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Trends in the use of tissue culture, applications and future aspects

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Abstract

Plant tissue culture has developed widely incorporated into biotechnology, the agricultural systems being a key factor to support many pharmaceutical and industrial outcomes. Since 1902 there is vast progress in plant culture and its application has emerged having a great diversity in the science filed. Due to development and desire to grow on high scale production in the past few decades, tissue culture techniques were manipulated for improvement of plant growth, biological activities, transformation, and secondary metabolites production. A significant advance in techniques has been sought to deal with problems of low concentrations of secondary metabolites in whole plants. The augmented use of plant culture is due to a superior perceptive of plant oriented compounds and secondary metabolites from economically important plants. Due to development in modern techniques, several particular protocols have been developed for the production of a wide array of secondary metabolites of plants on a commercial scale. Plant tissue culture has to lead to significant contributions in recent times and today they constitute an indispensable tool in the advancement of agricultural sciences and modern agriculture. This review would enable us to have an analysis of plant tissue culture development for agriculture, human health and wellbeing in general.

Introduction:

Plant tissue culture is a method to culture the cells, tissue organs and other components of the plant following the aseptic *in-vitro* culture under a well-defined environment. In a simple way, if a part of the plant body is dissected into a small part which is called explant and that can be grown into a complete plant. The explant exhibits a very high degree of plasticity *in-vitro*, thereby allow the explant to develop into another type and this way a whole new plant can be subsequently regenerated.[1, 2] To grow a full fledge plant; any portion of plant can be grown into explants using the growth media.

The commercial production of medicinal and ornamental plants is growing worldwide. Their monetary value has significantly increased over the last two decades and there is a great potential for continued further growth in both domestic and international markets. In recent years there has been renewed interest in natural medicines that are obtained from plant parts or plant extracts. On the order of 40% or more of the pharmaceuticals currently used in Western countries are already derived or at least partially derived from natural sources. Ayurveda, the indigenous system of Indian medicine, describes thousands of plant species in detail. With its varied climatic zones, India has a rich diversity of medicinal herbs. The forest harbour a large number of plant species, but deforestation has been responsible for the rapid loss of medicinal plant wealth, such that many valuable medicinal plants are under the threat of extinction. Pharmaceutical companies depend largely upon materials procured from naturally occurring stands that are being rapidly depleted. Plant tissue culture is an alternative method of commercial propagation[3] and is being used widely for the commercial propagation of a large number of plant species, including many

medicinal plants. Application of traditional medicinal plants for human use has also been reported.[4]

Major ornamental pot plants such as Begonia, Ficus, Anthurium, Chrysanthemum, Rosa, Saintpaulia, and Spathiphyllum are being produced in the developed countries. About 212.5 million plants including 157 million ornamental plants amounting to 78% of the total production were reported.[5,6] The Netherlands dominates the export of ornamental plants including pot plants like Begonia, Ficus, Cyclamen, Philodendron, Saintpaulia, Spathiphyllum and Rhododendron[7]. About 156 ornamental genera are propagated through tissue culture in different commercial laboratories worldwide. The shares of major producers are The Netherlands (33%), Japan (24%), Italy (11%), USA (12%), Thailand (10%) and others (14%). The major exporting countries are The Netherlands (59%), Colombia (10%), Italy (16%), Israel (4%), Spain (2%), Kenya (1%) and others (18%). The four leading exporters (The Netherlands, Colombia, Italy and Israel) constitute about 80% of the world market. The share of the developing countries of Africa, Asia and Latin America is less than 20%.[7] Planting material of ornamental plants is in great demand for commercial production as well as for domestic gardens and landscaping. The better quality of planting material is a basic need for growers for boosting productivity. Scientists reported the use of biotechnological approaches to improve horticultural crop production.[8]

Plant tissue culture has developed widely incorporated into biotechnology, the agricultural systems being a key factor to support many pharmaceutical and industrial outcomes. Plant tissue culture allowed using its several traits through transgenic breeds for the benefit of farmers and companies helped in the reduction of pesticide application having better nutritional quality. This review discusses the various aspects of theoretical and practical's uses of plant tissue culture and current status of the technology. This review would enable us to have an analysis of plant tissue culture development for agriculture, human health and wellbeing in general.

