

Investigation on Implications of Plant Tissue Culture

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ABSTRACT

Plant tissue culture is the most promising application areas for today's and potential agriculture of biotechnology methods in the very rapidly evolving biological science scenarios. The areas include micro propagating crops, ornamental or woodland, etc., developing medicinal substances and breeding plants to boost the nutritional value of staple crop plants, including trees for the cryopreservation of precious germplasm. Only micro propagation enables rapid development of high-quality, disease-free and standardized plant material. Anyway to the season and the weather, production can be done throughout the year. However, compared to traditional propagation methods by means of seed, cuttings and grafting, micro propagation technology is costly. Accordingly, steps to minimize manufacturing costs need to be implemented. Low cost plant production requires cost-effective practices and efficient use of equipment to decrease unit production costs. It can be accomplished by enhancing the performance of systems and efficient use of resources. The use of 'Bioreactor' in plant propagation will speed up crop propagation and growth, and reduce the requirements in space, energy and function. The cost of production can also be lowered by selecting several plants which offer the choice for the production of equipment and resource around the year and allow cost flow and optimal usage. Quality management is also extremely critical in order to ensure high-quality plant performance and the consumer's confidence. Selection of explants sources, disease-free products, authenticity of varieties and removal of somaclonal versions are some of the main criteria to guarantee the quality of seeds.

KEYWORDS: *Plant tissue culture, micro propagation, technology*

I. INTRODUCTION

The beginning of plant tissue culture research has its origins in cell discovery, preceded by cell theory propagation. Schleiden and Schwann suggested in 1838 that the cell was the fundamental structural unit of all living organisms. They visualized that the cell is capable of autonomy, so that each cell can be regenerated into the whole plant if it is given an atmosphere. In 1902, on this basis, Gottlieb Haberlandt attempted, for the first time, to cultivate single palisade cells in Knop salt solution enriched with sucrose, based on a German physiologist. The cells stayed alive for up to a month, grew in size, stored starch, but could not break up. Though unsuccessful, he laid the groundwork for technology for tissue culture for which he is called the "father" of tissue culture. Plant tissue culture is the *in vitro* aseptic culture, in controlled nutritional and environmental conditions, of cells, tissues, or organ or whole plant, also for plant clones (Thorpe, 2007). The clones result is true to the genotype picked. The regulated conditions establish a growing and multiplying climate for culture.

The requirements include the correct nutrient supply, medium pH, ample temperature and the right gas and liquid environment. The technology of plant tissue growing is generally used to multiply large-scale plants. In the field of seed propagation, diseases elimination, seed enhancement and development of second-hand metabolites by the use of small pieces of tissue (named explants) for hundreds of thousands of plants in a continuous process, despite their use as research tool, plant cultivation techniques have played an important role in recent years. In a relatively short time and under regulated conditions, a single explant can be multiplied into several thousand plants regardless of season and weather throughout the year (Akin-Idowu et al, 2009). Endangered, endangered and rare species were successfully cultivated and maintained through micro propagation due to high multiplication coefficients within less space. It is also considered by development of somaclonal and gametoclonal variants the most viable technology for improving crops.

Technological innovations in plant tissue culture

Plant tissues and organs are grown in *in vitro* in aseptic and regulated conditions, in the method of plant cell culture. The technique depends primarily on the conception of plant cell totipotentiality, which refers to a single cell's capacity for cell division of the entire genome (Haberlandt, 1902). Besides the plant cell's totipotential ability, cells are also equally necessary and essential to regenerate the entire plant for changing its metabolism, growth and development (Thorpe, 2007). The medium of cultivation of plant tissue contains all the nutrients essential for normal plants development and growth. It consists mainly of macronutrients, micronutrients, vitamins, organic ingredients, and regulators for plant growth, sources of carbon and some gelling agents for a solid medium (Murashige and Skoog, 1962). For the vegetative spread of several species of plants in *Vitro*, the

Murashige and Skoog medium (MS medium) is most commonly used. The media pH is also significant, both in plant growth and in plant growth regulators' behavior. For the cultivation of both solid and liquid media. The composition of the medium, in particular plant hormones and the source of nitrogen, has an important influence on the initial explants' response. The mechanism for production of plant cells and tissues in culture medium is determined by PGRs (Plant Growth Regulators). The most important uses of plant growth regulators are auxins, cytokinins and gibberellins. The type of hormones used and their concentrations depend primarily upon the plant, tissue or organ grown and the purpose of the experiment (Ting, 1982). The high auxin concentration usually promotes root formation, while the high cytokinin concentration promotes shooting regeneration. A balance between auxin and cytokinin leads to the production of masses of undifferentiated cells called calluses.

