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**Review Article**

# Biotechnology and *In Vitro* Conservation of Medicinal Plants

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**Abstract:** Medicinal and aromatic plants have been intimately linked with human health and culture since time immemorial. Herbal medicines are products of plant secondary metabolism and are involved in various aspects of a plant's interaction with its immediate environment. Besides all the progress in synthetic chemistry and biotechnology, plants are still an indispensable source of medicinal products of both preventive and curative nature. About 20,000 plant species are known to have worldwide use as drugs. Phytochemical tests have been performed in about 5000 and nearly 1110 species are extensively utilized in Ayurveda (80%) and Allopathic medicine (33%). Therefore, the conservation and sustainable utilization of medicinal plants must have a long term, integrated, scientifically oriented action programme. This should involve the pertinent aspects of protection, preservation, maintenance, conservation and sustainable utilization. Biotechnological tools are important to select, multiply and conserve the critical genotypes of medicinal plants. Plant cell tissue and organ culture system holds tremendous potential for the production of high quality plant based medicine *in vitro*. Elicitation, precursor feeding and genetic transformation are employed as potent tools for enhanced production of secondary metabolites. Recently, functional genomic strategies have been developed for differential gene expression analysis in plant cell culture towards production of designer secondary metabolites. This article reviews the achievements and advances in various tissue culture strategies for conservation of medicinal plants and for enhancement of secondary metabolite production *in-vitro*.

**Key Words:** Medicinal plants, Herbal medicine *in vitro*, Genetic improvement, Functional genomics.

## Introduction

Plants have been an important source of food, fibre and medicine for thousands of years and still continue to be so. Plant-derived medicines constitute a substantial component of present day human healthcare systems in industrialized as well as developing countries. In India, herbal medicines have been the basis of treatment for various diseases, physiological conditions in traditional systems such as Ayurveda, Unani and Siddha. Indian folk medicine comprises numerous herbal prescriptions for therapeutic purposes which may be as varied as healing wounds, treating inflammation due to infection, skin lesions, leprosy, diarrhea, scabies, venereal diseases, snake bite and ulcers. Genetic diversity of traditional medicinal species is continuously under threat of extinction as a result of habitat destruction and unmonitored trade of medicinal plants and their products [1].

Therefore, the conservation and sustainable utilization of medicinal plants are more desirable need of the hour [2]. There is no reliable figure for the total number of medicinal plants on earth, and numbers and percentages for countries and regions vary greatly. Estimates for the number of species used medicinally include: 35,000-70,000 or 53,000 worldwide; 10,000-11,250 in China; 7500 in India and 2237 in Mexico [3-5]. The United Nations Conference on Environment and Development (UNCED), held in 2010 at Rio de Janeiro, Brazil helped to place the loss of biodiversity and its conservation on the global agenda. It is inspiring to note that the Indian Postal Department during the last three decades has issued several postal stamps of important and endangered medicinal plants, which are not only educative but informative while dealing with conservation strategies [6].

To cope up with alarming situation, the recent exciting developments in biotechnology have come as a boon; one of them is the use of plant tissue culture technique. In recent

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years, tissue culture has emerged as a promising technique to obtain genetically pure elite populations under *in vitro* conditions rather than have in different populations. Tissue culture has become a well established technique for culturing and studying the physiological behavior of isolated plant organs, tissues, cells, protoplasts and even cell organelles under precisely controlled physical and chemical conditions. Most of the medicinal plants either do not produce seeds or seeds are too small and do not germinate in soil. Thus mass multiplication of disease free planting material is a general problem. In this regard micropropagation holds significant promise for true to type, rapid and mass multiplication under disease free conditions. Besides, the callus derived plants exhibit huge genetic variation that could be exploited for developing superior clones/varieties particularly in vegetatively propagated plant species.

***Conservation of Medicinal Plants In Vitro***

*In vitro* conservation can be effectively used to broaden germ plasm collection of medicinal plants and can be used as a source of material for both propagation and preservation of endangered *taxa [6]*. Tissue culture technologies have a major impact on *ex situ* conservation of plant genetic resources in addition to international germ plasm exchange. Lady's slipper orchid (*Cypripedium calceoius* L.) stands as an example as to how tissue culture approaches has been vital in the reestablishment of endangered plant species [7]. Major international germ plasm centers use *in vitro* conservation as their method of choice for vegetatively propagated crops, a few examples are United States Department of Agriculture; National Clonal Germplasm Repository, Oregon; the International Potato Center, Peru and National Bureau of Plant Genetic Resources (NBPGR), India [8].

