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Plant Cell Tissue and Organ Culture Biotechnology and Its Application in Medicinal and Aromatic Plants

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Background and Scope

Without claiming to deal with all features of this topic, this paper aims to provide a brief overview of the role of plant cell tissue and organ biotechnology in the field of the conservation and improvement of Medicinal and Aromatic Plants (MAPs). Several textbooks and review articles have been described this topic more comprehensively than is possible in this editorial [1,2].

MAPs are increasing worldwide and continue to attract growing interest for farmers, traders, economists, teachers, professionals, health officials and various industries. The MAPs are natural biological resources that have a great potential to synthesize a huge variety of important secondary metabolites, also referred to as natural products, far more than animal and even microorganisms. Are defined as secondary metabolites all specific compounds (characteristic of a species) which are not synthesized by metabolic pathways of primary metabolism that they are common to large groups of plants. Secondary metabolites are rather scarce, their structure is often very complex and their synthesis requires multistep enzymatic reactions. Their levels in plants are low, typically less than 0.1 to 5% of the biomass, and vary depending on numerous factors such as tissue type, plant developmental stage, environmental conditions, etc. These natural compounds are used as pharmaceuticals, agrochemicals, and cosmeceuticals. Recently, MAPs also are used as functional herbal food ingredients, nutraceuticals and health products [3]. The supply of the source plants however, is often limited due to diseases, changes in climate, and changes in the development in the growing regions.

It is well known that MAPs have been traditionally harvested in the wild state, in only few regions, and they are not used equally worldwide. To date, these phyto resources have been exploited nearly without any major limitation which may easily result in the exhaustion of plant genetic resources, biotope destruction and loss of wild populations and thus also threatening valuable incomes for rural households, especially in developing countries. Consequently, the sustainable use of natural resources has become an unavoidable necessity from both environment protection and socio economic points of view. Currently, between 4,000 and 10,000 MAP are on the list of threatened and/or endangered species and this number is expected to rise. These problems could be overcome through MAP selection and cultivation under agricultural conditions which also could respond to increasing demands in terms of plant security and traceability, socio-economic development, biodiversity conservation and sustainable use of genetic phyto resources as basic inputs for the future. Selection in wild population is the most common method of MAP breeding and cultivation means the use of traditional methods of cuttings, layering, grafting, etc., as well as the application of Plant Cell Tissue and Organ Culture (PCTOC) tools to produce a huge number of selected and genetically identical plants.

Furthermore research provides the prerequisites of efficiently breeding, for increasing the levels of desired compounds, decreasing the undesired compounds, more resistance, and most homogeneity uniformity of new varieties. Inheritable traits of MAPs can be adapted by breeding to the special demands of the costumers and of the supply chain. Evidence of the increasing importance MAP breeding has been demonstrated by the growing number registered varieties during the past decade [4]. Cultivated material is more appropriate for large scale uses such as the production of bioactive compounds by pharmaceutical, cosmeceutical and aromatherapy entities, which require standardized products of guaranteed or known content. The fully traceable MAP at the production chain has become necessary for a quality product. The plant characteristics should be identified, quantified and well defined with respect to the destination profile before plantation in the field.

The production script starts with the selection of the best quality of seeds, breeds and cultivars. In fact, cultivation decidedly guarantees a steady source of feedstock. Wholesalers and specialized companies could negotiate supply contracts regular and reasonable prices over time with producer and allows controlled post-harvest handling and, therefore, quality controls can be ensured, and product standards can be set with the regulations and consumer preferences [5-7]. Nonetheless, many problems persist including genetics (origin, variation), morphogenesis (leaf position and age, harvest, flowering), environment (temperature, photoperiod and light intensity), and finally agricultural practices (nutrition, irrigation, propagation, harvesting and extraction).

In order to overcome some of these various problems, the agro biotechnological approach by utilizing plant cell tissue and organ culture (PCTOC) technology has been expected to be an efficient and useful tools for the breeding (selection) of high-quality MAPs [8-10] and for the preservation of endangered species as well [10,11]. While providing an alternative source of mastered production of phytochemical products [11,12].

