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Review Article

Plant Tissue Culture and Its Application in Agriculture as Biotechnological Tool

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A B S T R A C T

In the very fast developing scenario of biological science, the plant tissue culture has taken lead because it is the most promising areas of biotechnological tools for today and tomorrow agriculture. The areas range from micro propagation of horticultural crops, ornamental and forest trees etc. Over the100 years ago, Haberlandt envisioned the concept of plant tissue culture and provided the bottom work for the cultivation and production of plant cells, tissues and organs in culture. Due to the changes in consumption patterns, demand for fruits, vegetables, dairy, meat, poultry and fisheries has been increasing. Hence, a need to raise crop diversification and improve allied activities. It may be noted that the slowdown in agriculture growth could be attributed to structural factors on the supply side, such as public investment, credit, technology, land and water management, etc., rather than to globalization and trade reforms. In this situation, plant tissue culture offers remarkable opportunities in vitro propagations, plant quality improvement and production of plants with desirable agronomical quality and quantity. It's now possible to develop virus-free plant regeneration, herbicide resistance, salinity tolerance, disease resistance, incorporation of high protein content and genetically engineered plants for desirable traits. Biotechnological approach has been introduced into agricultural to reinforce the productivity of different food crops at a rate without precedent. for in vitro regeneration, mass micro propagation techniques and gene transfer studies in several species. Genetic transformation in Plant Biotechnology is a crucial technique which is basically relies on the technical aspects of plant tissue culture and molecular biology for the production of improved varieties of different crops. In this review article studies some tissue culture practices of commercially food crops and highlights the challenges which is encountered in the tissue culture and explores the chances of optimization of the *in vitro* propagation techniques by using explants.

Keywords

Tissue culture, Micro propagation, Somatic embryogenesis, Genetic engineering

Introduction

Plant tissue culture technology is being widely used for large scale plant multiplication. Apart from their use as a tool of research, plant tissue culture techniques have in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. Small pieces of tissue (named explants) can be used to produce hundreds and thousands of plants in a continuous process. A single explant can be multiplied into several thousand plants in relatively short time period and space under controlled

conditions, irrespective of the season and weather on a year-round basis (Akin-Idowu PE *et al.,* 2009). Endangered, threatened and rare species have successfully been grown and conserved by micro propagation because of high coefficient of multiplication and small demands on number of initial plants and space.

In addition, plant tissue culture is considered to be the most efficient technology for crop improvement by the production of Somaclonal and Gametoclonal variants. The micro propagation technology has a vast potential to produce plants of superior quality, isolation of useful variants in well-adapted high yielding genotypes with better disease resistance and stress tolerance capacities (Brown *et al.,* 1995). Certain type of callus cultures give rise to clones that have inheritable characteristics different from those of parent plants due to the possibility of occurrence of Somaclonal variability (George *et al.,* 1993), which leads to the development of commercially important improved varieties. Commercial production of Recent Advances in Plant in vitro Culture plants through micropropagation techniques has several advantages over the traditional methods of propagation through seed, cutting, grafting and air-layering etc. It is rapid propagation processes that can lead to the production of plants virus free (Garcia-Gonzales R *et al.,* 2010). Meristem tip culture of banana plants devoid from banana bunchy top virus (BBTV) and brome mosaic virus (BMV) were produced (El-Dougdoug *et al.,* 2011). Higher yields have been obtained by culturing pathogen free germplasm in vitro. Increase in yield up to 150% of virus-free potatoes was obtained in controlled conditions (Singh *et al.,* 1992).

In plant cell culture, plant tissues and organs are grown in vitro on artificial media, under aseptic and controlled environment. The technique depends mainly on the concept of totipotency of plant cells, which refers to the ability of a single cell to express the full genome by cell division. Along with the totipotent potential of plant cell, the capacity of cells to alter their metabolism, growth and development is also equally important and crucial to regenerate the entire plant (Thorpe T, 2007).

Basic requirement for plant tissue culture

There are some important aspects of tissue culture. These are: (A) Aseptic condition (B) Aeration (C) Equipment's and (D) Nutrient medium.

Tissue culture is the method of '*in vitro'* culture of plant or animal cells, tissue or organ on nutrient medium under aseptic conditions usually in a glass container. Tissue culture is sometimes referred to as 'sterile culture' or 'in vitro' culture.

