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Plant Biotechnology Potential for Food Production: The Tyumen Region

Marina Viktorovna Nikolenko, Vera Viktorovna Trigub, Vladimir Grigorievich Popov, Elena Viktorovna Ragozinnikova, Galina Georgievna Cherentsova, Boisjoni Tokhiriyon*

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Abstract

A review of the research carried out by Russian and international scientists in plant biotechnology was performed. The potential application of plant biotechnology for commercial production of wild berries, and vegetables, as well as valuable medicinal raw materials and the design of functional food products, were explored. The composition and properties of biologically active compounds of the plants found in the West Siberian region were analyzed. Vaccinium vitis-idaea L., an evergreen shrub with a unique chemical composition, was explored. The conventional extraction procedures as well as cell cultures were analysed. Particular attention was drawn to micropropagation of plants as a way to obtain biologically active compounds. The experiments with the most common cell culture media were carried out. Vaccinium vitis-idaea L. grown in vitro was determined to be a promising source of food ingredients. The enriched food products can assist in improving the nutritional status and, thus, contribute to the improvement of public health per the state policy of the Russian Federation in the field of healthy nutrition.

Keywords: Biologically active substances, Plant micronutrients, Micropropagation, Plant biotechnology

Introduction

Plant biotechnology has recently attracted considerable attention thanks to its potential to use cells and tissues of plant-derived bioactive compounds (enzymes, nucleic acids, steroids, and complex organic compounds) (Yashchuzakova & Katargina, 2019). Plant biotechnology offers a set of techniques that enable scientists and producers to introduce and adapt plants to diverse climatic conditions, for example, severe weather in the Arctic regions, as well as to obtain supernatants for the food industry. Plant cells have unique structures. Plant cells are totipotent cells and can be cultivated in a liquid nutrient medium in the form of an unorganized mass (callus) (Elinov, 1995).

Over the last decades, mass production of plants with unique characteristics has evolved, which facilitated the obtainment of secondary plant metabolites like alkaloids and glycosides (Babikova *et al*., 2007; Timofeeva, 2007; Ivanova *et al*., 2014; Murashkina *et al*., 2015). Recently, many scientists have focused their research on studying the bioactive compounds of wild plants (Fedorova *et al*., 2020). Wild plants are considered especially valuable as they demonstrate high adaptability to environmental conditions and disease resistance. Many wild plants produce superior yields and offer better nutritional value when compared with cultivated varieties (Serba *et al*., 2018). Furthermore, wild plants, fruits, and berries are not treated with chemicals to control plant diseases. Therefore, the development of a methodology for the mass reproduction of wild ornamental plants, wild berries and vegetables, rare plants and valuable medicinal plant material is of great potential, since these plants can be used in the production of new functional foods (Lyutikova, 2013). The purpose of this study is to review and analyze the research conducted by Russian and foreign scientists in plant biotechnology.

Materials and Methods

Generalization and data mining, general scientific analysis tools, as well as statistical methods were applied in our research. The current diets of most city dwellers are deficient in micronutrients. To eat healthier and more balanced diets, meet energy requirements, and reduce the risks of developing long-term diseases, people have to follow the guidelines on the recommended intake of micronutrients (2008). Food fortification is a feasible solution that can be devised for public health benefit (Decree of the Government of the Russian Federation, 2016, Decree of the Government of the Russian Federation 2020).

The enormous diversity of plants in Western Siberia provides an unlimited source of biologically active compounds, cells, and tissues (Kapriyanova, 2018). Therefore, there are perfect opportunities for the development of new plant biotechnological techniques to be used in domestication. It should also be noted that the wild spaces of Western Siberia have not been explored properly, thus, many new plants are yet to be discovered (Kapitonova, 2019).

Results and Discussion

Marina Viktorovna Nikolenko, Vera Viktorovna Trigub, Vladimir Grigorievich Popov
Institute of Industrial Technology and Engineering, Tyumen Industrial University, Tyumen, Russia.