History of plant tissue culture

It was first in 1902; the first reports of tissue culture having success was that of Gottlieb Haberlandt who was able to develop and maintain mesophyll cells with totipotentiality in culture. Since then the tissue culture was developed constantly with reports suggesting its use in the application of breeding programs, biopharmaceutical production and genetic biodiversity conservation. German Botanist Golliob Haberlandt is regarded as the father of plant tissue culture. He later continued work in the area and developed palisade tissue grew on knob's salt solution. Just in other years Hanning (1904) excised matured embryos and grew them *in-vitro* on a mineral salt sugar solution. This was a turning point when embryo culture was developed. It was then 1950s when tissue culture was used on a large scale by the orchid industry. After many years in 1972, Carlson et al. 1972 created the first somatic hybrid of *Nicotiana gluca* and *N.langschorffii* by fusion of their protoplast.[9-11]

Requirements in plant tissue culture

Media in plant tissue culture

The two-state of media are used depending upon the stage of explants, a solid culture medium is used initially to establish the explants, but it can also be placed directly into a liquid medium, when cell suspension is desirable.[12] Very similar to other culture methods of microorganism, plant tissue culture medium is made up of artificial nutrients supplemented with organic and inorganic nutrients which are required for the growth of plant explants. Solid media is just a gelling phase of liquid media. In basic, plants or explants would require three fundamental nutrients which include various ions, organic supplements and indeed a source of fixed carbon. [13, 14]

In basic, the media contain three components as below (Figure: 1)-

1. Essential requirements- Ions in the form of a complex mixture of salts.
2. Additional supplement-organic supplements such as vitamins and amino acids.
3. Carbon sources- usually supplied as sucrose.

Apart from this, a complete media would also contain auxin and cytokinin which is used as a growth hormone for the plant.[15] The success of explants growth is determined by the appropriate composition of culture medium used for the in vitro cultivation of the plant cells. There are profound effects of the medium, composition on the morphology of the tissues that grow out of explants. Excess of growth hormones auxin may cause fast proliferation of roots, and cytokinin may yield shoots if provided in excess. So a fine balance to be maintained throughout the initial phase to produce an unorganized growth or callus.[16]

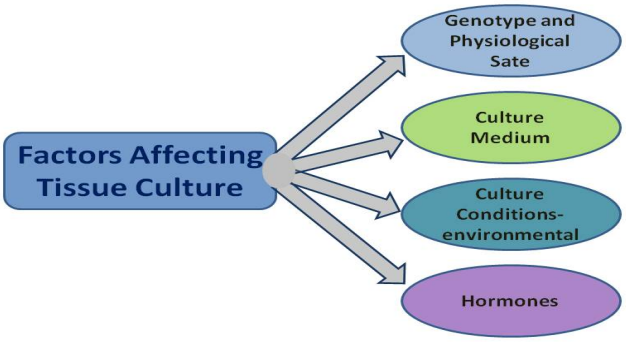

Figure 1: Basic requirement of plant tissue culture

To perform a successful culture the whole process required to be done under aseptic conditions using a laminar flow cabinet fitted with HEPA filtered. The source of explants is already heavily contaminated by the environment and therefore the part which is to be taken off for use is sterilized in disinfectant usually alcohol and sodium or calcium hypochlorite.[17-18]

Plant tissue culture process

The growth of explants rests on two fundamental properties of plant cells these are cell totipotency and cell plasticity.[18,19] These two properties of plant cells explore the capacity of living cells possess to develop into a new genetically identical cell and after differentiation processes would be able to form tissues, organs, systems and complete individuals.

In a well maintained controlled environment using defined culture media, a whole plant is generated from small tiny explants and this is now called "tissue-culture raised plants". The whole process is followed in aseptic conditions with raised plants are disease-free, having healthier root systems being more fibrous, and have a higher survival rate. While culturing most important is to have an appropriate medium together with auxin and cytokinin, which gives a good growth to explants into unorganized, growing mass of cells also called callus. Callus has a variable appearance in texture, and shape.[20] Explants follow through the mechanisms which trigger its growth from a cell or a tissue section and the rate of growth depends on various factors varies according to the age, species, type and of the tissue, the composition of the culture media and the environmental conditions manages its growth empirically.[21, 22]

Once the explants grow the required part of it is cut off and placed into an entirely fresh new media which will allow growth with altered morphology. All above an expert hand, skill together with the experience of the tissue culturist are other most important aspects required during a time when one needs to judge which pieces to culture and which to discard. For example, if shoots emerge it may be cultured freshly with auxin to produce plantlets which, if plotted in potting soil can grow further as normal plants.[23]

Important steps before starting tissue Culture Process

This section of the review will focus on the desirable steps required before starting plant tissue culture:

The first and basic steps of the culture are the requirement of nutritive media which can be liquid and solid based on the outcome uses.

