Plant tissue culture-Agriculture and health of man

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Abstract

Plant tissue culture offers tremendous opportunities in plant propagation, plant improvement and production of plants with desirable agronomical features. It is now possible to develop methods for virus free plant regeneration, salinity tolerance, herbicide resistance, disease resistance, frost resistance, incorporation of high protein content and genetically engineer plants for desirable traits. The medicinal plants are rich in secondary plant products (active principles) are termed medicinal; exert a profound physiological effect on man, curing many ailments and diseases of man. In vitro grown plant cells and tissues have been used extensively for the production of secondary metabolites, which are the source of various pharmaceutical and industrial products. Crop plants play an important role in the human nutrition and health by providing carbohydrates, proteins, fats, minerals, vitamins, antioxidants, phytosterols and dietary fibers. Plants can be used to produce pharmaceutical proteins for immunization, enzyme therapy or as a precursor for any pharmaceutical products. Transgenic plants can be used as bio-factories for producing pharmaceutical and industrial chemicals and raw materials. Plant tissue cultures were used in achieving a specific biotransformation to produce a cardiovascular steroid digoxin or methyl digoxin from digitoxin of *Digitalis lanata*. Cell cultures of *Stevia rebaudiana* can convert steviol into steviobiocide and stevioside which are 100 times sweeter than cane sugar. The role of plant tissue culture in meeting the ever increasing demand and requirements of man in the field of agriculture, forest, horticulture and medicine is highly impressive.

Keywords: Embryo culture, protoplast culture, somatic hybridization, secondary metabolites, micropropagation.

Introduction

Human population is likely to double in about 35 years and more than 10 billion will have to be fed, clothed and provided with jobs under the conditions of shrinking land and water resources, expanding abiotic and biotic stresses, increasing genetic erosion and raising cost of fuel energy reserves (Swaminathan, 1992). With a growing demand for the economic products of plant origin, relentless efforts are being made to enhance plant productivity and quality of production and also to develop elite plants of agronomic value through newer technologies as that of plant tissue culture. Plant tissue culture broadly refers to *in vitro* cultivation of plants, seeds, and plant parts (stem, root, leaf, flower, ovule, anther etc.). To date several plant species representing annuals and perennials, herbaceous and woody plants, monocots and dicots, self and cross pollinated plants have been cultured *in vitro* and regenerated into complete plants. The plant tissue culture techniques offer opportunities in plant propagation and plant improvement in all types of crop plants. Hence it has been possible to develop crops of pathogen resistance, increased tolerance, greater yield, and also to engineer plants with desirable characters. Plant tissue culture includes different methods/techniques like embryo culture, anther culture, endosperm culture, protoplast culture, somatic hybridization, synthetic seeds, *in vitro* secondary metabolite production, micropropagation, cryopreservation etc (Basavaraju, 2005).

Micropropagation: The multiplication of plants through tissue culture referred to as micropropagation offers many advantages over conventional methods of propagation such as rapid and large scale multiplication of productive plants under *in vitro* conditions, irrespective of season, conservation of space and time as well as production of virus-free plants. The technique essentially involves removal of a bit of tissue or cells from leaf, stem, root, floral parts etc. from a healthy donor plant and grow each of them into whole plants on an appropriate artificial nutrient medium in a culture vessel under controlled conditions of light, temperature, and humidity. Murashige (1974) outlined three major stages involved in micropropagation via. preparation and establishment, proliferation or multiplication, rooting and hardening. Plants developed under these conditions are transferred to green house for acclimatization and there after put forward to cultivation. Further, not all crop species need to be propagated *in vitro* by means of all these phases; they may be modified in nurseries and commercial labs all over the world. The urgent need for micropropagation has originated from the increased demand for flowers and indoor foliage plants. The world trade in flowers is to the tune of US $ 35-45 billion and this is growing at the rate of 15% annually, while the global demand is growing at the rate of 17%. Currently, the total area under floriculture is about 20,000 hectares and several companies set up tissue culture laboratories producing high value ornamentals and foliage plantlets such as chrysanthemums, lilies, gerbera, gladioli, rose, carnation, orchids, anthuriums, spathyphyllum, ficus and

