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Plant Tissue Culture: A Review

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ABSTRACT

In the last two decades plant biotechnology applications have been widely developed and incorporated into the agricultural systems of many countries worldwide. Tissue culture tools have been a key factor to support such outcomes. Current results have allowed plant biotechnology and its products –including transgenic plants with several traits- to be the most assimilated technology for farmers and companies, representing several benefits. The rapid and extensive assimilation for this technology has improved the competences of the agricultural systems both in industrial and in developing countries, based on the proper application of research programs. This review discusses the historical perspective, principles, basic requirement and stages of plant tissue culture. It also includes cryopreservation of cultured plants, use of biotechnology tools in plant tissue culture and production and propagation of disease-free plants by tissue culture.

INTRODUCTION:

The increase in food demand worldwide, as sociated with unequal distribution and the dis equilibrium in the distribution of wealth, has caused an increasingly important pressure on food producers who, in parallel, have increased their requirements for new technologies that allow greater yields and better quality of the products that they offer (Christou and Twyman, 2004). While at the same time, there has been an increasing consumer-led demand for lower environmental damage and greater sustainability in the food production chain. During the second half of the last century the development of genetic engineering and molecular biology techniques allowed the appearance of improved and new agricultural products which have occupied an increasing in the productive systems of several countries worldwide (Vasil, 1994a; Christou et al., 2006; Navarro Mastache, 2007; James, 2008). Nevertheless, these would have been impossible without the development of tissue culture techniques, which provided the tools for the introduction of genetic information into plant cells, the selection of the plants, which carried the genes of interest and at the same time, the massive and rapid multiplication of the genotypes that finally could be introduced into the production systems. The technology of transgenic plants is only one of the diverse applications that tissue culture has in plants because it is a tool of great versatility, which can contribute to solve various problems that affect humanity, not only necessarily related with food production (Pareek, 2005). Throughout this review, the basic principles over which tissue culture techniques and the practical applications of the technology rest upon will be approached, together with the advances made to date. Plant tissue culture background Tissue culture may be defined as the aseptic culture of cells, tissues, organs or whole plants under controlled nutritional and environmental conditions (Thorpe, 2007). The first reports regarding tissue culture date back to the beginning of the 20th century when Gottlieb Haberlandt (Haberlandt, 1902) developed experiments to maintain mesophyll cells in culture based on postulates which established the “totipotentiality of plant cells”. From this moment on, development has been constant and every year hundreds of results and reports regarding the application of tissue culture techniques, applied to breeding programs, genetic biodiversity conservation and biopharmaceutical production are documented. The development of tissue culture techniques rest upon two properties of plant cells: cell toti potency (Vasil and Hildebrandt, 1965) and cell plasticity (Thorpe, 2007). Cell totipotentiality is the genetically retained capacity that all living cells possess to originate a new genetically identical cell, and, after cellular division and differentiation processes, to be able to form tis sues, organs, systems and complete

individuals (Haberlandt, 1921; Takebe et al., 1971). Cellular plasticity is the characteristic which marks the difference between plant and animal cells in their capacity of multiplication, division, differentiation and formation of a new individual. As opposed to animals, plants are sessile organ isms often with long life cycles which has meant that they have been forced to develop defence and survival mechanisms in order to face different negative biotic as well as abiotic factors. This capacity of modifying response allows plant cells to respond to external stimuli directed towards the achievement of a determined response. The development of tissue culture techniques Plant tissue culture can begin once a genotype is selected on the basis of having identified a problem to be solved and the appropriate type of protocol to deal with it. Tissue culture techniques for plant micropropagation, genetic transformation, biotech assisted selection, mutagenesis, etc, rest on two fundamental morphogenesis processes: organogenesis and somatic embryogenesis. Organogenesis is the formation of plant organs from a determined tissue in order to form complete plants, characterized by being polar, which means that only one aerial organ or root is emitted and from this a new complete plant is regenerated. At the same time, organogenesis may be direct, if the organogenic shoot is directly obtained from the explants, or indirect, if the organogenic process occurs from previously formed callus in the initial explants (Vijaya and Giri, 2003). Somatic embryogenesis is the production of embryos from somatic plant cells (any non-sexual cell) to obtain a complete plant. Unlike organogenesis, this is a polar process where the aerial structures and roots of the plants are obtained from the somatic embryo. It can also be direct or indirect, if the process originates from the initial explants or from previously induced callus. Somatic embryogenesis consists of four fundamental stages: A) Callus induction; B) Embryo formation and proliferation; C) Embryo maturation; and D) Embryo germination. At the same time, the embryos may pass through four stages in their development, the globular form, the heart form, the torpedo and the cotiledonary forms (Ammirato, 1983). Each one of the stages of somatic embryogenesis, just as the different phases of normal embryo development, depend on the species and on the genotypes which a being cultured.