Elena Viktorovna Ragozinnikova, Galina Georgievna Cherentsova, Boisjoni Tokhiriyon *
Institute of Commerce, Food Technology and Service, Ural State University of Economics, Ekaterinburg, Russia.

***E-mail:** tohiriyoni@gmail.com

We analyzed the composition and properties of the plants found in the Tyumen region to identify those that can be used as sources for polyfunctional compounds and utilized in the commercial production of functional foods. It is known that plants provide primary metabolites, like amino acids, organic acids, fiber, vitamins, and minerals as well as secondary metabolites, like alkaloids, isoprenoids, flavonoids, essential oils, and saponins (Gavrilova *et al*., 2015). In the food industry, amino acids are used as flavor enhancers, food additives, and sources of protein. While organic acids are used as oxidizing agents and food preservatives (Khramova & Plisov, 2012; Spirichev *et al*., 2012). According to Zagoskina and Nazarenko, (2019), secondary metabolites impact taste and aroma and can be applied to alter and diversify seasonings, spices, and drinks. Several metabolites are employed in the production of dietary supplements. For example, flavonoids from Rhodíola rósea L. have adaptogenic and immunomodulating properties, arbutin from Vaccinium vitis-idaea L. and Arctosaphylos uva-ursi L. has antiseptic and diuretic properties, thymol from Thymus serpyllum L., Origanum vulgare L. has antiseptic and antimicrobial properties Spirichev *et al*., 2012). Crataegus laevigata L. is a valuable source of flavonoids (hyperoside, quercetin, vitexin, acetyl-vixin, pinnatifidine), essential oils, saponins, glycosides, and tannins (Surina *et al*., 2002; Khramova & Plisov, 2012). Berries of Vaccinium uliginosum L. and Vaccínium myrtíllus L. contain flavonoids (catechin, epicatechin, myrecetin, quercetin, kaempferol) and phenolic acids with bactericidal and antioxidant properties (Molan *et al*., 2010; Velichko & Berikashvili, 2016). The data on some common medicinal plants of the Tyumen region is presented in **Table 1** (Kulataeva *et al*., 2006; Shutova, 2007).

Table 1. The composition and benefits of common medicinal plants of the Tyumen region.

Plants	Micronutrients	Benefits
Sórbus aucupária, berries	Carotene, ascorbic acid, vitamins B and P, organic acids, tannins, bitterness, flavonoids	Supply vitamins, increase perspiration, the excretion of water, and the secretion of bile
Rósa majális and *Rosa acicularis Pall*, berries	Vitamins C, B, E, K, P, carotenoids, organic acids, pectin	Supply vitamins, increase the secretion of bile, have anti-sclerotic and adaptogenic activities
Vaccinium vitis-idaea, berries	Carotenoids, vitamins C, PP, E, potassium, phosphorus, iron, manganese, zinc, copper, organic acids	Supply vitamins have antiseptic, adaptogenic, and antioxidant activities
Chamaenérion angustifolium, flowers and leaves	Vitamins C, PP, carotenoids, potassium, calcium, magnesium, phosphorus, iron, manganese, zinc, tannins	Supply vitamins have immunomodulating and antioxidant activities
Prúnus pádus, berries	Tannins, organic acids, fatty oils, flavonoids	

Having analyzed the data on the plants, we chose Vaccinium vitis-idaea L., 1753, subfamily-Vaccinioideae, family-Ericaceae. First, the plant is found throughout the Russian Federation, from its western borders to the Far East, as well as in Central America and The Middle East. In the Tyumen region, the plant occurs in coniferous, mixed, and deciduous forests, in peat bogs, and in the tundra zone. Second, Vaccinium vitis-idaea is relatively resistant to parenteral and vascular bacterioses, and viral lesions; it is an easy-to-grow, high-yield plant, which is widely used in cooking and making medicine (Vereylen, 2002). The rhizome of the evergreen plant is horizontal with upward-growing shoots of 15–20 cm. The leaves are dark green above, light green below, shiny and leathery, obovate or elliptical, with rolled edges, 0.5–3 cm long, and up to 1.5 cm wide (Burmistrov & Nikitina, 1990). Bell-shaped bisexual flowers on short pedicels are arranged in clusters of 10-20 flowers.