syngonium. Protocols have been developed for fruit crops such as pineapple, papaya, grape, banana, and strawberry etc. The tree spp. identified covered a wide spectrum, which included trees of the desert and arid zone, tropical and temperate zone, leguminous trees and multipurpose tree species for timber fuel paper and pulp. To mention few of them are *Acacia nelotica, Alunus nepalensis, Dendrocalamus strictus, Tectona grandis, Shoria robusta, Dalbergia latifolia, Santalum album, Prosopis cineraria, Sapindus trifoliatus, Boswellia serrate, Eucalyptus, Populus, Pinus, Betuala* and *Cedrus sp. etc* (DBT Annual reports, 2000-2001, 02, 03, 04).

The plant species being studied have high economic potential for tissue culture regeneration systems and they include spices (turmeric, ginger, cinnamom, nutmeg, saffron, clove, cardamom, long pepper etc.), beverages, endangered plants (*Aegle*, *Ceropegia*, *Curculio*, *Hemidesmus*) forest trees, horticultural crops (banana, citrus, mango etc.), cash crop like sugar cane. India with its vast germplasm diversity and varied environmental conditions have benefitted tremendously by applying this technology which has influenced the production of food, fodder, flower, fruit, fragrance, flavor and herbal medicine (DBT Annual reports, 2000-2001, 02, 03, 04).

Embryo culture: Wild species of crop plants possess several useful genes for disease, insect/pest resistance, male sterility, quality, and stress tolerance. Attempts of conventional crosses among cultivated and wild species resulted in an aborted embryos in several cases due to crossability barriers. Embryo culture involving growth of isolated immature embryos on a nutrient medium produces successful interspecific and intergeneric hybrids (cotton, barley, tomato, rice, maize wheat, legumes, as well as wheat x rye, barley rye etc.) With recombined desirable genes for earliness, resistance to fungal, bacterial, nematode, pests and diseases in crops like tomato, maize, rice, brassica etc (Chawla, 2009).

Anther culture: Haploids can be produced by culturing anthers or haploid plant explants. Anther culture is the most efficient of these and has been widely used. Induction of haploidy was first reported in *Datura innoxia* by Guha and Maheshwari (1964). Since then anthers containing immature pollen have been successfully cultured for a wide range of economically important plants (about 200-350) to obtain haploids plants. Haploids are important sources of homozygous lines in the production of hybrid lines; this method has been used for introduction of disease and insect resistance and high yielding varieties of many crops including rice, wheat, tobacco, brassica, and rye etc. A conventional plant breeding program takes about 6-8 years to develop a pure homozygous line, whereas anther culture reduces the period to less than two years (Chawla, 2009).

Protoplast culture and somatic hybridization: Protoplast fusion is an alternative to conventional cross hybridization method for plant improvement. In this system fertilization is bypassed and new characters are introduced in plants by artificial fusion of two plant cells in the form of protoplasts *in vitro*. By this somatic hybridization method more than 400 plant species of 146 genera and 50 families of crop plants have been obtained and these include cereals, legumes, vegetables, fruits, medicinal plants and other important species. Somatic hybridization is also used for gene transfer for resistance to important diseases and to improve quality and higher yield of many crop plants. The families of Solanaceae and Brassicaceae contain the most commonly used species for somatic hybridization. Both interspecific and intergeneric hybrids have been obtained. Many disease resistance genes for potato leaf roll virus, leaf blight, verticillium etc have been transferred to *Solanum tuberosum* from their species normal crossings would not be possible due to a taxonomic barrier. Likewise many disease resistance genes are transferred from wild species to cultivated varieties of many crop plants of cereals and vegetables. Cytoplasmic male sterility has been successfully transferred in various crop plants like oryza, lycopersicum, brassica, nicotiana etc. resistance to herbicides and antibiotics has been introduced to some species of brassica (Chowdhury *et al*., 1997). The development of transgenic plants is the result of integrated application of rDNA technology, gene transfer and tissue culture techniques. These technologies have enabled the production of transgenic plants in more than 150 species, which include most major economic crops for food, fruits, vegetables, medicinal, tree and pasture plants.