1. **Media preparation**: The media should contain all basic components such as micro and microelements, amino acids, vitamins, iron source, and carbon source.[24]
2. **Aseptic culture propagation**: The whole entire culture requires the starting material normally shoot axillary or tip of a plant otherwise terminal bud or shoot.[25]
3. **Inoculation process**: as earlier said all the process till this to be done under septic and here in this step the desired explants are rooted into sterilized medium.[26]
4. **Explants development**: The media with explants are aseptically sealed and kept in a room for further development under diffused light 25±2^{0}C having 50 to 60% relative humidity.[26]
5. **Hardening of micro plants**: As in artificial condition the humidity and other requirement are conditioned developed and that because sometimes these plants from explants are not therefore ready for coping up in field conditions and they are hardened to catch up with field environment.[26,27]

These steps above are overall major steps to be followed during setting up a plant culture, but it finally begins depending on a genotype selected based on identifying a problem which is to solve as an outcome of this culture. For each specific type of genotype and target aim a particular protocol to be followed each time. In basic there are different moto plant cultures which are listed as plant micropropagation, mutagenesis, genetic transformation, etc, ad all

these are fundamentally required only two processes called organogenesis and somatic embryogenesis.[28]

Organogenesis

Organogenesis is a process of plant organs the formation from an explant with its determined tissue nature. This basically means that a single tissue organ is developed into a completely new plant. It is of two types the first one is a direct method if the shoots are obtained from the explants directly, and in the second method of indirect organogenesis occurs from formed callus of explants. In this, any organs such as roots, shoots or leaves are developed directly or indirectly from the meristem or undifferentiated cell masses (callus). This method involves callus production, adventitious meristem differentiation into organs.[29, 30]

Somatic embryogenesis

Somatic embryogenesis is *in vitro* plant regeneration method mainly used for sustained clonal propagation.[31] Here somatic cells or tissues are differentiated into embryos which consequently develop into whole plants by escaping the process of fertilization and no zygotic formation happens. Similar to organogenesis, somatic embryogenesis too initiated from explants directly or from a mass of unorganized cells indirectly. This follows the regeneration through the induction of embryogenic cultures and then germination takes place to plantlet developments that are transferred to soil.[32,33]

The somatic embryogenesis process has been successfully established in many plants of different families. Various factors affect the initiation and expansion of cultured somatic embryos. Various protocols are also tested for making this culture a growing event and protocol reported on grapevine using somatic embryogenesis showed higher plant sufficiently grows in a liquid medium. Somatic embryogenesis is not used for plant regeneration or mass plant propagation but it is a valuable genetic manipulation tool.[34] Genetic manipulation tools help the introduction of genes by genetic transformation.[35] Han et al. 2009 showed a successful protocol developed and used it for the regeneration of cotton cultivars resistance to *Fusarium* and *Verticillium* wilts.[36]

Stages of plant tissue culture

Plant tissue culture preparation

Though any section of the plant can be introduced as explants however its successful growth and establishment depend on sanity optimization and physiological environment requirement of the plant. To have maximum chances of growth explants should be provided with optimum cultivation conditions with sufficient nutrition, obligate temperature control with irrigation.[37,38] In culture condition explants can be more susceptible to infection and so fungicide, bactericide and/or insecticide are applied to prevent infection of microorganisms circulating throughout the tissues. According to requirement explants is also pretreated with appropriate growth regulators to improve the morphogenic response during the *in vitro* establishment.[39]

Stage of establishment

A most crucial and far difficult stage of an *in vitro* propagation system, as totally dependent on the physiological status of explants.[40] At this stage, the plant tissue is sterilized through disinfection (combined bactericide and fungicide products) superficial, and the chemical nature of disinfectant is decided based on the type of explants.[41] The most common disinfectants used for tissue culture disinfection are sodium hypochlorite, ethanol, and calcium hypochlorite.[37,38] Plant with more thick cellulose and lignin content which are woody or developed more in the soil would require extreme treatment of mercuric (II) and/or chloride ($HgCl_2$) wash.[32]

Propagation stage

At this stage, the numbers of culture units are increased in the desired number.[42] Again propagation stage requires a particular technique and protocols depending on species or the genotypes of a plant. For example, axillary shoots of sweet potato are propagated by organogenic propagation whereas coffee requires somatic embryogenesis for efficient propagation.[40,43] To achieve an optimum morphogenic response a perfect dose of plant growth regulators to be introduced.

