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Application of Biotechnology in Production of Medicinal Plants

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Abstract: Medicinal plants are the most important source of life saving drugs for the majority of the world`s population. 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 tumefacians*. 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 gen expression that becomes necessary for providing clues about regulatory mechanism, biochemical pathways and broader cellular functions.

Key words: Biotechnology % Secondary metabolites % Medicinal plants

INTRODUCTION

The current world population, approximately 6.8 billion, is expected to double by the year 2050 (Source: World Bank, World Development Indicators). The population increase in developing countries constitutes 97% of the global increase [1] and it is estimated that by 2050, 90% of the planet's population will reside in the developing countries of the southern hemisphere. The challenge for the future, therefore, lies in global food security that necessitates a doubling of food production in the next 50 years to meet the needs of the population [2].

World-wide, the beneficial impact of plant biotechnology has been almost exclusively on crops of high economic importance such as maize, wheat, soybean, sunflower, rice and potato [2].

It is estimated that 70-80% of people worldwide rely chiefly on traditional, largely herbal, medicines to meet their primary healthcare needs. The global demand for herbal medicine is not only large, but growing. Various technologies have been adopted for enhancing bioactive molecules in medicinal plants. 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 [3]. 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 evolving commercial importance of the secondary metabolites has in recent years resulted in a great interest, in secondary metabolism and particularly in the possibility to alter the production of bioactive metabolites by means of cell culture technology. The principle advantage of this technology is that it may provide continuous, reliable source of plant pharmaceuticals and could be used for the large scale culture of plant cells from which these metabolite can be extracted. Plant cell and tissue cultures hold great promise for controlled production of myriad of useful secondary metabolites on demand [4]. Advances in tissue culture, combined with improvement in genetic engineering

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techniques specifically transformation technology, have opened new avenues for high volume production of pharmaceuticals, nutraceuticals and other beneficial substances [3]. The utilization of plant cells for the production of natural or recombinant compounds of commercial interest has gained increasing attention over past decades [5]. Bioactive compounds currently extracted from plants are used as pharmaceuticals, agrochemicals, flavor and fragrance ingredients, food additives and pesticides. A number of plant species have been used for generation and propagation of cell-suspension cultures, ranging from model systems like *Arabidopsis*, *Catharanthus* and *Taxus*, to important monocotyledon or dicotyledonous crop plants like rice, Soya bean, alfalfa and tobacco. 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 [6]. Cell suspension cultures systems could be used for large scale culturing of plant cells from which secondary metabolites could be extracted. The advantages of this method are that it can ultimately provide a continuous reliable source of natural products. In recent years, traditional system ofmedicine has become a topic of global importance. Although modern medicine may be availablein developed countries, herbal medicines [phytopharmaceuticals] have often maintained popularity for historical and cultural reasons [4]. Recent advances in the molecular biology, enzymology and fermentation technology of plant cell culture suggest that these systems may become a viable source of important secondary metabolites [3].

***In vitro* Plant Regeneration and Micropropagation:** *In vitro* techniques considerably improve this potential by the application of nutritional and hormonal systems under aseptic conditions. Plant proliferation by this method is termed micro propagation because miniature shoots or plantletsare initially derived. There are a number of pathways for the regeneration of whole plants from excised plant parts. Two main pathways can be considered, that is, generation through shoot organogenesis and somatic embryogenesis [7].

Organogenesis is a developmental pathway in which shoots or roots (that is, organs) are induced to differentiate from a cell or group of cells. 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. Research has demonstrated that successful organogenesis in many plant species can be achieved by the correct establishment of medium components, selection of a suitable explant and control of the physical environment [8]. In somatic embryogenesis, somatic cells develop by division to form complete embryos analogousto zygotic embryos. The bipolar structure of the somatic embryo contains both shoot and root meristem. As the embryos develop, they progress through the distinct structural steps of the globular, heart, torpedo, cotyledonary and mature stages. Somatic embryogenesis can occur directly from cells of the explant tissue without anintervening callus phase. However, the indirect embryogenesis pathway, where somatic embryos are inducedand develop from a proliferated callus, is generally more common [9, 10]. Discoveries of cell cultures capable of producing specific medicinal compounds at a rate similar or superior to that of intact plants have accelerated in the last few years. The scheme of production of some important plant pharmaceuticals produced in cell cultures [11]. New physiologically active substances of medicinal interest have been found by bioassay. It has been demonstrated that the biosynthetic activity of cultured cells can be enhanced by regulating environmental factors, as well as by artificial selection or the induction of variant clones. Some of the medicinal compounds localized in morphologically specialized tissues or organs of native plants have been produced in culture systems not only by inducing specific organized cultures, but also by undifferentiated cell cultures. The possible use of plant cell cultures for the specific biotransformation of natural compounds has been demonstrated [12]. Due to these advances, research in the area of tissue culture technology for production of plant chemicals has bloomed beyond expectations [4].