HISTORICAL PERSPECTIVE OF PLANT TISSUE CULTURE:

Plant tissue culture is the science of growing plant cells, tissues, organs, or any plant parts isolated from the mother plant, on artificial media under aseptic conditions (*in vitro*). The science of plant tissue culture takes its roots from path breaking research in biology like discovery of cell followed by propounding of cell theory. It was Gottlieb Haberlandt who in the first decade of this century pioneered the field of plant tissue culture. His idea was to

achieve continued cell division in explanted plant tissue culture. Following the discovery and use of auxins, the work of Gautherel, Nobecourt and White ushered in the second phase of plant tissue culture over 30 years ago. These and other scientists determined the nutritional and hormonal requirements of the cultured plant tissues. Rapid advances in diverse aspect of plant tissue culture have been made during the last few years and plant tissue culture techniques have been extensively applied to agriculture and industry.

PRINCIPLES OF PLANT TISSUE CULTURE:

As a principle, plant cells, tissues, organs and any other parts of the plant are cultured *in vitro* on artificial nutrient media, under aseptic and controlled environment. It is well known that starting plant materials from the field are naturally contaminated on their surfaces (and sometimes interiors) with microorganisms, thus surface sterilization of starting materials (explants) in chemical solutions (usually alcohol or bleach) is very important. After that the explants are usually placed on the surface of a solid culture medium, but are sometimes placed directly into a liquid medium, particularly when cell suspension cultures are desired. Plant tissue culture depends mainly on the concept of totipotentiality of plant cells which refers to the ability of a single plant cell to express the full genome by cell division and/or to grow and develop into a fully differentiated plant. In addition to the totipotent potential of plant cell, the capacity of cells to alter their metabolism, growth and development is also equally important to regenerate the entire plant. The culture medium contains all the necessary nutrients required for the normal growth and development of plants. It is mainly composed of macro and micro nutrients, vitamins, amino acids, plant growth regulators, carbon source and some gelling agents in the case of solid culture medium. The pH of the media is also very crucial since it affects both the growth of plants and activity of plant growth regulators, thus it has to be adjusted at the critical value. The culture medium composition, especially the plant growth regulators and the nitrogen source have profound effects on the response of the initial explant. In general, there are a number of tissue culture techniques and they can be employed for different purposes. More recently, plant tissue culture has been given the highest priority in plant breeding work since it provides immense potential for crop improvement programs such as selecting disease/insect, or stress.

BASIC REQUIREMENT OF PLANT TISSUE CULTURE:

There are some important aspects of tissue culture. These are: (A) Aseptic condition (B) Aeration (C) Equipments 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 equipments 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.

STAGES OF PLANT TISSUE CULTURE:

1. Preparation of nutrient medium: A semi-solid medium is prepared in double distilled water containing macro-elements, micro elements, amino acids, vitamins, iron source, carbon source like sucrose and phyto-hormones. The medium is heated for dissolving the agar and 25 to 50 ml is dispensed into each wide mouth bottles. The vessels containing culture media are then sealed and sterilized by autoclaving.

2. Establishment of aseptic culture: The starting material for the process is normally an actively growing shoot tip of auxiliary or terminal bud or shoot tip of a plant. The process of tissue culture starts from the selection of mother plants having the desired characteristics. Ex-plant preferably the meristematic tissue of the selected mother plant is isolated. The excised tissue/explant is washed with water and then rinsed with a disinfectant such as savlon or dettol solution followed by a sterile-water wash. The tissue is then dipped in 10% bleach solution for ten minutes for disinfecting the plant tissue material, killing most of the fungal and bacterial organisms. Sterilization process of explants depends on the plant species and types of explants.

3. Inoculation: Inoculation is carried out under aseptic conditions. In this process explants or micro shoots are transferred on to the sterilized nutrient medium.