Preparation of explants for the* ex vitro *conditions

This stage can run through together with propagation and multiplication of the explants in the same old or new media. Sometimes it's become necessary that media is to be changed and in such cases, it is necessary to change media with nutritional and growth regulator modification to induce rooting and/or shoot as per desirable protocol and requirement. Plants for the *ex vitro* phase are prepared by modifying media composition and exchange of gas inside the culture set up.[44]

Plant acclimatization

This is the adaptation stage of *in vitro* plants which face outside laboratories environment. Therefore it is of utmost importance that light intensity, temperature, the humidity of the room and substrate moisture is strictly maintained at optimum requirement. In vitro, a constant high humidity is maintained so that active roots are formed which facilitate adaptation in a real environment where water loss would be more.[45]

Uses and potential of plant tissue culture

The use of plant tissue culture can be explored based on plant cell behavior, plant modification, clonal propagation, product formation, germplasm storage and pathogen-free plants (Figure: 2). Plant tissue cultures have wide uses and being essential for much academic research of applied plant science. In academia plant tissue culture was used to explore for investigating its totipotency nature and analyzing the cytodifferentiation hormones roles in organogenesis. Tissue culture-based plant gives freedom to study genetically engineered species plants exploring the idea of gene regulation in transgenic species. Tissue culture techniques have many other

applications in the field of applied plant science, agriculture and plant biotechnology.[17] The very basic idea of using plant tissue culture is that it's easy to grow the cloned plants as suspended cells and can be harvested.[46] Genetically engineered cells are developed form transgenic whole plants also require tissue culture process; apart from this formation of somatic haploid embryos need the help of these techniques thus it has been, prominent in academic and applied plant science.[42,47]

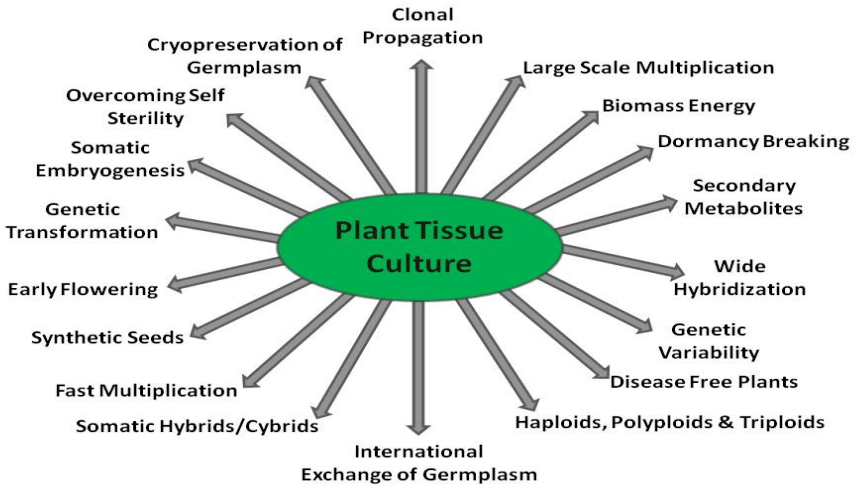

Figure 2: Different use and prospective of plant tissue culture

Applications of plant tissue culture

Meristem and shoot culture of the plant is used to produce large numbers of identical explants to be used in commercial production for potting, florist subjects, and landscape. This helps in conserving the one species which is rare and endangered. Explants or tissue culture and/or cells can be used to screen cells for herbicide resistance/tolerance, stress-tolerant plants which is time saving rather than plants.[46] A large scale of explants growth in liquid culture is done to scale up the production of plant-derived secondary metabolites and biopharmaceuticals through recombinant proteins.[48] This is even more useful when there is a need to produce related species by regeneration of the novel hybrid and/or protoplast fusion. Plant tissue culture is also tested for cross-pollination to get distantly related species to rescue the rare species.[49,50]