Micropropagation, using somatic embryo and shoot tip culture techniques assists many crop plant improvement programmes and these methods are being used for the conservation of endangered medicinal plant species. Crop plants which are vegetatively propagated present particular conservation problems, as their seeds are not available for banking. Whilst field gene banks provide important conservation options, germ plasm maintained in this manner can be at risk from pathogen attack and climatic damage. For vegetatively propagated species, *in vitro* conservation using tissue culture methods is the only reliable, long-term means of preservation. *In vitro* propagation of plants holds tremendous potential for the production of high quality plant based medicines [9]. This can be achieved through different methods including micropropagation, in which the multiplication rate is greatly increased. It also permits the production of pathogen free plant material. High frequency plant regeneration from shoot meristem has been achieved in important medicinal plants like *Catharanthus roseus, Cinchona ledgeriana and Digitalis spp., Rehmannia glutinosa, Rauvolfia serpentina, Isoplexis canariensis, Citrullus colocynthis, Zephyranthes bulbous, Plectranthus vetiveroids* and *Glossocardia bosvallea [10-15]*.

***Micropropagation of Rare Medicinal Plants***

Micropropagation has many advantages over conventional methods of vegetative propagation, which suffer from several limitations [16]. Using *in vitro* propagation methods several rare and endangered plant species can now be quickly and successfully propagated and preserved from a minimum plant material and with low impact on wild population [17,18]. During the last three decades, various medicinal plants have been successfully propagated and reestablished by means of media optimization and supplementation of plant growth regulators [19-33]. Micropropagation of rhizome producing turmeric varieties (Duggirala and tekkurpetta) was achieved using rhizome bud explants [34]. Direct micropropagtion without callus phase has been described in medicinal plants like *Leucojum aestivum, Eryngium foetidum* and *Lilium rhodopaeum Catharanthus roseus, Cinchona ledgeriana Digitalis spp, Rauvolfia serpentina, Isoplexis canariensis* and *Eryngium foetidum [35,36,20]*. Mass multiplication through tissue culture was achieved in several threatened and endemic medicinal plants such as *Gentiana kuroo, Picrorhiza kuroa, Coleus forskohlii, Saussurea lappa, Atropa acuminate, Salvadora persica, Paedaria foetida, Tylophora indica, Ginkgo biloba, Bacopa monnieri, Eclipta alba, Decalepis hamiltonii, Janakia arayalpathra* and *Picorhiza kurroa [*25,29,37-41]. Using axillary buds mass clonal multiplication has been successfully achieved in several

Himalayan medicinal plants including *Potentilla fulgens [42]*.

***Somatic Embryogenesis***

Micropropagation through somatic embryogenesis is an option for the rapid production of uniform plants. Somatic embryogenesis is a type of vegetative propagation based on plant cell totipotency which offers a powerful alternative to other vegetative propagation methods. Somatic embryogenesis is the process by which embryo like structures similar in structure and function originate directly or indirectly in large numbers through a callus phase from different types of explants. Somatic embryogenesis (SE) ultimately is the developmental pathway by which somatic cells develop into structures that resemble zygotic embryos (i.e., bipolar and without vascular connection to the parental tissue) through an orderly series of characteristic embryological stages without fusion of gametes [43]. Somatic embryos obtained by direct embryogenesis are less numerous than embryos derived from indirect embryogenesis. Thus, the second type is generally preferred for large-scale propagation.

Regardless of the plant species, somatic embryogenesis protocols generally follow a similar process flow: a) induction of embryogenic calli followed by their identification and selection by physical isolation; b) multiplication of the embryogenic cells (undifferentiated phase); c) regeneration of large numbers of embryos from these cells (embryogenic phase); d) conversion of these embryos into mature embryos able to regenerate a plant. The ontogeny, physiological, biochemical and media properties required for somatic embryogenesis have been reported by several authors [44-49]. Typical globular, heart, torpedo and cotyledonary stages of somatic embryos differentiate from various kinds of explants. True-to-type nature of the somatic embryo-derived plantlets has been reported in important medicinal plants [50, 51]. Interestingly explants of juvenile and immature nature and the ones which were part of zygotic embryo and cotyledons result in direct embryogenesis. Direct somatic embryogenesis arise either from epidermal or sub epidermal cells of both intact and cut ends of the explants. Immature inflorescence of cereals and grasses are of potential explants to regenerate a large number of somatic embryos. As the ontogeny commence from a single cell which differentiates into a pro-embryogenic mass and later organize themselves as individual somatic embryos whole plant without the involvement of sexual fusion this process exactly fitting the term "totipotency" of the plant cells [52, 45].

