

Biotechnology based on cell cultures of higher plants

Amira Nourani^{1,*}, *Elena Popova*² and *Maria Titova*²

¹ Peoples' Friendship University of Russia (RUDN University), 117198, 6 Miklukho-Maklaya Street, Moscow, Russian Federation

² K.A. Timiryazev Institute of Plant Physiology of Russian Academy of Sciences, 127276, 35 Botanicheskaya st., Moscow, Russia

Abstract. This paper reviews the role of plant cell culture as a biotechnological tool in preserving the botanical diversity of higher plants while meeting the growing demand of the commercial market for large volumes of plant raw material. The prospects of plant cell-based technology are discussed in the framework of creating an economy of sustainable development in the short and long term.

1 Introduction

Conservation of biodiversity of higher plants in ex situ collections, such as botanical gardens, seed and plant banks in vitro, is one of the most promising and actively developing strategies for the conservation of plant genetic resources worldwide [1].

Modern biotechnological tools enable the formulation and development of new solutions for the protection of botanical plant biodiversity. One of such solutions is the in vitro method of cultivation of plant cells and organs, which is based on the idea of totipotency - the possibility of preserving the "memory" of the functional potential of the whole organism in individual cells. In vitro cultures of plant cells and organs can serve on the one hand, as a renewable source of biologically active secondary metabolites [2] and, on the other hand, can become the basis for developing new varieties with valuable traits.

In addition, establishment of in vitro collections of cultured plant cells represents a promising alternative method of providing renewable plant sources for Hi-Tech industrial production based on cell cultures [1].

2 Cell culture and conservation of plant biodiversity

Plants produce about 100,000 kinds of known metabolites, and many new compounds from plants continue to be discovered every year [3]. The demand for higher plants for food production and for the needs of the pharmaceutical industry has increased in recent decades.

* Corresponding author: 1032194279@pfur.ru

Pharmaceutical companies rely heavily on plant materials from rapidly depleting natural sources. Plants collected in the wild account for up to 80-90% of plant raw materials for commercial production [2], so the need for alternative renewable sources of plant biomass has increased significantly in recent decades, especially for rare and endemic plant species. The traditional cultivation of medicinal plants on plantations is time-consuming and requires the use of pesticides, which often discredits the quality of the produced plant biomass.

For this reason, the search for alternative and self-sustaining biotechnological methods for the production of plant raw materials of high quality is actively developing all over the world. Application of such methods would reduce the collection of wild plants and thus contribute to the preservation of their biodiversity.

Plant cell culture allows the production of plant biomass of controlled quality to meet the needs of the commercial market, with the ability to scale up the production process and increase the yield of the desired product without the use of toxic insecticides and herbicides [4]. It should be noted that cell-based biotechnologies are dependent heavily on the development and use of cell strains with the ability to accumulate biologically active compounds in greater quantities compared to wild plants [5]. The presence of active metabolites, including various isomeric forms, that are unusual or seldomly presented in wild plants is another asset of cell culture that recently attracted researchers' interest [1].

The commonly discussed advantages of cell cultures compared to wild and plantation cultivated plants is the possibility to produce biomass with guaranteed quality and desired characteristics, regardless of season, climatic changes and environmental conditions. In addition, cell-based technologies are ecologically friendly and fit well in the most recent trends of sustainable production systems and responsible consumptions [1].

3 Cell culture in the economic market

From the very start of the research on plant cell cultures, their commercial use has attracted attention [6] whether for pharmacological, cosmetics, food production or for the industrial use of natural dyes, and the demand for cell-derived products in the market is growing.

3.1 Cell cultures for pharmaceuticals

The richest sources of medicinal substances are medicinal plants. In recent decades, suspension plant cell cultures have been successfully used for the production of valuable secondary metabolites with high biological activity [7].

The development of plant cell culture methods has significantly diversified the production of secondary metabolites of commercial interest, such as alkaloids, phenolic components, isoprenoids, and indole alkaloids. The latter are one of the most diverse groups of secondary metabolites used in medicine as an anticancer agent (camptothecin, vincristine, and vinblastine, as well as the antiarrhythmic alkaloid aymalin) [2].

By 1989, the sales of plant derived products on the North American Market increased on an average by three billion US dollars annually, moreover, in Europe 2000, in China 10,000 species are used as medicinal plants [2].

For instance, culturing of plant cells has been considered as a promising alternative for the efficient production of ginseng biomass and its active ingredients, which pharmacological functions include antiplatelet, hypocholesterolemic, antitumor, immunomodulatory functions, and help improving the central nervous system [8].

According to Transparency Market Research estimates, by 2025 the global market for anticancer drugs will reach 155.6 billion US dollars [9]. For example, paclitaxel which is produced from the *Taxus sp.* in Canada, USA, Germany and South Korea [1].

3.2 Cell culture for the dye industry

Cell cultures can be used to obtain natural dyes [6]. It should be noted that the market for natural dyes is one of the fastest growing markets for the food and cosmetics industry with an estimated volume of the global anthocyanin market of \$ 291.7 million in 2014 and a forecast of \$ 387.4 million for 2021 [6,9]. For example, cell cultures are a promising commercial source of shikonine [5]. It has been shown that the amount of shikonine produced by the cell culture of *Lithospermum erythrorhizon* was 20 times greater than that existing in the intact plant [2,10].

3.3 Cell cultures for the cosmetic industry

The global cosmetics market is increasing every year, and the demand for raw materials for the cosmetics industry is high.

The emerging trend of manufacturing cosmetics and food products in a natural and sustainable manner has brought a new wave in plant cell culture technology over the past 10 years. More than 50 products based on extracts from plant cell cultures have made their way into the cosmetics industry during this time, whereby many are produced with plant cell suspension cultures [11].