The major advantages of a cell culture system over the conventional cultivation of whole plants are: (1) Useful compounds can be produced under controlled conditions independent of climatic changes or soil conditions; (2) Cultured cells would be free of microbes and insects; (3) The cells of any plants, tropical or alpine, could easily be multiplied to yield their specific metabolites; (4) Automated control of cell growth and rational regulation of metabolite processes would reduce of labor costs and improve productivity; (5) Organic substances are extractable from callus cultures. In order to obtain high yields suitable for commercial exploitation, efforts have been focused on isolating the biosynthetic activities of cultured cells, achieved by optimizing the

cultural conditions, selecting high producing strains and employing precursor feeding, transformation methods and immobilization techniques [13]. Transgenic hairy root cultures have revolutionized the role of plant tissue culture in secondary metabolite production. They are unique in their genetic and biosynthetic stability, faster in growth and more easily maintained. Using this methodology a wide range of chemical compounds have been synthesized [14]. Advances in tissue culture, combined with improvement in genetic engineering of pharmaceuticals, nutraceuticals and other beneficial substances [15].

Exploration of the biosynthetic capabilities of various cell cultures has been carried out by a group of plant scientists and microbiologists in several countries during the last decade. Most applications of plant-cell-suspension cultures in biotechnology are aimed at the production of naturally occurring secondary metabolites. This has included production of shikonin, anthocyanins and ajmalicine and, recently, important anti-tumor agents like taxol, vinblastine and vincristine [16]. In the last few years promising findings have been reported for a variety of medicinally valuable substances, some of which may be producedon an industrial scale in the near future. Today, the expression of recombinant antibody'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 [17]. The aim of the present review is to focus on the importance of tissue culture technology in production of some of the plant pharmaceuticals.

Tissue Culture Producing Pharmaceutical Products of Interest Case Studies: Research in the area of plant tissue culture technology has resulted in the production of many pharmaceutical substances for new therapeutics. Advances in the area of cell cultures forthe production of medicinal compounds has made possible the production of a wide variety of pharmaceuticals like alkaloids, terpenoids, steroids, saponins, phenolics, flavanoids and aminoacids. Successful attempts to produce some of these valuable pharmaceuticals in relatively large quantities by cell cultures are illustrated.

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 [16]. 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 [18].

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 [19].

Ginsenosides: The root of *Panax ginseng*, so-called ginseng, has been widely used asa tonic and highly prized medicine since ancient times [20]. 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 Rg 1 is one of the major active molecules from *Panax ginseng*.

Berberine: Berberine is an isoquino line alkaloid found in the roots of *Coptis japonica* and cortex of *Phellondendron amurense*. This antibacterial alkaloid has been identified from a number of cell cultures, notably those of *Coptis japonica*, *Thalictrum* spp and *Berberis* spp [21]. The productivity of berberine was increased in cell cultures by optimizing the nutrients in the growth medium and the levels of phytohormones.

Diosgenin: Diosgenin is a precursor for the chemical synthesis of steroidal drugs and is tremendously important to the pharmaceutical industry [22]. Tal *et al*., [23] 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 dimericindole alkaloids vincristine and vinblastine havebecome 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 [16].

Bioprocess Technology for Production of Plant Secondary Metabolites: In literature plant cellsare described as extremely sensitive for shear forces, necessitating the use of special low-shearbioreactors, e.g. air lift bioreactors. However, in industry such bioreactors are not common; most processes are runned in stirred-ank. As a consequence, such a bioreactor is preferable for plant cell culture; it is the lowest cost process-unit [24]. 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 [20]. 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. Plant cells have even been cultured in a 60 m^3 stirred tanks [16]. The technology being, feasible, how about the economy? A number of papers have appeared on this [25]. Assuming a yearly production of 3000 kg/year of compound produced by a cell culture at a level of 0.3 g/l, resulted in a calculated price of 1500 US $/kg. An increase of productivity with a factor 10 (i.e, 3 g/l) results in a price of 430 $/kg [26]. In both cases a fed-batch type of process was applied. These prices are high, but a number of natural products have even much higher prices (e.g taxol, vinblasine and vincristine) [27]. However, most of the high-value specialty chemicals are produced at too low levels in the plant cell cultures [28]. Their production must thus be increased to make an industrial process possible [4].

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: Plant transformation mediated by *Agrobacterium tumefactions*, a soil plant pathogenic bacterium, has become the most commonly used method for the introduction of foreign genes into plant cells and the subsequent regeneration of transgenic plants. 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. The rapid progress in the area of crop biotechnology is mainly because of the development of efficient regeneration and suitable Agrobacterium mediated transformation protocols for different crop species. Similar success could also be achieved in the medicinal plants, which in turn could be used for the enhancement of secondary metabolites content. Transformation systems based on *A. tumefaciens* are well established for *Taxus* (yew), *Echinacea*, *Scrophularia* (figwort), *Digitalis* (foxglove), *Thalictrum* (meadowrues) 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 (1-4 μm) 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*).

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, *Solatium dulcamara*, to a voltage of 250 to 1250 V cmG^{1} for three successive pulses, each of 10-50 μs duration, stimulated growth of protoplast-derived tissues.

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.

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 cellculture 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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