The fruits are shiny, spherical, multi-seeded, sweet and sour, red berries up to 8 mm in diameter, with a dried calyx at the top. The fruits ripen in August-September. The seeds are reddish-brown and slightly crescent-shaped.

All parts of the plant are unique in their chemical composition. The main bioactive compounds are phenol glycosides, condensed tannins, and hyperoside (Fedoseeva *et al*., 2009). The leaves contain gallic, ellagic, quinic, tartaric, and ursolic acids. The berries contain sugars (up to 10%), organic acids (up to 2%), including citric, malic, benzoic, oxalic, acetic, glyoxylic, pyruvic, hydroxypyruvic, α-ketoglutaric acids, and vaccinin glucoside (up to 0.1%); idaein chloride, lycopene, and zeaxanthin. The seeds contain fatty oil (up to 30%) composed of glycerides, linoleic and linolenic acids. Arbutin, a well-known phenol glycoside, is broken down into glucose, hydroquinone, and water **(Figure 1)**.

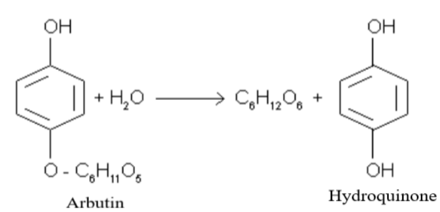

Figure 1. The fermentation.

Arbutin is known to suppress linoleic acid peroxidation and utilise free radicals in cell-free systems in vitro (Zamyatina, 2006). There is evidence that arbutin is able to protect the skin from damage induced by free radicals. It is explained by the effect of hydroquinone on certain metabolic processes, like oxygen consumption by tissues, and blood sugar levels (Lubsandorzhieva, 2008).

Experiments performed on rats have shown that arbutin and hydroquinone can influence the processes of free radical oxidation.

The pro-oxidant effect of arbutin and the antioxidant effect of hydroquinone is attributable to the accumulation of oxidant phenol glycoside derivatives, which are formed when free radicals are neutralized (Zamyatina, 2006; Lubsandorzhieva, 2008; Kovalev, 1997). Arbutin has anti-inflammatory, antimicrobial, and mild diuretic effects, without causing electrolyte imbalance. Arbutin is favorable when compared to synthetic drugs, since, like most natural compounds, it can have multiple positive effects. Hyperoside is a flavonol glycoside found in plants and characterized by diverse pharmacological activities. It is cardiotonic and sedative, and it lowers blood pressure. Hyperoside is used as a dietary supplement to prevent heart diseases and enhance cardiovascular health. Flavonoids have adaptogenic and immunomodulating properties. Organic acids are a group of compounds that are involved in energy metabolism by lowering the pH (Traubenberg *et al*., 2008). These biologically active compounds are important additives used for food preservation and enrichment.

Technologies applied for the extraction of biologically active compounds are different. The conventional methods include extraction procedures that are performed with the use of extractants, for example, ethanol and water (Belyanovskaya & Korobkov, 2005; Kulataeva *et al*., 2006; Shutova, 2007). The advantages of conventional extraction methods are easy-to-follow instructions and simple equipment. The drawbacks include lower extraction efficiency (less than 90%), longer extraction time, and high content of pectin, mucus, and proteins left. However, in the past decades, new extraction methods like ultrasound-assisted extraction (Vinatoru, 2001), supercritical fluid extraction, microwave-assisted extraction, and others have gained popularity (Chemat, 2013). It is important to use the part of the plant that contains the largest amount of biologically active substances (Grigorieva, 1996).

For many years, most biologically active compounds have been extracted from wild plants. However, a vast variety of plants remains undomesticated due to different reasons (Petrov *et al*., 2019). For this reason, there has been an increased interest in applying cellular technologies to the mass production of biologically active substances (Paseshnichenko, 2001; Nosov, 2010).

