Georgiev, Biochem Pharmacol (Los Angel) 2015, 4:5
DOI: 10.4172/2167-0501.1000e180

Biochemistry & Pharmacology: Open Access

Editorial Open Access

Mass Propagation of Plant Cells – An Emerging Technology Platform for Sustainable Production of Biopharmaceuticals

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Editorial

Plants have been used as a source of natural compounds with unique chemical structures and wide range of biological activities since time immemorial, and to this day, 11% of the essential drugs for human application originate in plants [1]. The expanded demand for medicinal plants raise issues of concern about sustainability, conservation and the preservation of natural habitats. Overharvesting of some species for commercial uses reduced the stocks of wild populations and has placed some species under threat [2]. Moreover, most of the medicinal plants are rare or endemic species, growing under specific climate, soil or latitude, which complicates their domestication and breeding. To address the above limitations, the recent researchers' efforts have been focused on plant tissue culture technique as a new reliable and environmental friendly platform for production of plant metabolites. Mass propagation of plant cells, tissues, organs or genetically modified plants in a controlled environment can be viably up-scaled for commercial production of wide range biopharmaceutical as: plant-made pharmaceuticals (PMPs, including secondary metabolites and recombinant therapeutic proteins), plant-made vaccines (PMVs) and plant-made industrials (PMIs, including technical enzymes, antibodies, cosmetic active ingredients, food supplements and nutraceuticals) [3].

Plant cell culture technology (PCCT) is a tissue culture technique that deals with large scale cultivation of plant cells under submerged conditions. PCCT is a powerful technology platform for production of bioactive phytochemicals, active cosmetic additives, food supplements and nutraceuticals, which in general utilizes non-genetically modified plant cells, grown under controlled microenvironment in a form of suspended single cells and/or small aggregates. Several pharmaceutically active compounds, including: tropane alkaloids atropine, hyoscyamine and scopolamine [4]; benzylisoquinoline alkaloid protopine [5]; diterpenoids as paclitaxel [6]; triterpenoids as ursolic and oieanolic acids [7,8]; pentacyclic triterpenoid saponins as centellosides [9]; stilbenoids as resveratrol [10]; aryl tretralin lactone podophyllotoxin [11]; phenylethanoid glycosides as verbascoside and leucosceptoside A [12]; etc. have been found to accumulate in plant cell suspensions. However, only limited number of PCCT have been successfully commercialized and the following PMPs are available on the market: anti-inflammatory drug shikonin from *Lithospermum erythrorhizon*; anticancer drug paclitaxel from *Taxus* spp.; anticholinergic alkaloid scopolamine from *Duboisia* spp.; anti-inflammatory protoberberines from *Coptis japonica*; anti-inflammatory phenolic rosmarinic acid from *Coleus blumei*; anticancer compound geraniol from *Geramineae* spp.; anticancer and antiviral agent podophyllotoxin from *Podophyllum* spp.; cardioprotective triterpene saponins mix Ginseng from *Panax ginseng*; tyrosinase inhibitor arbutin from *Catharanthus roseus*; and immunostimulant polysaccharides from *Echinaceae* spp. [13,14]. A breakthrough in commercialization of PCCT has been made with adaptation of technology for production of bioactive phytochemicals and plant cell biomass used as active cosmetic ingredients, where the natural origin and the lack of genetic modifications are of special importance for consumers [15,16]. By using PCCT the cosmetic industry can rely on continuous supply of high quality biomass from exotic, rare, protected, or endangered plants, or plants growing in remote, barely accessible areas [15]. Recently, several PMIs obtained by PCCT have been released, including anti-ageing, calming, and protecting cosmetic supplement "Resistem™" Sederma (www.sederma.fr); UV-protective additive PhytoCellTec™ Solar Vitis, anti-aging and delaying the senescence of hair follicles supplement PhytoCellTec™ Malus Domestica, and anti-aging and UV-protective supplement PhytoCellTec™ nunatak® from cell culture of rare and protected plant species *Saponaria pumila* (recognized as an Eco breakthrough at the UN Conference Rio+20) by Mibelle Biochemistry (www.mibellebiochemistry.com). Because PCCT utilizes non-genetically modified plant cells, the biomass obtained by that production platform could be used to improve nutritional and medicinal value of food, when added as nutraceutical supplement [17]. Recently, the company DianaPlantSciences released Cocovanol™ - a dietary supplements consisting of freeze dried biomass of *Theobroma cacao* cell suspension. According to company web site Cocovanol™ is caffeine and theobromine free product, with improved flavonoids pattern, standardized 15% total polyphenols content, and high total antioxidant score (www.diana-group.com). Cocovanol™ is the first commercial food additive produced by PCCT and granted with the status of GRAS (Generally Recognized as Safe), which paved the way for development of PMIs nutraceuticals by using the PCCT technology platform. However, PCCT still have some limitations that slowed down its scale-up and commercial realization. The main speed breakers are the low and variable yields of targeted biopharmaceutical and the high level of somaclonal variability of production clones, which results in product loss and reduce the economic feasibility of the process [14]. To address the above limitations, cambial meristematic cell (CMC) cultures have been developed and adapted for production of biopharmaceutical [18]. Production of PMPs vindoline, catharanthine, and ajmalicine has been reported in CMC culture of *Catharanthus roseus* [19]. Recently, the company Unhwa secured the world's first patent for plant CMC isolation and culture (www.unhwa.com). The company developed PCCT for commercial PMP by *P. ginseng* CMC culture and medicinal ginsenosides (gypenoside XVII, ginsenoside F2, insenoside Rg3, insenoside Rg5 and compound K) have been produced in high yields (17, 54, 75, 70 and 50 mg/g DW, respectively) [20]. Currently, Unhwa investing in research for development of CMC