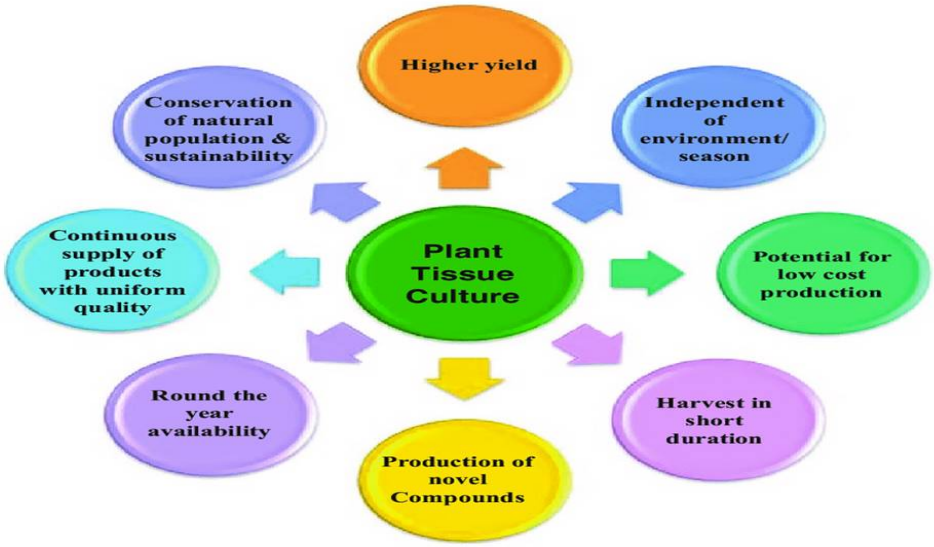

Figure 3: Applications of Plant tissue culture

The in-depth information on the use of plant tissue culture (Figure: 3) is provided below-

Transgenic plants in agriculture

To enhance the cultivation capability of crop and growth transgenic plants continues to be cultivated together with the massive propagation for agriculture worldwide.[51] *Disease-free plants:* Tissue culture allows *production and propagation of* disease-free plant material genetically homogeneous, thus this technique eliminates pathogenic organism's sensitivity of plants.[52]

Genetic transformation

One of the most used, developed techniques and most relevant tissue culture applications because of its practical use. The genetic transformation uses two methodologies; one is that direction in which desired genetic traits are introduced into one which is to be harvested. This method may or may not use a biological vector to carry and deliver genetic information. Another is an indirect way to introduced into the cell through natural mechanisms using a vector of biological origin and allows its integration in the plant's genome.[53]

Plants as bioreactors

Transgenic plants are the source for recombinant proteins of pharmaceutical and industrial interest in an industry with the potential of innovative capacity.[54]

Propagation and conservation

Tissue culture provides an alternative for managing valuable resources such as secondary metabolite through micropropagation helps in multiplication of the endangered species and conservation of plant biodiversity from a minimum available plant material.[55]

Other applications

In other application of plant culture, the *somaclonal variation* is one useful tool which helps in the induction of minute variation or differentiation within species.[56] Such techniques are required when there is a need to expose the explants with exact does to acquire the desired mutation.[57] Management and modification of the ploidy levels in plants can determine the expression of many traits and allows the generation of haploids homozygous lines (Scheid 1996), genetic transformation programs and hybridization.[58,59] *E*mbryo rescue has a potential role in breeding and genotype selection after fertilization otherwise individuals present abortion at early stage.[52] Species that lost the reproduction capacity and prevent seed germination can be grown using *e*mbryo rescue. Germplasm conservation enables to covers the loss of high rate disappearance of species and safeguards the floristic patrimony of the countries.[60-64]

Future aspects

Due to development and desire to grow on high scale production in the past few decades, tissue culture techniques were manipulated for improvement of plant growth, biological activities, transformation and secondary metabolites production. A significant advance in techniques have been sought to deal with problems of low concentrations secondary metabolites in whole plants, The sterile plantlets will overcome the contamination problem and reduce the time for the sterilization process. *In vitro* propagation for selective metabolite production is found highly useful for secondary metabolites and medicinally important compounds.

Summary and Conclusions

The augmented use of plant culture is due to a superior perceptive of plant oriented compounds and secondary metabolites from economically important plants. Plant tissue culture has become the most appreciable method when it is used in the production of metabolites and phytoconstituents often difficult to regenerate and conserve the species saving them from extinction. Due to development in modern techniques, several particular protocols have been developed for the production of a wide array of secondary metabolites of plants on a commercial scale. Plant tissue culture has to lead to significant contributions in recent times and today they constitute an indispensable tool in the advancement of agricultural sciences and modern agriculture.

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