Generally the somatic embryos do not have a strong attachment with mother tissue indicating its arousal from single cells and ultimately producing clones of the cultured plant species. As a result, any plant which needs to be altered by genetic engineering and transgenesis requires a pre developed protocol for successful regeneration through somatic embryogenesis [53,54]. Because, the other methods of micropropagation in reality will not produce "true to type" plants which will be a draw back for transgenic plant technology. Hence, developing a protocol for plant regeneration through somatic embryogenesis will immensely benefit the plant conservation programmes too as it is a shorter and viable method for producing large number plantlets. Bioreactor based production of somatic embryos have been reported for several plant species including alfalfa, carrot, potato and coffee. Plant regeneration via somatic embryogenesis from single cells has been demonstrated in many medicinal plant species. Bhojwani and Arumugam [55] reported the development of somatic embryos in *Podophyllum hexandrum* on MS medium supplemented with NAA and Kinetin. Somatic embryogenesis among medicinally important and red listed plants have been widely known in *Crataeva nurvala [56], Bunium persicum [57]*, *Hemidesmus indicus [58]*, *Hardwickia binata* [59], *Gymnema sylvestre [*60] and *Holostemma ada-kodien* [61].

The pivotal role of plant growth regulators in controlling somatic embryogenesis has been well established in different plant species. According to Zimmerman [62], removal of auxin from the culture medium is a prerequisite to inactivate genes or synthesis of its products necessary for embryo development. The success of micropropagation through somatic embryogenesis relies on the maturation and conversion of the embryos. *In vitro* studies and somatic embryogenesis have been carried out on many members of Asclepiadaceae such as *Tylophora indica [63]*,

*Calotropis gigantean, Hemidesmus indicus [58]*, *Araujia sericirfera* and *Ceropegia spp*. [64].

***Suspension Cultures and Production of Secondary Metabolites In vitro***

Callus and suspension cultures have been exploited commercially for the production of active principles in many medicinally important plant species. Callus induction requires an environment where the cells can divide and proliferate rapidly. With prolonged culture in nutrient medium supplemented with plant growth regulators callus cells may undergo some degree of differentiation and may change the spectrum of secondary metabolite synthesis. For many years, callus cultures have been employed in the study of secondary metabolism *in vitro [65]*. Conditions for callus induction and growth are not conducive for secondary metabolite production and the tissues need to be transferred to a new medium with different composition. Production methods tend to be contingent upon defining conditions that allow maximum product accumulation rate. Generally the plants which accumulate relatively high quantity of specific secondary metabolites, give rise to tissue cultures producing high levels of secondary metabolites and vice versa. This is because the capacity for the biosynthesis of secondary metabolites is genetically determined. The chemical gradients, cell-to-cell contact and small degrees of differentiation also seem to favor secondary metabolite production.

When callus is suspended in liquid growth medium, the cells disperse producing cell suspensions that can have faster and uniform growth rates coupled with secondary metabolite production. With their relatively faster growth, ease of manipulation and comparative homogeneity, suspension cultures are the most widely employed systems in secondary metabolite production. Liquid cultures represent various degrees of cellular aggregation and generally, the more aggregated, slower growing cultures display the highest productivities. Suspension cultures can be 'induced' by media manipulations and elicitors to produce compounds of interest. Suspension cultures are also amenable for growth in fermenters however, these cultures show genetic and biochemical heterogeneity. Explorations on cell cultures capable of producing medicinal compounds at a rate similar or superior to that of intact plants have accelerated in the last decades. It has been demonstrated that the biosynthetic activity of cultured cells and differentiated organs like roots can be enhanced by regulating environmental factors as well as by artificial selection or the induction of variant clones. The possible use of plant cell cultures for the specific production of natural compounds has been reported. Due to these advancements, research in the area of tissue culture technology for the production of phytochemicals has bloomed beyond expectations [65].