Potential of Plant Cell Tissue and Organ Culture Biotechnology

PCTOC biotechnology is a corpus of techniques designed for the growth and multiplication of plant cells tissues and organs using nutrient solutions under aseptic and in a defined physical and chemical environments *in vitro*. Provided that an appropriate phytohormone regime is chosen, explants such as plant meristems, buds, leaves, stems, roots, etc cultivated *in vitro* can undergo coordinated division and development resulting in the formation of various and complex structures such as cotyledons, shoot tips, hypocotyls, anthers,

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internodes, leaf disks, roots, stem and thin cell layers and ultimately complete plants. PCTOC is based on the principle of totipotency, which states that every cell within the plant has the potential to regenerate into a complete plant [11,13,14].

Briefly, PCTOC is now a proven technology for the rapid clonal propagation [15,16], preservation of endangered species and valuable regeneration and multiplication of genetically modified fertile clones [10,11,15-17] enzymes production [14] and economically valuable chemicals [11,17-19]. The empirical approach that has been extensively used in numerous studies on PCTOC has shown that success is largely dependent on three factors: explant choice, medium composition, especially phytohormone or synthetic growth combinations and control of the physical environment. The composition of a nutrient medium, preferable to a certain plant species, is nearly the main task for the establishment of successful plant PCTOC techniques. This biotechnology has been developed to such a level that any MAP species can be *in vitro* regenerated through one of the following methods: embryo culture, anther culture, callogenesis, somatic or asexual embryogenesis, and organogenesis. The choice of either method depends on the species, the success rate of the method for producing plants at a realistic cost, and local production conditions.

PCTOC tools may offer certain advantages over traditional agricultural methods of propagation, including: 1-the rapid production and propagation of high quality disease-free and uniform planting material; 2-the MAP propagation can be done under a controlled environment, anywhere, independent of climatic changes and soil conditions, on a year-round basis; 3-for slow-growing and sparsely distributed species, poor production of seeds or seeds with poor germination rate, rapid multiplication is possible; 4-the easy selection of desirable traits directly from the *in vitro* culture, thus reducing spaces needed for field testing; 5- the reduction of the selection cycle required in the MAP is possible, no need to wait for the lifecycle of seed development; 6-it allows for the avoidance diseases of plants by careful selection and the use of sterilization techniques; 7-it allows the preservation of the gene pool by storing pollen, organs, tissues and cells (as a seed bank); 8-it enables the reduction of deposits for a large number of viable plants; and 9-it guarantees national and international trade and exchanges of diseases-free plant material (Quarantine is not required).

In addition, use of PCTOC systems offers a tremendous tool for dissecting the physiological, biochemical and molecular regulation of plant development and stress response phenomena. They also are extensively utilized for clonal propagation, as a gateway for genetic engineering of a vast majority of valuable economic plants and as an economical and large scale production platform for various pharmaceutics, drugs, flavors colors, enzymes and medicine based compounds under controlled conditions [9-14].

Many plant species containing high-value compounds are difficult to cultivate and the chemical synthesis of plant-derived compounds is often not economically feasible because of their highly complex structures. The PCTOC of MAPs can provide an alternative way of consistent medicinal chemical isolation from plant materials [18,19].

After approximately four decades of the study and numerous attempts by several laboratories around the world, it has been demonstrated that PCTOC systems will produce many unusual secondary metabolites. Compared to the conventional methods for propagation of whole plants, the advantages of PCTOC systems are:

- the production of useful compounds can be produced under controlled physical and chemical environments independent of climatic changes or soil conditions;
- organ, tissue and cell culture are free of diseases and pathogens and insects;
- the plant cells of any species could easily be *in vitro* cultivated to produce their specific metabolites;
- organ, tissue and cell can be selected for high production of many compounds;
- organ, tissue and cell can be stored for long periods,
- plant cells can be grown in automated bioreactors, containing thousands of liters of medium, with improved productivity and reduced labor costs;
- vitro plant cells often produce different quantities with different profiles of secondary metabolites and this production might be more manageable reproducible and reliable;
- harvest the product can be quick, clean, concise and accurate compared to the extraction of complex whole plants,
- organ, tissue and cell cultures may provide a source of defined standard phytochemical in large amounts.