4. Development of plants in growth room: After the inoculation of the plant tissue, the bottles are sealed and transferred into growth room to trigger developmental process under diffused light (fluorescent light of 1000-2000 lux) at $25 \pm 2^{o}C$ and 50 to 60% relative humidity. Light and temperature requirements vary from species to species and sometimes during the various stages of developments. The cultures are observed daily for growth and any signs of infection/contamination. Cultures, that do not show good growth or infected, are discarded. The healthy cultures grow into small shoot buds. These are sub-cultured on the fresh medium after 4 weeks. The number of subcultures required is specific to the plant species, which are standardized. The shoots generally develop after 4 weeks. After enough number of shoots is developed in each container (10 to 15), to a minimum height of 2 cm they are transferred to another medium for initiating the process of rooting. The constituent of rooting medium for each plant species are specific. Roots are generally formed within 2 to 4 weeks. Plants at this stage are delicate and require careful handling.

5. Hardening of micro plants: Due to very high humidity inside the culture vessel and artificial conditions of development, the plantlets are tender and are therefore are not ready for coping up with the filed conditions. The plants removed from the sterile medium are washed and are maintained under intermittent mist or are covered with clean transparent plastic. After 10 to 15 days under high humidity, the plants are transferred to green house and maintained for another 4 to 6 weeks. They are then ready to be transferred to net house or the field. Normally, the tissue culture plants are sold either as ex-agar plants or hardened plants from the green house.

A: Ex-agar plants: Depending on the parameters such as location/the site of planting, soil quality and the climatic conditions defined by the customer, the ex-agar plant for sale could be *in vitro* rooted plants or only the shoots. When the tissue culture plants are sold at this stage, the plants are washed in sterilized water to remove the agar medium. The washed plants are sorted into 2 to 3 grades and packed in corrugated plastic boxes lined with sterilized tissue paper as per specifications of the Plant Quarantine Authority, Government of India for exports. The number of plants per box depends on the customer's requirement. Depending on the final destination and the preference of the customer, the plants are treated with specific fungicides and antibiotics to avoid infection. The ex-agar plants are preferred for export or for destinations where hardening facility are available. The plants after being removed from nutrient media should preferably be transplanted within 72 hours.

B: Hardened plants: The plants are transferred to net pots/ pro tray for acclimatization after they fully develop shoots and roots in the bottles. The rooted plantlets are transferred to pots filled with suitable substrate and are watered. This operation is carried out on an open bench. These pots are then transferred to the green house for 4 to 6 weeks. During this process, they are given fertilizers and treated like plantlets obtained by any other means of propagation. After the plants are acclimatized fully, they are transferred to poly-bags. At this stage the plants are completely hardened and are ready to be planted in the field for cultivation. Hardening units can be set up in sites away from the micro-propagation unit.

SLOW GROWTH OF CULTURED PLANTS:

Slow growth is usually achieved by reducing the culture temperature, by modifying culture media with supplements of osmotic agents and growth inhibitors, or by removing growth promoters to reduce the cellular metabolism of the material, striving to maximize the time between subcultures (Gonçalves and Romano, 2007 and Scherwinski-Pereira et al., 2010).

Osmotic regulators, such as sucrose and mannitol, act as growth retardants by causing osmotic stress to the material under conservation. When added to the culture medium, these carbohydrates reduce the hydric potential and restrict the water availability to the explants (Fortes and Scherwinski-Pereira, 2001). Besides temperature and osmotic regulators, growth regulators are also routinely used for *in vitro* germplasm conservation, with abscisic acid (ABA) being one of the most used.

MICROPROPAGATION:

Micropropagation has the potential to provide very high multiplication rates of selected tree genotypes, with resulting short-term silvicultural gains. Aseptic cultures have been established from seeds, seedlings, shoots, flowers and lignotubers. Callus cultures have been established from a wide range of tissue sources for at least 30 species of Eucalyptus. Plant regeneration from callus was successful for 12 of these species. Micropropagation through axillary proliferation, or adventitious shoot proliferation on nodal explants, or both, has been successful. An agar-based medium of Murashige and Skoog with a low auxin/cytokinin ratio is most commonly used for shoot multiplication. Vitrification and shoot senescence remain problems. Gibberellic acid was added in some media to stimulate shoot elongation. Various media are used for *in vitro* root initiation. Suspension and protoplast cultures have been achieved and plants have been regenerated from protoplasts. *In vitro* techniques are presently being applied to Eucalyptus to achieve genetic transformations.