(a) Aseptic condition

Tissue culture should be done in completely aseptic condition. Dry heat is used to sterilise equipment's in an incubator. Wet heat sterilization is done in an autoclave at 120°C at 15 lb pressure for 15 minutes. Liquid media, which are unstable at high temperature are sterilised by ultrafiltration. Chemicals, such as alcohol is used to sterilise working area and instruments. The tissue to be cultured is surface sterilised chemically some of the commonly used sterilising agents are:

(I) 9-10% calcium hypochlorite, (I) 2% sodium hypochlorite solution, (III) 10-12% hydrogen peroxide, (IV) 1-2% bromine water. Some other sterilising agents are: 1% chlorine water, mercuric chloride, silver nitrate, antibiotics etc.

(b) Aeration

Proper aeration of the tissue in the culture medium is essential. Those tissues, which are cultured on semi-solid medium do not require any special method for aeration. But those tissues, which are cultured in liquid medium require special device for aeration.

(c) Equipment

Glassware used for tissue culture should be of borosilicate glass (Pyrex glass), because soda glass may hamper the growth of the tissue.

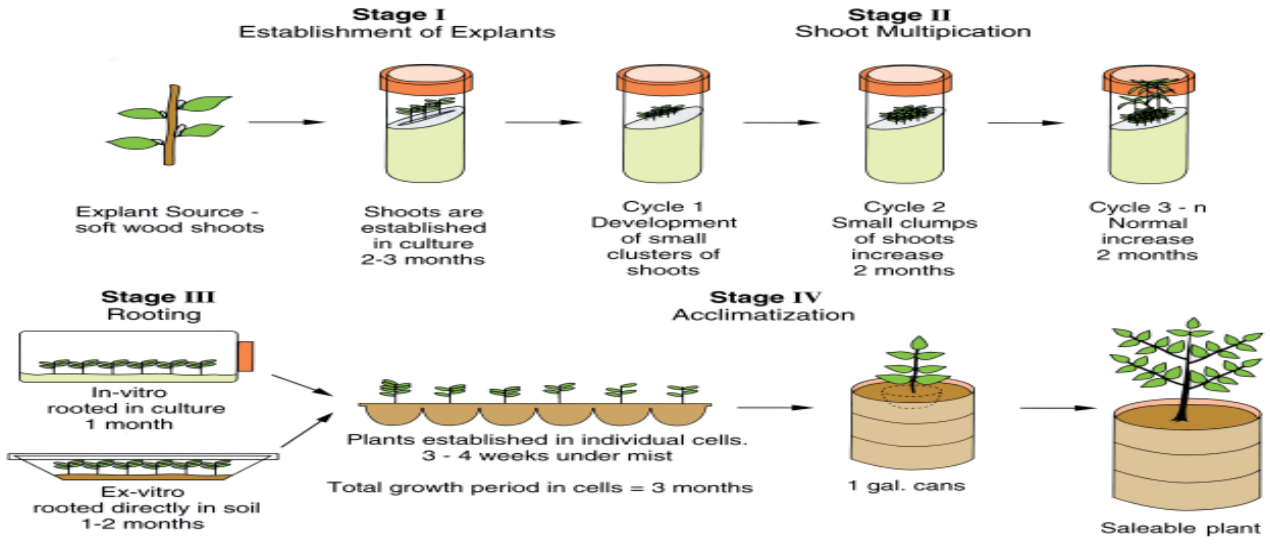
(d) Nutrient media

Plant tissue culture medium contains all the nutrients required for the normal growth and development of plants. It is mainly composed of macronutrients, micronutrients, vitamins, other organic components, plant growth regulators, carbon source and some gelling agents in case of solid medium (Murashige and Skoog, 1962). Murashige and Skoog medium (MS medium) is most extensively used for the vegetative propagation of many plant species in vitro. The pH of the media is also important that affects both the growth of plants and activity of plant growth regulators. It is adjusted to the value between 5.4 - 5.8. Both the solid and liquid medium can be used for culturing. The composition of the medium, particularly the plant hormones and the nitrogen source has profound effects on the response of the initial explant.

Methods of plant tissue culture

1. Micropropagation: which is a form of tissue culture, increases the amount of planting material to facilitate distribution and large-scale planting. In this way, thousands of copies of a plant can be produced in a short time. Micropropagated plants are observed to establish more quickly, grow more vigorously and are taller, have a shorter and more uniform production cycle, and produce higher yields than conventional propagules.

Stage 0: Preparation of donor plant- To enhance the probability of success, the mother plant should be ex vitro cultivated under optimal conditions to minimize contamination in the in vitro culture (Cassells and Doyle, 2005).