The synthetic capacity of dedifferentiated tissues often differs substantially from that of fully differentiated tissues, both qualitatively and quantitatively. The differing synthetic capacities are usually a direct result of differences in enzyme profiles which mirror the organ-specific expression of biosynthetic genes [66]. The differentiated cultures often show biochemical and genetic stability and hence offer a predictable and high-productivity system which does not require extensive optimization. For example, the accumulation of isoprenoids usually dependent upon plastid differentiation, as many of the enzymes in the pathway are plastid-related. In other cases, formation of glandular hairs or other storage organs is necessary for metabolite production to proceed. For example, vindoline, a major alkaloid of *Catharanthus roseus* is scarcely produced in suspension cultures but is produced in high quantity in shoot cultures derived from the same [67]. Callus cultures of *Taraxacum officinale* synthesize and accumulate α and γ-amyrins while differentiated tissues synthesize and accumulate taraxasterol and lupeol because these differentiated tissues develop laticifers where these compounds are stored [68]. Secondary product formation is an expression of a particular state of cell differentiation, which can be influenced by a number of signals. In some cases, initiation of morphological differentiation represents such a triggering signal. Different classes of secondary compounds require different degrees of cell or tissue differentiation. Formation of physical and biochemical gradients due to cellular organization is also an important contributing factor.

Secondary product synthesis by cell suspension cultures is typically low in a non-

manipulated medium. A number of approaches are available for enhancing the productivities of such cultures. Development of cell lines and selective culture of cell lines have lead to higher production of secondary compounds than simple mass culture of source tissues [69]. Addition of plant growth regulators, elicitors and precursors to enhance the production of target secondary compounds have been successful in several medicinal plant species [70-73]. Cell immobilization strategies have been optimized to maximally enhance the synthesis and secretion of secondary compounds in the culture systems [74]. Altering the genetic make up and engineering the respective genes to synthesize and accumulate secondary compounds are of modern interest among molecular biologist to mitigate continuous production of first priority secondary compounds [75].

**Commercialized Herbal Medicines from Plant Cell andTissue Culture**

| Metabolite | Plant cell culture |
|---|---|
| Shikonin | *Lithospermum erythrorhizon* |
| Ginsenosides | *Panax ginseng* |
| Anthroquinones | *Morinda citrifolia* |
| Ajmalicine, Serpentine | *Cathranthus roseus* |
| Rosmarinic acid | *Coleus blumei* |
| Ubiquinone-10 | *Nicotiana tobaccum* |
| Diosgenin | *Dioscoria deltoids* |
| Colchicine | *Gloriosa superpa* |
| Littorine | *Atropa belladonna* |
| Rosmarinic acid | *Coleus blumeii* |
| Artimisinin | *Artimisia annua* |
| Gymnimic acid | *Gymnema sylvestre* |
| Taxol | *Taxus brevifolia* |

***Specialized Organ Cultures for Secondary Metabolite Production***

Besides cell, protoplast and tissue culture, specific organ cultures such as roots, tubers and rhizomes have been maximally exploited for *in vitro* synthesis and extraction of secondary compounds. Culture of isolated roots has long been feasible, root having the advantage of being a fully differentiated and organized unit and its growth is not affected by other organs [76,77]. Root cultures provide an efficient means of biomass production due to the fast growth rate and stable metabolite productivity. These cultures show biochemical and genetic stability and usually exhibit the full biosynthetic capacity associated with the organ in *planta*. Root cultures of *Hemidesmus indicus* have been utilized as a potent tool for the *in vitro* production 2-hydroxy 4-methoxy benzaldehyde [78]. Several authors have reported the long term genetic stability of root cultures; comparing other form of cell aggregates, rhizoids or adventitious tuberous roots could be a better alternative for accumulating a higher quantity of secondary metabolites [9,41,79,80]. As hairy roots are not connected with any other plant organs, metabolites produced cannot be transported to other plant parts for modification, degradation or storage. Many of the so-called novel compounds may be present in minor, undetectable quantities in roots *in planta* and these get accumulated in higher levels in hairy roots due to optimized culture conditions. *In vivo* sites of synthesis for many secondary compounds are unknown and many leaf based compounds may be synthesized in roots *in vivo* and so hairy roots can be manipulated for the production of various secondary compounds. Several reports are available on bioreactor based mass production of secondary compounds from hairy roots induced by *Agrobacterium rhizogens* mediated transformation [81].

