF3888

Rai M.K. (2001). Current advances in mycorrhization in micropropagation. *In vitro: Cell Dev Bio Plan.* **37:** 158-167. https://doi.org/10.1007/s11627-001-0028-8

Barden K.A., Smith S.S., Murakishi H.H. (1986). Regeneration and screening of tomato somaclones for resistance to tobacco mosaic virus. *Plant Sci* **45:** 209-213.

Fujimura T., Komamine A. (1975). Effects of various growth regulators on the embryogenesis in a carrot cell suspension culture. *Plant Science Letters* **5:** 359-364.

Sylvia D. (1998). The Promise (and obstacles) of AMF inoculation. In: Gianinnazzi S and Schuepp H (eds.), Arbuscular mycorrhizas in sustainable soil-plant systems. Report of 1997 activities, Cost Action 821, Iceland. **152.**