Plants such as *Rhodiola rosea*, *Aloe barbadensis*, *Ajuga reptans*, *Echinacea spp.*, and *Calendula spp.* are most popular in cosmetology [11]. Recently, cell cultures of crop and model plants such as *Nicotiana glauca*, *Nicotiana glauca*, *Malus domestica*, *Rubus idaeus*, *Vitis vinifera*, *Daucus carota*, *Oryza sativa*, *Coffea bengalensis*, *Syringa vulgaris*, *Rhododendron hirsutum*, *Camellia sinensis* have been also utilized [1]. It was shown that extracts of cell biomass have anti-inflammatory, antioxidant, and UV-protective activity; and activate the processes of skin regeneration [12].

3.4 Cell culture for the production of food supplements

The food supplements and additives industry represent a great opportunity in the global economic market. In addition, individual food ingredients are the basis of major global industries [13].

Such an approach has been implemented in a Zurich University of Applied Sciences (ZHAW) study which investigated the potential of callus and suspension cell lines of *Theobroma cacao* for the cocoa ingredient in chocolate production [11]. Another study on plant cell cultures from cloudberry, lingonberry and stoneberry validated the great potential of plant cell cultures for food purposes [14]. In Russia, cell culture biomass of a medicinal plant *Polyscias filicifolia* is used in a series of food supplements including “Vitagmal”, which was demonstrated to have anti-teratogenic and immunomodulating actions.

The annual world market for plant-derived vanilla flavor is estimated at more than US \$ 250 million, while the total annual worldwide herbal extracts market was recently estimated at US \$ 17.5 billion [14,15].

Table 1. Examples of historical and present biotechnologies based on plant cell cultures

Company (country)	Species	Product	Reference
Bristol-Myers Squibb USA/Global	<i>Taxus sp.</i>	Paclitaxel	[16]
Phyton Catalytic formed in 1990 in Ithaca, NY, USA	<i>Taxus sp.</i>	Paclitaxel	[17]

Company (country)	Species	Product	Reference
Samyang Biopharmaceuticals Corp., Seoul, Korea	<i>Taxus sp.</i>	Paclitaxel	[18]
ESCA genetics (USA), CA, USA	<i>Taxus sp.</i>	Paclitaxel	[19,20,21]
	<i>Vanilla planifolia</i>	Vanilline	[22]
Mitsui Petrochemical Industries (Japan)	<i>Lithospermum erythrorhizon var. erythrorhizon Sieb. et Zucco</i>	A mixture of shikonin derivatives	[23,24 25,26,27 [28,29,30]
	<i>Coptis japonica</i>	Berberine	[22, 31, 32,33]
	<i>Catharanthus roseus</i>	Formation of vinblastine from vincristine Catharanthine Arbtin	[34, 35]
DIVERSA Gesellschaft für Bio-und Verfahrenstechnik mbH (Germany)	<i>Rauwolfia serpentina</i>	Indole alkaloids Raucafficine Ajmaline	[36,36,37]
	<i>Echinacea purpurea, Echinacea augustifolia</i>	Immunostimulating polysaccharides	[38]
PCC Technology Inc. Japan (500 l)	<i>Aralia cordata</i>	Anthocyanins	[39]
Sumitomo Chemical Co., Ltd. (Japan)	<i>Duboisia</i>	Scopolamine	[35, 40]
Nippon Oil (Japan)	<i>Podophyllum</i>	Podophyllotoxin	[35]
A. Nattennann & Cie. GmbH, Biotechnology/Plant Cell Culture, Postfach 350120, 5000 Köln 30, FRG (Germany)	<i>Coleus blumei</i>	Rosmarinic acid	[41]
Nitto Denko (Japan)	<i>Panax ginseng</i>	Cell biomass	[42]
Kibun (Japan)	<i>Carthamus tinctorius</i>	Cartamine	[34,44,45]
Nippon Shinyaku (Japan)	<i>Beta vulgaris</i>	Betacyanins	[38,46]
Nippon Paint (Japan)	<i>Euphorbia milli</i>	Anthocyanins	[38]
PCC Technology Inc. Japan (500 l)	<i>Aralia cordata</i>	Anthocyanins	[40]
Boehringer Mannheim Co. (Germany)	<i>Digitalis lanata</i>	Biotransformation products of cardiac glycosides	[47]

Company (country)	Species	Product	Reference
Japan Tobacco Inc. Tokyo, Japan Later The Japan Tobacco and Salt Public Corporation	<i>Nicotiana tabacum</i>	Nicotine, tobacco biomass	[48-55]

4 Problems and prospects

In vitro plant cell cultures are a biotechnological strategy widely used for the production of biomass and metabolites with medicinal value for pharmaceuticals, cosmetology and food industries. Plant cell culture is a renewable, environmentally friendly and safe source of target substances that can be adapted for large-scale production.

However, the industrial application of plant cell cultures for the production of secondary metabolites is still not widespread, mainly due to the high costs of production process, the duration and high cost of certification of the resulting products, particularly for the pharmacological application [6].

In order to improve biomass production, several strategies have been used to increase the product yields at the production stage which include changes in medium composition, addition of inducers or precursors, elicitation [56], stress exposure, genetic engineering approaches or mutagenesis [57] which affect the qualitative and quantitative composition of the compounds synthesized [1].

Recent developments in this area allow to assume that, in the foreseeable future, industrial cultivation of plant cells combined with the development of superproducer cell lines will make a solid base for the biotechnological production of biomass and secondary metabolites to meet the growing needs of the commercial market. In turn, the transition to environmentally friendly, renewable raw materials in the form of cell cultures will help

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