Tissue culture in pharmaceuticals

The culture of plants and tissues is highly promising to produce myriad of useful secondary metabolites in controlled manner. Plant cell crops are combined to produce useful secondary therapeutic metabolites in combined plant system with that of microbial and animal cultures. Biotechnological approaches, in particular plant tissue cultures, have been found to be potential as addition to conventional agriculture in the industrial development of bioactive plant metabolites to look for alternatives to the production of medicinal compounds from plants. In the last decade, a group of plant scientists and microbiologists has been investigating the biosynthetic capabilities of different cell cultures in several countries.

Tissue culture in agriculture

As an evolving technology, the culture of plant tissue impacts both on agriculture and the industry by supplying plants needed to fulfil the world's rising demand. In recent times it has made important contributions in the promotion of agriculture and is now an integral tool for modern agriculture.

In agricultural practise, biotechnology has been implemented at an unprecedented pace. Tissue culture enables genetically standardized, disease-free plant material to be produced and propagated. A valuable method for inducing somaclonal variation in cells and tissue in vitro culture. As a source of variability new stable genotypes might be used to achieve genetic variability caused by tissue culture. In vitro regeneration, mass micro propagation techniques and gene transfer experiments in tree species were motivating to intervene in the biotechnological approaches. A plant is applied in vitro to retrieve the plants from the intergeneric crosses of mature and/or immature zygotic embryos that do not contain fertile seeds. A variety of enhanced crop varieties with high yield potential and pest tolerance can be made possible by genetic engineering. Genetic transformation technology is focused on the scientific aspects of tissue cultivation and molecular biology for:

- Produce of plants without diseases (virus)
- Genetically transformed
- secondary metabolite production •
- Production of salinity, drought and heat stress tolerant varieties

Technological Protocols

Recent advances in micropropagation of plant cells in the field of their viable commercial applications have been rapidly established. The collection of the plant tissues (explants) from a healthy, vigorous mother plant begins for the micro propagation of planting materials (Murashige, 1974). Any part of the plant can be applied as explants (leaf, apical meristem, bud and root). The following steps can be summed up as

Stage 0: Preparation of donor plant- The mother plant should be cultivated ex vitro under optimum conditions in order to reduce contamination in in vitro culture to increase likelihood of success (Cassells and Doyle, 2005).

Stage I: Initiation stage- An explants is sterilized in the surface and transferred to the nutritive medium at this point. The combined use of bactericides and fungicides is commonly recommended. The product range depends on the type of explants to be used. Explants surface sterilization in chemical solutions is an essential move towards the removal of pollutants with minimal plant cell harm (Hussain and Anis, 2009). Sodium hypochlorite (Tilak et al, 2009), calcium hypochlorite (Garcia et al, 1999), ethanol (Singh and Gurung, 2009), and mercurical chloride are among the most widely used disinfectants (Hussain and Anis, 2009). The cultures are incubated by propagation method in a rising chamber under light and dark conditions.

Stage II: Multiplication process – This phase will increase the number of propagules by multiplying several subcultures, until the required number of plants is reached (Saini and Jaiwal, 2002).

Stage III: Rooting- The rooting stage will occur in the same culture media used to multiply the explants at the same time. In certain cases, however, a change in media, including dietary changes and the composition of growth regulators is required to induce rooting and the growth in strong root growth.

Stage IV: Acclimatization stage- At this time, weaning and hardening of the in vitro plants. High to medium humidity and low intenseness of light to high light intensity are used gradually to harden. The plants are transported to a suitable substratum (sand, peat, compost etc.) and hardened under greenhouse gradually.

Explants Size and Thin Section Culture System

The most important advance in plant tissue culture will likely be the inducing of a desired morphogenic event in vegetative tissues by effective *in vitro* manipulation. The effectiveness of such driven morphogenic events is largely determined by the tissue itself. Various factors associated with explants seem to affect the organizational capacity of the cultivated tissue (Benson, 2000). These include growth situations, whole plant physiology and the source plant genotype. Furthermore, there has been a negative association between the size of the explants and the number of cells theoretically available for organographies (Lakshmanan et al, 1995 & 1996,). Lakshmanan et al (1995) showed in a previous report that *in vitro* orchid protocorms development can be improved considerably if explants size alone is manipulated. For example, a single shooting tip (6-7 mm long) created by small, transverse sections (0,6 mm thick) had 5 times the number of protocorms produced by an intact shooting point (6–7 mm in length) grown under the same conditions. There has also recently been a related observation on sugar cane. Leaf explants have produced several plants in this crop (>50 per explants) when their explants thickness has been reduced to 1 to 2 mm. This result shows clearly that the size of the explants plays a major role in the organogenic ability of the cultivated tissue. This examines the size gap in organogenic ability, which has since been successfully applied in developing a new approach in plant regeneration, a small section culture method.

In vitro Environmental Conditions

There are some important basic research points that could strengthen our understanding and thus our ability to manage regeneration and growth in *in vitro* facilities. It is possible to track or constantly regulate the medium composition by recirculate the liquid culture systems. We have to learn more about mineral nutrition dynamics *in vitro* (Williams, 1995). Light quality is one of the potentially significant environmental factors, as it has been shown that it influences the course of plant morphogenesis *in vitro* (Morini et al, 2000). Tissue cultivation processes often involve extensive cuts and tissue stress damage, which can trigger physiological changes in plants which affect success (Leon et al, 2001).