Stage I: Initiation stage: In this stage an explant is surface sterilized and transferred into nutrient medium. Generally, the combined application of bactericide and fungicide products is suggested. The selection of products depends on the type of explant to be introduced. The surface sterilization of explant in chemical solutions is an important step to remove contaminants with minimal damage to plant cells (Hussain and Anis, 2009). The most commonly used disinfectants are sodium hypochlorite (Tilak *et al.,* 2009), calcium hypochlorite (Garcia *et al.,* 1999), ethanol and mercuric chloride (Hussain and Anis, 2009). The cultures are incubated in growth chamber either under light or dark conditions according to the method of propagation.

Stage II: Multiplication stage: This phase is to increase the number of propagules under which the number of propagules is multiplied by repeated subcultures until the desired number of plants is attained (Saini and Jaiwal, 2002).

Stage III: Rooting stage: The rooting stage may occur simultaneously in the same culture media used for multiplication of the explants. However, in some cases it is necessary to change media, including nutritional modification and growth regulator composition to induce rooting and the development of strong root growth.

Stage IV: Acclimatization stage: At this stage, the in vitro plants are weaned and hardened. Hardening is done gradually from high to low humidity and from low light intensity to high light intensity. The plants are then transferred to an appropriate substrate (sand, peat, compost etc.) and gradually hardened under greenhouse.

Somatic embryogenesis: It is an in vitro method of plant regeneration widely used as an important biotechnological tool for sustained clonal propagation (Park *et al.,* 1998). It is a process by which somatic cells or tissues develop into differentiated embryos. These somatic embryos can develop into whole plants without undergoing the process of sexual fertilization as done by zygotic embryos. The somatic embryogenesis can be initiated directly from the explants or indirectly by the establishment of mass of unorganized cells named callus (Suman and Kumar, 2016). Plant regeneration via somatic embryogenesis occurs by the induction of embryogenic cultures from zygotic seed, leaf or stem segment and further multiplication of embryos. Mature embryos are then cultured for germination and plantlet development, and finally transferred to soil. Somatic embryogenesis has been reported in many plants including trees and ornamental plants of different families. There are various factors that affect the induction and development of somatic embryos in cultured cells. A highly efficient protocol has been reported for somatic embryogenesis on grapevine (Jayasankar *et al.,* 1999) that showed higher plant regeneration sufficiently when the tissues were cultured in liquid medium. Plant growth regulators play an important role in the regeneration and proliferation of somatic embryos. Highest efficiency of embryonic callus was induced by culturing nodal stem segments of rose hybrids on medium supplemented with various PGRs alone or in combination (Xiangqian *et al.,* 2002). The embryonic callus showed high germination rate of somatic embryos when grown on abscisic acid (ABA) alone. Somatic embryogenesis is not only a process of regenerating the plants for mass propagation but also regarded as a valuable tool for genetic manipulation. The process can also be used to develop the plants that are resistant to various kinds of stresses

(Bouquet and Terregosa, 2003) and to introduce the genes by genetic transformation (Maynard *et al.,* 2003). A successful protocol has been developed by using this tool for regeneration of cotton cultivars with resistance to Fusarium and Verticillium wilts (Han *et al.,* 2009).

Organogenesis: It refers to the production of plant organs i.e. roots, shoots and leaves that may arise directly from the meristem or indirectly from the undifferentiated cell masses (callus). Plant regeneration via organogenesis involves the callus production and differentiation of adventitious meristems into organs by altering the concentration of plant growth hormones in nutrient medium. (Skoog and Miller, 1957) were the first who demonstrated that high ratio of cytokinin to auxin stimulated the formation of shoots in tobacco callus while high auxin to cytokinin ratio induced root regeneration.

Embryo culture: It is a type of plant tissue culture that is used to grow embryos from seeds and ovules in a nutrient medium. In embryo culture, the plant develops directly from the embryo or indirectly through the formation of callus and then subsequent formation of shoots and roots. The technique has been developed to break seed dormancy, test the vitality of seeds, production of rare species and haploid plants (Holeman, 2009). It is an effective technique that is employed to shorten the breeding cycle of plants by growing excised embryos and results in the reduction of long dormancy period of seeds. Intra-varietal hybrids of an economically important energy plant "Jatropha" have been produced successfully with the specific objective of mass multiplication (Mohan *et al.,* 2011). Somatic embryogenesis and plant regeneration have been carried out in embryo cultures of Jucara Palm for rapid cloning and improvement of selected individuals (Guerra and Handro, 1988). In addition, conservation of endangered species can also be attained by practicing embryo culture technique. A successful protocol has been developed for the in vitro propagation of Khaya grandifoliola, a plant of high economic value for timber wood and for medicinal purposes as well, by excising embryos culture from mature seeds (Okere and Adegey, 2011). Plant tissue culture technology has an important application in forestry by offering a mean of propagation of elite individuals where the selection and improvement of natural population is not feasible and viable too.