In addition, new molecules that have never been found in plants or were not chemically synthesized are produced by cell cultures. Thus, this technology is a true path of producing new molecules and constitutes powerful tool of achieving production of novel secondary metabolites with new and improved biological activities that will be of great value for the pharmaceutical industry. Thus, the discovery and development of novel, more efficient, and safer phyto-therapeutics that will contribute to cure, at lower costs to the healthcare system, a variety of human diseases. In this context, the development of bioprocess for industrial scale production of plant secondary metabolites is thus of first importance. Several attempts were undertaken to produce secondary metabolites of major importance, through the use of cell or tissue cultures as an alternative to whole plant extraction with limited success because of the slowness and low yield of the culture process. [1,12,18,19].

Cell suspension cultures are the most culture type in the research and production of secondary metabolites. Unfortunately, in many cases low quantities of the desired compounds accumulated by undifferentiated cell cultures and often the biosynthesis of a certain compound is not produced in cell culture and remains organ or tissue specific [20,21]. In such cases the organ cultures, i.e. shoot or root cultures, may provide a better alternative than undifferentiated cell cultures. For example, cardenolide biosynthesis was achieved in cultures regenerating shoots or somatic embryos more than undifferentiated cells of *Digitalis sp*. The growth and the alkaloid content of *in vitro* cultured *Fritillaria unibracteata* [22,23] were higher than found in the wild plant specie [24].

Otherwise, the pharmaceutical industry is highly interested in the secured availability and biotesting of natural but also of novel phyto-molecules for the discovery of new drugs. In particular, demand is high for new families of phyto-molecules displaying improved properties, including lower toxicity and higher solubility in water, and for feeding the many new molecular targets resulting from the intensive research in genomics and proteomics.

Transformed hairy roots induced by using *Agrobacterium rhizogenes*, that can be excised from the explant and placed on a

medium containing a suitable antibiotic to free them from the bacteria, are characterized by high growth in hormone free media and remains stable genetically. In many cases they show higher product yields and can be used as a promising source for the continuous and standardized production of secondary metabolites under controlled conditions without losing genetic or biosynthetic stability [25,26].

These new trends in the biopharmaceutical industry have stimulated research interest in various areas for the supply of new chemo-diversity including elicitation, genetic transformation to manipulate specific genes involved in secondary metabolism biosynthetic pathways. Whatever *in vitro* systems used for secondary metabolites production or harvest, and depending on the usage in the in different approaches and strategies are being used to obtain higher yields of secondary metabolites in cultures through biotic and/or abiotic elicitors (methyl jasmonate, salicylic acid, chitosan, autoclaved pathogen phytoalexins, heavy metal ions, osmotic stress, ultraviolet or gamma irradiations, high salinity,…), gene technology, media manipulation, adsorption of the metabolites to overcome feedback permeation of metabolites, to facilitate downstream processing, phytohormone combinations, precursor feeding and immobilization, many of them are completely inter active. The basic idea behind the usage of elicitors focuses on that they induce upon administration in the cell cultures, which concomitantly affects the yield and quality parameters of the secondary products accumulated [11,12,18,19].

Attempts have been made to manipulate pharmaceutically important medicinal plants for their secondary metabolic pathways by using transgenic technique. The development of transgenic plants is the result of integrated application of rDNA technology, gene transfer and PCTOC techniques. These technologies have enabled the production of transgenic plants in more than 150 species, which include MAPs and most major valuable crops. Examples of direct DNA transfer methodology to engineer medicinal plants and cultures have been reviewed [27]. Direct genetic transformation, a technology that is progressing fast in other areas of plant improvement, can only be exploited for secondary metabolite formation if we have information about the gene(s) controlling the synthesis of the desired product or genetic markers associated with it. Knowledge and mastery of the biosynthetic pathways of secondary metabolites, their regulation and genetic control is the keystone to significantly increase yields in production of molecules in cells. Since secondary products however are often biosynthesized in multi-step enzymatic reactions in specifically differentiated cells manipulations of such pathways to alter metabolic production is complex, complicated and unpredictable.