Advantages of Micro-propagation Technology:

Micro-propagation has several advantages over conventional methods of propagation such as:

1. Rapid multiplication: Micro-propagation offers rapid multiplication of desired plant species.

2. Requirement of only limited number of explants: Small pieces of plant (explants)/tissue can be used to produce a large number of plants in a relatively small space.

3. Uniform or true to type plants: Micropropagation provides a high degree of phenotypic/physical uniformity. Since the production cycle takes place under controlled conditions, proper planning and scheduling based on the market demand is possible. The resulting product has very high degree of uniformity compared with traditionally propagated plants.

4. Germplasm storage: Plants can be stored *in vitro* in a small space and less labour is required for maintenance of stock plants.

5. Disease free planting material: Plantlets produced by tissue culture are usually disease free. With proper diagnosis and treatments, elimination of fungus, bacteria and virus prior to large scale propagation is possible. With the help of serological and molecular technique it is possible to index virus of mother plant/explant which is to be used for mass multiplication.

6. Growth manipulation: Nutrient levels, light, temperature and other factors can be more effectively controlled to manipulate the growth, multiplication and regeneration.

7. Round the year production: Micro-propagation is independent of season. As micro - propagation could be carried out throughout the year; production cycle can be scheduled to meet peak demands.

CRYOPRESERVATION OF CULTURED PLANTS:

Cryopreservation is a storage method of plant genetic resources at ultra-low temperature, for example, that of liquid nitrogen (LN; -196°C). It is a preservation method that enables plant genetic resources to be conserved safely, and cost-effectively. For successful cryopreservation, it is essential to avoid intracellular freezing and induce the vitrification state of plant cells during cooling in LN. In addition, the cryopreservation method should be a simple protocol for everyone to use easily. Since the 1970"s, cryopreservation techniques have been researched using different plant organs, tissues and cells. As a result, different cryopreservation procedures have been developed (for example, slow-prefreezing method, vitrification method, dehydration method). With the development of these cryopreservation methods, tissues of tropical plants, which have been conventionally thought to be not cryopreserved, also were successfully preserved in LN (Bajaj, 1995; Towill and Bajaj, 2002). Cryobionomics is a new approach to study genetic stability in the cryopreserved plant materials (Harding, 2010). The embryonic tissues can be cryopreserve for future use or for germplasm conservation (Corredoira et al., 2004).

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 cryoinjury 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 „trueto-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). According to Sengar et al., plant tissue culture technique offers an alternative source for the conservation of endangered genotypes and/or species. Filho et al. reported that germplasm conservation 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. 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. 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 technique 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. It is desirable to assess the genetic integrity of recovered germplasm to determine whether it is 'true-to-type' following cryopreservation.

SOMACLONAL VARIATION:

In nature, the genetic diversity and variability within a population are generated via recombination events. Factors such as natural selection, mutation, migration and population size influence genetic variability in different ways. However, genetic variation arising from tissue culture of plants has been termed somaclonal variation. Variation has been observed in a wide range of species from plants derived from a variety of explants, using different tissue culture techniques. Crop improvement is a multi-disciplinary activity concerned with the optimization of genetic attributes within the constraints of the environment and of environmental factor within the constraints of the genetic material. Conventional breeding exploits the natural variation existing in plant populations to recover elite crops. However, the