Culture of tuber and rhizome pieces either to induce callus in order to obtain secondary compounds or to multiply such organs *in vitro* have been experimented. The success of such experiments is very limited however that holds promise for alternate production of source tissues in mass using suspension or bioreactor based methods. Micro-rhizomes have been induced from shoot cultures of *Curcuma aromatica*. Induction of micro-rhizome required transfer of shoots to BAP supplemented medium; quality of micro-rhizome was found to be determined by both the concentration of BAP and sucrose [82]. Besides the use to extract secondary compounds micro-rhizomes are feasible to facilitate germ plasm exchange across national borders. Among different forms of plant cell and tissue culture systems, specific organ cultures such as roots, tubers and rhizomes hold tremendous potential for *in vitro* production of uniform, high quality plant based medicines [6,9,77,78,83].

**Limitations over Secondary Metabolite Production *In Vitro***

Intact plant-based production systems have the problem that in most cases the natural product is present at low levels, or accumulates only in a specific tissue and at a

specific vegetative growth stage or upon certain environmental conditions. Furthermore, collecting material from the wild, not always in a sustainable way, can lead to over exploitation of endangered species as well as to habitat destruction. Nevertheless, about two thirds of all medicinal plants are still collected from the wild. The problem with whole plant extraction is also that the pharmaceutical industry prefers homogeneous samples with more or less constant levels of the active ingredient, which cannot be ensured from random wild sampling. Domestication of these plants would be a valuable alternative, giving rise to a more controlled environment, and thus more stable production. Plant tissue culture was believed to be the answer to these production problems, but unfortunately this has not delivered up to the expectations. The first problem encountered is that in cultures the natural product often does not accumulate at all, or at very low concentration. One obvious reason here can be that the natural product itself has a high intrinsic cellular toxicity. Different kinds of culture systems have been exploited, with varying success. Hairy roots have traditionally often been used, because they give high biomass that is easy to maintain, but again not all natural products will accumulate in these root cultures [65]. Tissue cultures also pose the problem that they sometimes have a slow growth rate, and that they are vulnerable to epigenetic changes, so-called somaclonal variation, and thus are not stable in their production level. The limited knowledge on the biosynthesis of natural products and its regulation forms a bottleneck for using tissue culture in combination with other approaches to improve resistance [84]. Hence, genetic transformation is also needed for metabolic engineering.

### *Genetic Transformation for Improved Secondary Metabolite Production*

Recent advances and developments in plant genetics and recombinant DNA technology have helped to boost research into secondary metabolite biosynthesis. A major line of research has been to identify enzymes of a metabolic pathway and then manipulate these enzymes to provide better control of that pathway [85]. Transgenesis is currently used for genetic manipulation of more than 120 species of at least 35 families, including the major economic crops, vegetables, ornamental, medicinal, fruit, tree and pasture plants using vector mediated or direct transformation methods [53]. However, *Agrobacterium*-mediated transformation offers several advantages over direct gene transfer methodologies (particle bombardment, electroporation, etc.), such as the possibility to transfer only one or few copies of DNA fragments carrying the genes of interest at higher efficiencies with lower cost and the transfer of very large DNA fragments with minimal rearrangement [54,85]. Genetic transformation has been reported for various medicinal plants. Successful regeneration of transgenic neem plants (*Azadirachta indica*) was reported using *Agrobacterium tumefaciens* containing a recombinant derivative of the plasmid pTi A6. The genetic transformation of *Atropa belladona* has been reported using *Agrobacterium tumefaciens,* with an improved alkaloid composition [86]. *Agrobacterium* mediated transformation of *Echinacea purpurea* has been demonstrated using leaf explants [87]. Genetic transformation would be a powerful tool for enhancing the productivity of novel secondary metabolites of limited yield.

