SECONDARY METABOLITES PRODUCTION IN PLANT TISSUE CULTURE: A REVIEW

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ABSTRACT

Plant secondary metabolites are the economically important as drug, fragrances, pigments, food additives and pesticides. The biotechnological tools are important to select, multiply, improve and analyze medicinal plants. Plant cell culture systems represent a potential renewable source of valuable medicinal compounds, flavours, fragrances and colorants, which cannot be produced by microbial cells or chemical synthesis. In-vitro production of secondary metabolites in plant cell suspension culture has been reported from various medicinal plants and bioreactors are the key step towards commercial production of secondary metabolites by plant biotechnology. Genetic transformation is a powerful tool for enhancing the productivity of novel secondary metabolites; especially by Agrobacterium tumefaciens. Combinatorial biosynthesis is another approach in the generation of novel natural products and for the production of rare and expensive natural products. DNA profiling techniques like DNA microarrays save as suitable high throughput tools for the simultaneous analysis of multiple genes and analysis of gene expression that becomes necessary for providing clues about regulatory mechanism, biochemical pathways and broader cellular functions.

KEYWORDS: Secondary metabolites, Tissue culture, Medicinal plants.

INTRODUCTION

Biotechnological tools are important for the multiplication and genetic enhancement of the medicinal plants by adopting techniques such as in vitro regeneration and genetic transformation. It could also be harnessed for the production of secondary metabolites using plants as bioreactors(Yaseen and Aliabbas, 2009). Plant cell culture systems represent a potential renewable source of valuable medicinal compounds, flavours, fragrances and colorants, which cannot be produced by microbial cells or chemical synthesis. The utilization of plant cells for the production of natural or recombinant compounds of commercial interest has gained increasing attention over past decades. Bioactive compounds currently extracted from plants are used as pharmaceuticals, agrochemicals, flavor and fragrance ingredients, food additives and pesticides. The secondary metabolites are known to play a major role in the adaptation of plants to their environment, but also represent an important source of pharmaceuticals. Although modern medicine may be available in developed countries, herbal medicines phytopharmaceuticals have often maintained popularity for historical and cultural reasons.

Recent advances in the molecular biology, enzymology and fermentation techniques considerably improve this potential by the application of nutritional and hormonal systems under aseptic conditions. Plant proliferation by this method is termed micropropagation because miniature shoots or plantlets are initially derived. Two main pathways can be considered, that is, generation through shoot organogenesis and somatic embryogenesis. Plant regeneration through organogenesis generally involves induction and development of a shoot from explant tissue, followed by transfer to a different medium for the induction of root formation and development. In somatic embryogenesis, somatic cells develop by division to form complete embryos analogous to zygotic embryos. Somatic embryogenesis can occur directly from cells of the explant tissue without an intervening callus phase. The scheme of production of some important plant pharmaceuticals produced in cell cultures. The possible use of plant cell cultures for the specific biotransformation of natural compounds has been demonstrated. Due to these advances, research in the area of tissue culture technology for production of plant chemicals has bloomed beyond expectations(Ramachandra Rao and Ravishankar, 2002).

Advances in tissue culture, combined with improvement in genetic engineering of pharmaceuticals, nutraceuticals and other beneficial substances (Brown and Thorpe, 1995). This has included production of shikonin, anthocyanins and ajmalicine and, recently, important anti-tumor agents like taxol, vinblastine and vincristine. Today, the expression of recombinant antibiotic's and antibody fragments in plants is a well-established technique and the advantages of plants over bacterial or mammalian production systems have been reviewed (Rashid, 1988).

Tissue Culture Producing Pharmaceutical Products: Research in the area of plant tissue culture technology has resulted in the production of many pharmaceutical substances for new therapeutics like alkaloids, terpenoids, steroids, sapo- ninis, phenolics, flavonoids and aminoacids. Successful attempts to produce some of these valuable pharmaceuticals in relatively large quantities by cell cultures are illustrated.

Morphine and Codeine: Latex from the opium poppy, Papaver somniferum is a commercial source of the analgesics, morphine and codeine. Callus and suspension cultures of P. somniferum are being investigated as an alternative means for production of these compounds. Production of morphine and codeine in morphologically undifferentiated culture has been reported.

Ginsenosides: The root of Panax ginseng, so-called ginseng, has been widely used as a tonic and highly prized medicine since ancient times. Ginseng has been recognized as a miraculous promoter of health and longevity. The primary bioactive constituents of ginseng were identified as ginsenosides, a group of triterpenoid saponins. Among them, ginsenoside Rg1 is one of the major active molecules from Panax ginseng (Yu et al., 2002).

Berberine: Berberine is an isoquinoline alkaloid found in the roots of Coptis japonica and cortex of Phellodendron amurense. This antibacterial alkaloid has been identified from a number of cell cultures, notably those of Coptis japonica, Thalictrum longifolium and Berberis spp. The productivity of berberine was increased in cell cultures by optimizing the nutrients in the growth medium and the levels of phytohormones (Srivastava and Srivastava, 2007).