available genetic variability in gene pools is one of the limits to crop improvement. The evolution of the theoretical aspect of *in vitro* culture paved the way for the emergence of its practical applications which reached exploding proportions in the past decade or so. Initially used for clonal propagation of plants, it later pioneered several new possibilities like removal of sexual incompatibility by embryo rescue techniques, somatic hybridization through protoplast technology, transgenic plant through genetic engineering, production of haploids via anther culture and most important in the context of the present issues i.e. the induction of new genetic variability and selection of desirable traits like salt tolerance, disease resistance and pest resistance. Thus, *in vitro* culture, no longer sacrosanct, has emerged as biotechnological tool to widen the germplasm base. *In vitro* culture is a rich source of genetic variation. The best available germplasm may be subjected to a tissue culture cycle with or without selection pressure and regenerants selected for superiority of one or more traits while retaining all the original characters. Such incremental improvement in desirable traits could therefore lead to the formation of new alleles spontaneously generated *in vitro*. Thus, tissue culture techniques leading to somaclonal variation could be capitalized upon to accelerate progress in conventional breeding since in the plant breeder's perspective however, the bottom line, remains ultimately that the genetic variability recovered from tissue culture regenerated plants should result in a phenotype that is agriculturally useful. It has also been most successful in crops with limited genetic systems and/or narrow genetic bases, where it can provide a rapid source of variability for crop improvement. Cell and tissue *in vitro* culture is a useful tool for the induction of somaclonal variation because the tissues may be cultured with little differentiation or allows the culture of isolated cells (Marino and Battistini, 1990). This makes it easier to correctly dose the concentrations of the mutagenic agents, locate the mutagenic activity in the tissues with greater potential to be mutated, prolong the exposure to mutagens and regenerate plants with high levels of efficiency (Ravindra et al., 2004). The downside is that some tissues more readily mutate even when simply exposed to plant growth regulators or other "normal" media components (Rzepka Plevnes et al., 2009). Whether genetic variability induced by tissue cultures could be used as a source of variability to obtain new genotypes, or whether this variation can be disadvantageous for keeping the genetic fidelity of the *in vitro* propagated material, it is very important to assess the genetic stability of the plants during or after tissue culture.

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). The morphogenic response of tissues to determined stimulus, resulting in the production of organs or complete plants, is known as plant regeneration (Tisserat, 1985). Plant regeneration is one of the sine qua nom factors from which the establishment of a genetic transformation protocol depends.

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.

USE OF BIOTECHNOLOGY TOOLS IN PLANT TISSUE CULTURE:

Secondary metabolite production from medicinal plants has accelerated in the last decade. In land conditions, both internal factors such as the genotype, plant organ and age of the plant, and external factors such as photoperiod, temperature, soil type, light intensity and wavelength, and amount of water available and climatic conditions affect the concentration and content of compounds obtained from medicinal plants. The reaction of plants to stress factors varies depending on the intensity and duration of stress. The above-mentioned factors may also affect the content of medicinal plants produced *in vitro*. In a study by Kapoor et al., it was revealed that the quality of light in callus cultures of the medicinal plant Rhodiola imbricata is an important factor in the growth rate of callus biomass and in the production of industrially important secondary metabolites. Biomass and alkaloid production of

Catharanthus roseus increased by the inclusion of 200 mg/L tryptophan or phenylalanine as a nitrogen source in the B5 nutrient medium with a pH of 5.82. The response of plants to abiotic stress factors consists of four stages in general: 1- Initial alarm phase, 2- Acclimation phase, 3- Maintenance phase and 4- Exhaustion phase.

PRODUCTION AND PROPAGATION OF DISEASE FREE PLANTS BY TISSUE CULTURE:

Tissue culture allows the production and propagation of genetically homogeneous, disease-free plant material. For these, “cleanup” techniques to eliminate plant pathogenic organisms have been developed, such as meristem cultures or explant disinfection treatments through chemical or physical methods (Chatenet et al., 2001). Meristems are the growing points of the plants and are located in the apices, lateral buds and roots. Meristems have low development of vascular tissues which means that virus, bacteria or fungi presences in this tissue are lower compared to other tissues in the plant. Using meristem isolation culture in nutritive medium it has been possible to obtain a high percentage of plants free of bacterial or fungal diseases, which can then be propagated disease free (Rzepka-Plevnes et al., 2009). In the case of berries, specially Fragaria sp., nique in order to eliminate viral, -i.e. Fragaria sp.: Strawberry mild yellow-edge virus (SMYEV), Strawberry vein banding virus (SVBV), Strawberry crinkle virus (SCV), or fungal diseases -i.e. Fragaria sp.: V. dahliae, Sphaeroteca macularis, S. humili, Botrytis cinerea, relevant to Chilean farmers by allowing the production and large scale propagation of disease-free plants (McInnes et al., 1992). For woody species, meristem cultures have also been efficient for the elimination of virus and the consequent massive multiplication of disease-free plants (Popesku et al., 2010; Tan et al., 2010). However, the technology does have some limitations since it has been found that meristems can be strongly dependent on the cytokinin: giberellin ratio as demonstrated for beans cv. Zorin (Phaseolus vulgaris L.). In this case, plant growth regulators in the basal medium had a significant influence on plant survival during ex vitro acclimatization - also called acclimatationproducing more vigorous plants (Benedicic et al., 1997). Thus it means the techniques sometimes take longer to develop initially.

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