*Agrobacterium rhizogenes* has been used regularly for gene transfer in many dicotyledonous plants. Plant infection with this bacterium induces the formation of proliferative multi branched adventitious roots at the site of infection; the so-called 'hairy roots'. Transformed hairy roots mimic the biochemical machinery present and active in the normal roots, and in many instances transformed hairy roots display higher product yields. Hairy roots, transformed with *Agrobacterium rhizogenes*, have been found to be suitable for the production of secondary metabolites because of their stable and high productivity in hormone free culture conditions [6,70,73]. *Agrobacterium rhizogenes* mediated hairy root culture system have been established in several medicinal plants such as *Artemisia annua [88]* and *Aconitum heterophyllum [89]* by infection with *Agrobacterium rhizogenes.* An efficient protocol for the development of transgenic opium poppy (*Papaver somniferum* L.) and California poppy (*Eschscholzia californica* Cham.) root cultures using *Agrobacterium rhizogenes* is reported [90]. Bonhomme *et al [*91] have reported the tropane alkaloid production by hairy roots of *Atropa belladonna* obtained after transformation with *Agrobacterium rhizogenes*. Argolo *et al [*92]

reported the regulation of solasodine production by *Agrobacterium rhizogenes*-transformed roots of *Solanum aviculare*. Souret *et al [93]* have demonstrated that the transformed roots of *A. annua* are superior to whole plants in terms of yield of the sesquiterpene artemisinin. Shi and Kintzios [94] have reported the genetic transformation of *Pueraria phaseoloides* with *Agrobacterium rhizogenes* and puerarin production in hairy roots. Thus, these transformed hairy roots have great potential as a commercially viable and an alternative form of raw material for the extraction of secondary metabolites.

***Functional Genomics and Secondary Metabolism***

In principle each natural product is formed by chemical transformations of small and larger molecules through a number of enzymatic reactions. To understand how a natural product is synthesized, the enzymes involved in these reactions need to be identified and the complex network of regulations and interactions to be understood. This identification can be done on a gene and genome level [95-99]. This might, however, have the disadvantage that it does not always give information on the nature of encoded enzyme and which reaction is being biochemically performed by the enzyme. Functional genomic strategies can thus be used in combination with metabolomics to elucidate biosynthetic pathways of natural products [96]. The basic question is to identify all the players involved in the biosynthesis of a natural product, both on the enzyme level and on the regulation level, so that the road is paved for metabolic engineering. The general concept behind metabolic engineering is that certain pathways within a biosynthesis network could be stimulated or favoured over others, by over expressing a crucial, for example rate limiting enzyme. In simple cases this approach has led to good results. Lee *et al* have reported higher biosynthesis of triterpenes and phytosterols in *Panax ginseng* upon over-expression of squalene synthase gene [97]. Increased synthesis of flavonoids and carotene in tomato obtained through RNAi-mediated suppression of the DET1 gene [98]. Over-expression of a transferase, has shown to give rise to accumulation of chlorogenic acid, an antioxidant which protects animals against age-related degenerative diseases [84]. In addition, transcription factors regulating a whole pathway could also prove effective role to determine the synthesis of the product of interest[99]. Alternatively, silencing a gene which give rise to a specific enzyme of certain pathway, can also lead to the accumulation of specific metabolite. Allen *et al*. have shown that when the pathway leading to morphine in *Papaver somniferum* was silenced, reticuline and its methylated forms have been accumulated as a positive side effect [95]. Reticuline is a potential substrate for the synthesis of various antimalarial and anticancer products. Specific genomic tools have been developed to improve and speed-up functional analysis of candidate genes in transgenic plant cells. Medium through-put strategies for isolation of full length ORFs, super-transformation of plant cells with reporter gene constructs, transient protoplast expression assays and micro-array facilities, have been designed and their potential also been validated.

## Conclusion

Plants have been an important source of medicine for thousands of years. Tissue culture is useful for multiplying and conserving the species, which are difficult to regenerate by conventional methods and save them from extinction. Production of secondary metabolites can be enhanced using bioreactors. Genetic transformation may provide increased and efficient system for *in-vitro* production of secondary metabolites. Biosynthetic potential of cell and organ cultures will be enhanced by means of elicitation, precursor feeding, *A.rhizogenes* mediated transformation. New transgenic varieties could be created as efficient green production lines for pharmaceuticals, vaccines and anticancer drugs. Functional genomic tools are helpful to analyze the differential gene expression pattern of cell cultures and to produce designer secondary metabolites. So, the improved *in-vitro* plant cell, organ and tissue culture systems have higher potential for conservation of medicinal plants and commercial exploitation of herbal medicines.

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