Anther culture: Anther culture has become the most popular method for production of homozygous lines for rice cultivars worldwide. Haploids can be produced by culturing anthers or haploid plant explants. Induction of haploidy was first reported in Datura Innoxia by Guha and Maheshwari (1964). Research on pea (*Pisum sativum* L.) haploidy began in the 1960–1980s. Calli, roots, shoots and embryos were produced in anther culture (Gupta *et al.,* 1972; Gupta 1975). More recently haploid plant recovery from culture of isolated anthers and microspores is attempted. Calli, embryo-like structures, regenerated shoots and plants were produced in anther culture (Sidhu and Davies 2005).

Haploid production: By use of the tissue culture techniques it is possible to produce homozygous plants in relatively short time period through the protoplast, anther and microspore cultures instead of conventional breeding (Morrison and Evans, 1998). Haploids are sterile plants having single set of chromosomes which are converted into homozygous diploids by spontaneous or induced chromosome doubling. The doubling of chromosomes restores the fertility of plants resulting in production of double haploids with potential to become

pure breeding new cultivars (Basu *et al.*, 2011). The term androgenesis refers to the production of haploid plants from young pollen cells without undergoing fertilization. (Sudherson *et al.*, 2008) reported haploid plant production of sturt's desert pea by using pollen grains as primary explants via tissue culture. Now a day the haploidy technology has become an integral part of crop improvement programmes through plant breeding by speeding up the production of inbred lines (Bajaj, 1990) and overcoming the constraints of seed dormancy and embryo non-viability (Yeung *et al.*, 1981). The technique has a remarkable use in genetic transformation by the production of haploid plants with induced resistance to various biotic and abiotic stresses. Introduction of genes with desired trait at haploid state followed by chromosome doubling led to the production of double haploids inbred wheat and drought tolerant plants were attained successfully (Chauhan and Khurana, 2011).

Use of biotechnology tools in plant tissue culture

Biotechnology has been introduced into agricultural practice at a rate without precedent. Tissue culture allows the production and propagation of genetically homogeneous, disease-free plant material (Chatenet *et al.*, 2001). Cell and tissue in vitro culture is a useful tool for the induction of somaclonal variation (Marino G *et al.*, 1990). Genetic variability induced by tissue culture could be used as a source of variability to obtain new stable genotypes. Interventions of biotechnological approaches for in vitro regeneration, mass micropropagation techniques and gene transfer studies in tree species have been encouraging. In vitro cultures of mature and/or immature zygotic embryos are applied to recover plants obtained from intergeneric crosses that do not produce fertile seeds (Ahmadi *et al.*, 2010). Genetic engineering can make possible a number of improved crop varieties with high yield potential and resistance against pests. Genetic transformation technology relies on the technical aspects of plant tissue culture and molecular biology for:

- Production of improved crop varieties
- Production of disease-free plants (virus)
- Genetic transformation
- Production of secondary metabolites
- Production of varieties tolerant to salinity, drought and heat stresses

Germplasm conservation

In vitro cell and organ culture offers an alternative source for the conservation of endangered genotypes (Sengar *et al.*, 2010). Germplasm conservation worldwide is increasingly becoming an essential activity due to the high rate of disappearance of plant species and the increased need for safeguarding the floristic patrimony of the countries (Filho *et al.*, 2005). Tissue culture protocols can be used for preservation of vegetative tissues when the targets for conservation are clones instead of seeds, to keep the genetic background of a crop and to avoid the loss of the conserved patrimony due to natural disasters, whether biotic or abiotic stress (Tyagi *et al.*, 2007). The plant species which do not produce seeds (sterile plants) or which have 'recalcitrant' seeds that cannot be stored for long period of time can successfully be preserved via in vitro techniques for the maintenance of gene banks.