Scale-up of *In Vitro* Mass Production of Secondary Metabolites

Large-scale PCTOC offers a smooth and controlled sourcing of phytochemical products independent of the plant availability. The environment in witch plant cell tissues and organ grow usually changes when cultures are scaled-up from shake flasks to bioreactors, and this may result in reduced productivity. With the ultimate aim of implementing an industrial-scale process, the behavior of cell and tissue culture in bioreactor has received much attention. The researchers are currently developing efficient bioreactors to improve the cell growth and production of secondary metabolites. It is noteworthy to point out that PCTOC have proven successful for the commercial production of several important plant compounds. Scale up of the process from laboratory scale, through pilot to industrial scales was successful, adopting either continuous or semicontinuous cultures and using stirred bioreactors with a capacity of up to 75,000 liters, with full automation of medium preparation and sterilization, transfers, stirring, growth monitoring and harvesting [28]. This has been successfully achieved for the production of immunostimulant polysaccharides by *Echinacea purpurea* cultures [29] and several other plant constituents by commercial or semi-commercial large scale procedures [30,31].

It should be mentioned that literature reports provide only an incomplete picture of the actual commercial progress, since current industrial investigations fall well under the realm of proprietary and patentable research [31]. Briefly, in 1983, the anti-inflammatory drug shikonin produced by plant cell cultures of *Lithospermum erythrohizon* on an industrial scale for the first time by Mitsui Petrochemical Industries Ltd. [31]. Screening for clones derived from individual high-producing cells, and the use of two state cultures one for growth and the second for production has resulted in the commercial production of the antihypertensive drug, ajmalicine by *Catharanthus roseus* cultures [32]. Rosmarinic acid from cell suspension cultures of *Coleus blumei* [33,34], digoxin by cell cultures from Digitalis [35]. Ginsenosides from *Panax ginseng* cultures [36]. The successful industrial production of paclitaxel, an anti-cancer drug originally extracted from *Taxus brevifolia*, by plant cell cultures [37] will trigger research into other plant-based chemotherapeutics such as podophytoxin and comptothecin etc.

Concluding Remarks and Outlooks

Even with the advances in microbial and chemical productions process, plants remain an indispensable source for a number of chemical substances that are difficult to synthesize chemically owing to their complicated structures. They represent a huge source of untapped chemicals, with a wide variety of proteins and secondary metabolites that have already been isolated from plant. Nowadays most of MAPs are still harvested from the wild in a no sustainable way. Such practice can lead to over-exploitation of endangered and vulnerable species as well as to biotope destruction. Therefore, cultivated material is more suitable for large scale uses and breeding of MAPs is undoubtedly linked to the future success of the exploitation. MAP cultivation is a promising way to respond to existing and future needs in terms of biomass and by-products. Likewise, it decidedly accounts for a sustainable solution for reducing harvest pressure on wild populations and preservation of biotopes. In this context, PCTOC are of great importance in selection and propagation of valuable genotypes and obtaining of new genotypes , and therefore both in obtaining of uniform plant material (for economic purposes) and increasing of diversity (for conservation purposes). PCTOC has been established for a wide variety of species, a useful strategy for secondary metabolites production from endangered or rare plants. Despite the enormous progress achieved in secondary metabolites production into PCTOC systems, several important challenges remain. These include the low productivity, only a small fraction of plant natural products can be expressed at industrially useful levels in culture, recalcitrance of some plant genotype or cell line systems to express their biosynthetic potential, knowledge of the genetic control and mastery of the biosynthetic pathways of secondary metabolites and their regulation are the keystones to significantly increase yields in production of molecules.

There has been remarkable progress in understanding of the biochemistry of MAPs due to the development of new analytical tools such as metabolomic proteomics, and genomics. These new techniques that not only provides information on phenotypic variation, caused by *in vitro* environment, but also includes data about genes involved in metabolic pathways of biosynthesis. As well, these new tools could

facilitate discovery and development of novel, more efficient, and safer phyto-therapeutics that will contribute to cure, at lower costs to the healthcare system, a variety of human diseases.

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