Cellular technologies enable scientists to take advantage of genetic diversity and create environmentally sustainable plants in vitro. Entire plants can be regenerated from a single cell for the design of multifunctional food ingredients as well as their further use in food enrichment, after freeze-drying or biochemical synthesis of ingredients in a biochemical reactor. Freeze drying (ice-to-vapour conversion in high vacuum) is an effective way to extend the shelf life of a product as well as to increase the content of biologically active substances by the reduction of moisture. In addition, the removal of moisture at a low sublimation temperature (20-40°C below zero) can significantly reduce the oxidation of vitamin C, flavonoids, and carotenoids, and also save approximately 95% of biologically active substances. Freeze-dried products maintain their natural color, smell, and original shape.

According to Spirichev V.B., Trikhin V.V., Pozdnyakovsky V.M., enriched vitamins demonstrate a higher bioavailability, i.e. they are absorbed and used better than those present in foods naturally. For example, the bioavailability of vitamin B6 in food of plant origin is from 5 to 75% (depending on the type of the product). Therefore, this factor must be taken into account when enriching products with functional substances (Spirichev *et al*., 2012).

Micropropagation is another effective and convenient method of plant propagation in vitro (Timofeeva, 2007; Fedorova, 2020). Cell cultures of higher plants have several advantages compared to lower plants, namely, (a) the desired amount of a product with reproducible characteristics can be obtained under aseptic conditions; (b) adverse climatic factors and seasonal conditions are evaded; (c) cropland is reduced; (d) and there are opportunities to make changes in plant tissue.

When developing methodological approaches to micropropagation, it is necessary to take into account the genotype of the original plants, aseptic conditions, nutrient media, and growth regulators, as well as physical factors of cultivation. Elinova (1995) and Serba *et al.* (2018) experimentally determined the conditions for the sterilization of raw material, the concentration, and exposure of the sterilizing agent. To remove autochthonous microbiota, the following sterilizing agents are used: Ethyl Alcohol 95% (C2H5OH), 0.08% solution of silver nitrate (AgNO3), and Tween 80 or Tween 20.

We studied and experimented with nutrient media. The experiments were designed with the most common cell culture media: Minimum Essential Medium (MEM) and Dulbecco's Modified Eagle's Medium (DMEM) which is a modification of Basal Medium Eagle (BME). MEM contains 13 amino acids, 6 water-soluble vitamins, choline, and inositol. There are also modifications of MEM with Earle's Salts or Hanks' salts as well as an alpha-modified one. DMEM contains four-fold concentrations of amino acids and vitamins along with additional supplements to enhance cell growth. To induce cell proliferation, kinetin and α-naphthylacetic acid were added to MEM, while DMEM was enriched with 6-benzylaminopurine. Enriched nutrient media actively stimulated the division of individual cells of isolated protoplasts.

Fedorova *et al*., (2020) experimented with cell suspensions that were obtained from loose, hydrated callus tissues (2-3 g per 60-100 ml of nutrient medium), treated with pectinase, and placed in a liquid nutrient medium, without calcium ions, but with 2, 4-dichlorophenoxyacetic acid. Growing conditions were maintained either in periodic or continuous modes, depending on a particular biological object. Protoplasts with reconstituted cell walls can also be used as suspension cultures. Single-cell colonies (clones) can be subcultured one or more times (if necessary) and then transferred to suspension cultures. For plant tissues, microporous polypropylene membranes are used (Tokhiriyon *et al*., 2019; Ulugboev *et al*., 2020; Chelnakova *et al*., 2021). Membranes offer a range of advantages. They are better for cell growth and removal of phenolic compounds, and it is easier to prevent cell damage. Therefore, fewer raw materials are necessary and the production costs are lower.

Conclusion

Growing Vaccinium vitis-idaea L. in vitro has enormous potential, can enable commercial production of plant-derived compounds that do not depend on weather conditions, and improve nutritional status by introducing essential macro- and micronutrients into food products.

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