Cryopreservation plays a vital role in the long-term in vitro conservation of essential biological material and genetic resources. It involves the storage of in vitro cells or tissues in liquid nitrogen that results in cryo-

injury on the exposure of tissues to physical and chemical stresses. Successful cryopreservation is often ascertained by cell and tissue survival and the ability to re-grow or regenerate into complete plants or form new colonies (Harding, 2004). It is desirable to assess the genetic integrity of recovered germplasm to determine whether it is 'true-to-type' following cryopreservation (Day, 2004). The fidelity of recovered plants can be assessed at phenotypic, histological, cytological, biochemical and molecular levels, although, there are advantages and limitations of the various approaches used to assess genetic stability (Harding, 2005). Cry-bionomics is a new approach to study genetic stability in the cryopreserved plant materials (Harding, 2010). The embryonic tissues can be cryopreserved for future use or for germplasm conservation (Corredoira, 2004).

Genetic transformation

Genetic transformation is the most important aspect of plant cell tissue culture that provides the mean of transfer of genes with desirable trait into host plants with ultimate recovery of transgenic plants (Hinchee *et al.,* 1994). It has a great potential of genetic improvement of various crop plants by integrating with plant biotechnology and breeding programmes. It has a prioritized promising role for the introduction of agronomically important traits such as increased yield, better quality and enhanced resistance to pests, diseases and abiotic stresses (Sinclair *et al.,* 2004; Sharma *et al.,* 2010; Kumar *et al.,* 2016). Genetic transformation in plants can be achieved by either vector mediated (indirect gene transfer) or vector less (direct gene transfer) method (Sasson, 1993). Among vector dependant gene transfer methods, Agrobacterium mediated genetic transformation is most widely used for the expression of foreign genes in plant cells. Successful introduction of agronomic traits in plants was achieved by using root explants for the genetic transformation (Franklin and Lakshmi, 2003). Regeneration of disease or viral resistant plants is now achieved by employing genetic transformation technique. Successfully transgenic plants of potato resistant to potato virus Y (PVY) has been developed thus resolving a major threat to potato crop worldwide (Bukovinszki *et al.,* 2007).

Protoplast fusion

Somatic hybridization being achieved by protoplasm fusion is an important tool of plant breeding and crop improvement by the production of inter-specific and inter-generic hybrids. It involves the fusion of protoplasts of two different genomes followed by the selection of desired somatic hybrid cells and regeneration of hybrid plants (Evans and Bravo, 1988). Practically in the crop improvement programmes protoplast fusion is efficiently used as a mean of gene transfer with desired trait from one species to another (Brown and Thorpe, 1995). Somatic hybrids were produced by fusion of protoplasts from rice and ditch reed using electrofusion treatment for salt tolerance (Mostageer and Elshihy, 2003).

In the recent years, in vitro protoplast fusion tool has opened a way of developing unique hybrid plants by overcoming the barriers of sexual incompatibility. It has been applicable in horticultural industry to create new hybrids with increased fruit yield and better resistance to diseases. Successful viable hybrid plants were obtained when protoplasts from citrus were fused with other related Citrinae species (Motomura *et al.,* 1997). The potential of somatic hybridization in important crop plants is best illustrated by the production of intergeneric

hybrid plants among the members of Brassicaceae (Toriyama, 1987). In wheat crop for the purpose of gene pool recovery and improvement by resolving the problem of loss of chromosomes and decreased regeneration capacity, successful protocol of protoplasm fusion has been established and used for the production of somatic hybrid plants by using two types of wheat protoplast as recipient and protoplast of *Haynaldia villosa* as a fusion donor (Liu *et al.,* 1988).

In conclusion, plant biotechnology has the potential to play a key role in the sustainable production of fruit crops. However, there is enormous potential for genetic manipulation of some vegetative propagated Banana fruit crops in order to improve their disease and pest resistance. Plant tissue culture represents the most promising areas of application at present time and giving an out look into the future.

The areas range from micropropagation of ornamental and forest trees, production of pharmaceutically interesting compounds, and plant breeding for improved nutritional value of Banana crop plants, including trees to cryopreservation of valuable germplasm. All biotechnological approaches like genetic engineering, haploid induction, or Somaclonal variation to improve traits strongly depend on an efficient in-vitro plant regeneration system.

The rapid production of high quality, disease free and uniform planting stock is only possible through micropropagation. It may also be possible to incorporate other characteristics such as drought tolerance, thereby extending the geographic spread of some fruit crops for production, and thus contributing substantially to enhanced food security and poverty alleviation.

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