Secondary metabolites: Plants produce two types of metabolites i.e. primary and secondary metabolites. Primary metabolites are essential for the growth and development of the plants. Secondary metabolites are considered as end products, not involved in metabolic activity of growth, are mostly accumulated by plant cells in smaller quantities than primary metabolites. The medicinal plants are those rich in secondary metabolites are termed as medicinal; exert a profound physiological effect on man. The physiological effect of the active principle is used for curing many ailments and diseases of man. *In vitro* grown plant cells and tissues have been used extensively for the production of secondary metabolites. Depending on the objectives, biotechnological methods are used for understanding metabolic pathways and improvement of plants for the production of secondary metabolites. Plants are major source of various pharmaceutical and industrial products. Nearly 30% of the drugs produced are of plant origin. Tissue culture of medicinal plants provides a continuous and reliable source of natural products round the year without the destruction of the entire plant. It also enables easy purification of the compound in the absence of significant amounts of pigments, production of higher

quantities of desired compound through cell line selection and /or addition of precursors into the production medium.

Improving the product quality and quantity

Compounds like phytoalexins (a category of secondary metabolites of plants) are the stimulators that induce the production of desired secondary metabolites *in vitro*. They are called elicitors by Keen *et al.* (1972). Depending on the usage in the *in vitro* studies, elicitors are of two types 1. abiotic and 2. biotic. The former being physical (UV, IR or gamma irradiations) or chemical (alkalinity, osmotic pressure, or heavy metal ions etc.) in nature and the latter being homogenates of fungal or bacterial cultures. Potential abiotic elicitors include: diethylaminoethyl dichloro phenyl ether catharanthin in the production of Ajmalacine (*Catharanthus roseus*), activated carbon for echinofuron (*Lithospermum erythrorhizon*), UV irradiation for flavonoid glycosides (*Haplopappus gracilis*), Ethephon for caffeine (*Coffea arabica*). Biotic elicitors (Mycelial/cell extracts or filtrates): *Fusarium conglutinane* in the production of thiophene (*Tagetus patula*), *F.oxysporum* in the synthesis of sanguinarine (*Eschscholtzia*), *Phytophthora megaspore* in the development of tropane alkaloids (*Datura stromonium*), *Micromucor isobellina* in ajmalacine (*Catharanthus roseus*), *Pseudomonas solanacearum* in phyturberine (*Nicotiana tabacum*) etc. The basic idea behind the usage of elicitors focuses on the stress that they induce upon administration in the cell cultures, which concomitantly affects the yield and quality parameters of the secondary products accumulated (Pullaiah & Rao, 2009).

Vegetables play an important role in the human nutrition and health by providing minerals, micronutrients, vitamins, antioxidants, phytosterols and dietary fibres. Biotechnological studies have shown that several agriculturally important traits can be used to improve different vegetable crops and for biotic, abiotic stresses and also for pharmaceutical proteins for immunization, enzyme therapy or as precursors for any pharmaceutical product. Plant derived antibodies have extensive medicinal uses such as passive immunization and targeted drug delivery. Antibody production has been successfully demonstrated in tobacco, soy bean and potato. In addition to pharmaceutical production, transgenic plants can also be used as biofactories for producing industrial chemicals and raw materials (Monika and Ananda Kumar, 2005). Polyamines participate in the regulation of plant growth and development, including *in vitro* morphogenesis and a number of other developmental processes such as flower and fruit development and senescence. Genetic manipulation of polyamine metabolism has produced many transgenic plants in rice, tobacco, carrot, eggplant, pea etc. (Rajam, 2005). Thus plant tissue culture plays a prominent role in the development of sustainable agriculture though micropropagation, embryoculture, anther culture, protoplast culture, secondary metabolites and takes health care of mankind.

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