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Received November 01, 2015; **Accepted** November 03, 2015; **Published** November 07, 2015

Citation: Georgiev V (2015) Mass Propagation of Plant Cells – An Emerging Technology Platform for Sustainable Production of Biopharmaceuticals. Biochem Pharmacol (Los Angel) 4: e180. doi:10.4172/2167-0501.1000e180

cultures of *Taxus cuspidata*, *Ginkgo biloba* and *Solanum lycopersicum* for cosmetic active ingredients application [20].

With the advance of synthetic biology molecular biology, metabolomics and genetic engineering plant tissue culture become an attractive production platform for molecular farming [21]. In contrast to PCCT, plant cell molecular farming deals only with genetically modified plant cells and uses them as a host to produce recombinant protein therapeutics such as: vaccines, antibodies, and industrial proteins. Molecular farming by mass propagation of plant cell suspensions has several advantages than the agricultural-scale production [21,22]. Plant cell suspensions are amenable to good manufacturing practice (GMP) procedures and can be cultivated relatively easily in large volume bioreactors similar in design to that used in industrial microbiology [22]. Wide range of PMPs - proteins of medical relevance [22] and cytokines [23,24]; PMVs - vaccines [23,25-27]; PMIs - antibody [23] and antimicrobial peptides [28] have been produced by utilizing plant tissue culture production platform. The breakthrough in commercialization has been made on 2006 when The US Department of Agriculture (USDA) issued the first market license to veterinary vaccine produced by plant cell suspension culture [29]. The vaccine against Newcastle disease virus was developed by using tobacco BY-2 cells as production platform as a proof a concept by the Dow AgroSciences and was never released on the market but paved the way for future development of PMVs [21,29]. The next important step in development of plant cell suspension for the needs of molecular farming have been made in 2012, when Israel company Protalix Biopharmaceutics received the first US Food and Drug Administration (FDA) approval for plant cell-based recombinant therapeutic protein for human use (ELELYSO™ - taliglucerase alfa) produced by company patent protected platform ProCellEx® utilizing genetic engineered carrot and tobacco cell cultures (www.protalix.com). However, despite the commercial success of ELELYSO™, development and commercialization of other recombinant therapeutic proteins for human use, produced by plant cell culture platform is going slow. Major constraints arising from some limitation of production platforms, concerning the low yields and the nonmammalian glycosylation pattern of produced proteins [22]. Recently, a successful proof of concept for improving yields and recovery of recombinant protein by combining the hydrophobin fusion technology and surfactant-based aqueous two-phase separation (ATPS) in tobacco BY-2 suspension cell platform have been reported [30]. Moreover, it was demonstrated that glycosylation pattern of human secreted alkaline phosphatase produced by recombinant tobacco NT1 cell suspension cultures can be modified by manipulating nutrient medium composition [31]. The yields of biopharmaceuticals could be also improved by proper optimizations of environmental parameters, nutrient medium and downstream process [32].

In summary, this editorial highlights the recent advance in use of plant cell suspension systems as production platform for biopharmaceuticals. The current lifestyle pushes the consumers to search for pharmaceuticals and nutraceuticals with natural origin and the tendency is the demand to continue growing in the near future. Plant cell suspension offers powerful technology platform for sustainable production of biopharmaceuticals in both non-genetically and genetically modified systems and could be considered as a bio-factory of the future.

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