Scientific-cum-commercial applications of plant tissue culture technology

Today applications for plant tissue culture include much more than micro propagation and clonal propagation. Somatic embryogenesis, somatic hybridization, and viral removal as well the application of bioreactors to mask and generate secondary metabolites have been extended to include the spectrum of routine technologies. Two fields of general interest can be classed in the applications of plant tissue cultivation:

Culture of plant cells and tissue Basics Plant tissues and organs are cultivated under aseptic and controlled conditions *in vitro* on artificial media. The technique is mainly based on the idea of plant cell totipotentiality that refers to a cell's ability to express the complete genome by cell division. Together with the totipotent potential of a plant cell, it is also essential and critical to regenerate the entire plant to change the cells' metabolism, growth and development. The medium of cultivation of plant tissue contains all the nutrients essential for normal plants development and growth. It mainly consists of macronutrients, micronutrients, vitamins, other organic ingredients, regulators for plant growth, the source of carbon, and gelling agents for solids. For the vegetative spread of several species of plants *in vitro*, the Murashige and Skoog medium (MS medium) is most commonly used. The media pH is also significant, both in plant growth and in plant growth regulators' behavior. For the cultivation of both solid and liquid media. The composition of the medium, in particular plant hormones and the source of nitrogen, has an important influence on the initial explants' response.

Embryo culture

Embryo culture is a kind of tissue plant culture used in the development of embryos in a nutrient medium out of seeds and ovules. In the area of embryo cultivation, the plant grows from the embryo directly or indirectly, by callus formation and then root and root formation. The technology for breaking seed dormancy, for checking seeds vitality, development of rare species and haploid plants has been developed. It is an efficient technique used by growing excised embryos to reduce the reproductive cycle of plants and to reduce the dormancy time of seeds. With a clear objective of mass multiplication, intra-varietal hybrids of an economically significant power plant "Jatropha" were successfully developed. In embryo cultivation of Jucara Palm for rapid cloning and enhancement of selected individuals somatic embryogenesis and plant regeneration has been performed. Moreover, it can also be done through the embryonic culture method to protect endangered species. A procedure to spread *Khaya grandifoliol* *in vitro* by excising embryos from mature seed has recently been developed. For wood and medicinal purposes, the plant has high economic value. This technique provides a significant application in forestry, by providing a means to disperse elite persons, where natural populations are difficult to pick and improve.

Current and future status of plant tissue culture

The previous many years of plant cell biotechnology has developed as another period in the field of biotechnology, zeroing in on the creation of an enormous number of auxiliary plant items. During the second 50% of the only remaining century the advancement of hereditary designing and sub-atomic science strategies permitted the presence of improved and new rural items which have involved an expanding request in the beneficial frameworks of a few nations overall. By and by, these would have been incomprehensible without the improvement of tissue culture strategies, which gave the instruments to the presentation of hereditary data into plant cells. These days, perhaps the most encouraging techniques for creating proteins and other restorative substances, for example, antibodies and immunizations, is the utilization of transgenic plant. Transgenic plants address a prudent option in contrast to aging based creation frameworks. Plant-made immunizations or antibodies (plantibodies) are particularly striking, as plants are liberated from human infections, along these lines decreasing screening costs for infections and bacterial poisons. The quantity of ranchers who have consolidated transgenic plants into their creation frameworks in 2008 was 13.3 million, in contrast with 11 million out of 2007.

II. OBJECTIVE OF THE STUDY

1. To investigate technical advances in tissue cultivation
2. To research the main factors that influence in vitro growth

III. CONCLUSION

Culture of plant tissue reflects currently the most promising areas of application and offers a potential outlook. These areas range from the micro propagation of ornamental and forest trees, pharmaceutical compounds processing, and plant breeding, to improved nutritive value of staple crop plants, such as trees, to a valuable germplasm cryopreservation. An effective in-vitro regeneration system is a crucial element in all biotechnological methods such as genetic modification, haploid induction and somaclonal variations. Only micro propagation enables rapid development of high-quality, disease-free and standardized plant material. New opportunities for growers, farmers and nursing owners for high quality fruit, ornamental, forest tree and vegetable planting material were established. Farm production throughout the year b c Plant fabric culture: current status and opportunities 19 regardless of weather and season. However, compared to traditional propagation methods by means of seed, cuttings and grafting, micro propagation technology is costly. Accordingly, steps to reduce production costs are important. Low cost plant production requires cost-effective practises and efficient use of equipment to decrease unit production costs. It can be accomplished by enhancing the performance of systems and efficient use of resources. Plant propagation based on bioreactors can speed up the propagation and growth of crops and reduce space, energy and labour demand when commercial propagation begins.

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