Diosgenin: Diosgenin is a precursor for the chemical synthesis of steroid drugs and is tremendously important to the pharmaceutical industry. Tal et al., 1984 reported on the use of cell cultures of Dioscorea deltoidea for production of diosgenin. They found that carbon and nitrogen levels greatly influenced diosgenin accumulation in one cell line.

Vinblastine and Vincristine: The dimeric indole alkaloids vincristine and vinblastine have become valuable drugs in cancer chemotherapy due to their potent antitumor activity against various leukemias and solid tumors. These compounds are extracted commercially from large quantities of Catharanthus roseus. Since the intact plant contains low concentrations (0.0005%), plant cell cultures have been employed as an alternative to produce large amounts of these alkaloids (Ravishankar and Ramachandra, 2000).

Taxol: Taxol (plaxitaxol), a complex diterpene alkaloid found in the bark of the Taxus tree, is one of the most promising anticancer agents known due to its unique mode of action on the microtubular cell system. At present, production of taxol by various Taxus species cells in cultures has been one of the most extensively explored areas of plant cell cultures in recent years owing to the enormous commercial values of taxol, the scarcity of the Taxus tree and the costly synthetic process(Vijayasree et al., 2010).

Bioprocess Technology for Production of Plant Secondary Metabolites: In literature plant cell cultures described as extremely sensitive for shear forces, necessitating the use of special low-shear bioreactors, e.g. air lift bioreactors. As a consequence, such a bioreactor is preferable for plant cell culture; it is the lowest cost process-unit. More recent studies on the shear sensitivity of plant cells, among others in laboratories, have shown that in fact plant cells ingeneral are quite shear-stress tolerant. This is supported by the fact that a series of large-scale processes have been reported with plant cell cultures, e.g. shikon in production. These prices are high, but a number of natural products have even much higher prices (e.g taxol, vinblastine and vincristine). However, most of the high-value...
specialty chemicals are produced at too low levels in the plant cell cultures (Hansen and Wright, 1999).

Genetic Transformation Technology and Production of Transgenic Plants: Genetic transformation has been proved to be a powerful tool for the production of plants with desired traits in many crops. It promises to overcome some of the substantial agronomic and environmental problems that have not been solved using conventional plant breeding programs.

Agrobacterium and Non-agrobacterium Mediated Gene Transfer: This soil bacterium possesses the natural ability to transform its host by delivering as well-defined DNA fragment, the transferred (T) DNA, of its tumour-inducing (Ti) plasmid into the host cell. Transformation systems based on A. tumefaciens are well established for Taxus (yew), Echinacea, Scrophularia (figwort), Digitalis (foxglove), Thalictrum (meadowrue) and Artemisia. Thus, Agrobacterium transformation provides a method for routine genetic transformation of many important medicinal species.

Direct Gene Transfer
Generation of Transgenic Medicinal Plants by Particle Bombardment: Particle bombardment procedure was introduced in 1987, which involves the use of a modified shotgun to accelerate small (1-4µm) diameter metal particles into plant cell wall. There is no intrinsic limitation to the potential of particle bombardment since DNA is governed entirely by physical parameters. Efficient transformation of the tropane alkaloid-producing medicinal plant, Hyoscyamus muticus, was also achieved by particle bombardment. An efficient and stable transformation has been achieved in garlic plants (Allium sativum)(Martin et al., 2008).

Generation of Transgenic Medicinal Plants by Electroporation: Electroporation uses brief pulses of high voltage electricity to induce the formation of transient pores in the membrane of the host cell. Exposure of cell suspension protoplasts of the woody medicinal plant, Solanum dulcamara, to a voltage of 250 to 1250V cm⁻¹ for three successive pulses, each of 10-50µs duration, stimulated growth of protoplast-derived tissues(Chattopadhyay et al., 2002).

Generation of Transgenic Medicinal Plants by Chloroplast Transformation: Stable transformation of the chloroplast by inserting foreign genes into the chloroplast genome was first achieved in the single cell green alga, Chlamydomonas reinhardtii in 1988, soon to be followed by tobacco plant and more recently, Arabidopsis thaliana more than 40 Trans (Brown and Thorpe, 1995).

CONCLUSION
Plant cell and tissue culture play important roles in the manipulation of plants for improved crop varieties. In vitro propagation of medicinal plants with enriched bioactive principles and cell culture methodologies for selective metabolite production is found to be highly useful for commercial production of medicinally important compounds. To improve yields metabolic engineering offers promising perspectives, but requires the understanding of the regulation of the secondary metabolite pathways involved on the levels of products, enzymes and genes, including aspects as transport and compartmentation. In vitro propagation of medicinal plants with enriched bioactive principles and cell culture methodologies for selective metabolite production is found to be highly useful for commercial production of medicinally important compounds. To improve yields metabolic engineering offers promising perspectives, but requires the understanding of the regulation of the secondary metabolite pathways involved on the levels of products, enzymes and genes, including aspects as transport and compartmentation.

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