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Plant Growing

Bioresources and Qualitative Indicators of Blackberry (*Rubus fruticosus*) in Georgia

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The berry crops, namely blackberries (*Rubus fruticosus*), have been found mainly in the wild habitats of Georgia since ancient times. The country has a rich ethnobotanical tradition of collecting and using their fruits. However, cultivated varieties are now preferred, as modern agricultural technologies make their cultivation and maintenance more convenient. These cultivars are highly productive and are in great demand both locally and on the world market. The creation of industrial blackberry plantations will contribute to the conservation of Georgia's bioresources, especially in the highlands, where blackberry yield and quality are high. Improving seedling production technologies, including the *in vitro* method has become essential. In turn, the qualitative indicators of raw materials obtained from plants propagated by this technology determined the direction of our research. Based on our experiments, we found that the content of biologicaly active substances is higher in mid-highland areas, and raw materials and products from plants propagated by *in vitro* technology are not inferior to those obtained from wild plants and plants propagated by conventional technology. *© 2025 Bull. Georg. Natl. Acad. Sci.*

bioproduction, ethnobotanical tradition, qualitative indicators, productivity, berry bioresources

Blackberry (*Rubus fruticosus*) has been found in Georgia mainly in the wild habitats since ancient times. The qualitative indicators and productivity of raw materials obtained from plants propagated with *in vitro* method determined the goal of our research: to enrich the gene bank with wild forms, cultivated varieties and obtained *in vitro* technology of blackberries in the different regions of Georgia under various ecosystem conditions for growing cultivated plantations (raw fruits: frozen at -45^{0}C, ripe, unripe) to differentiate the qualitative value of fruits in order to produce a different range of demanded products at the next stage, which is the basis of the viability of research results [1].

Blackberry is widespread and popular in Mediterranean countries. Our research findings were compared with the physicochemical parameters of blackberries commonly grown in this area (Morocco, Spain), which further confirm the beneficial properties of these berries and prospects of its spread [2].

To achieve the set goals, the following tasks were defined: differentiation of biomorphological, qualitative and agricultural characteristics of wild blackberry forms, cultivated varieties and plants propagated using *in vitro* technology under different ecosystem conditions; determination of biologically active compounds in fruits (ripe, unripe, raw and frozen): carbohydrates, among them pectins, organic acids, total phenols, total flavonoids, anthocyanins,vitamins [3].

Materials and Methods

The phytochemical compounds of blackberry fruit were analyzed using the HPLC-PDA-MS method, with a focus on their antioxidant, antimicrobial, anti-cold, and anticarcinogenic properties. Additionally, marketing aspects were taken into consideration [4].

Monitoring of wild blackberry forms, cultivated varieties and and in vitro-propagated plants was conducted across different ecosystems and full ripening conditions from 2019 to 2023. Approved methods, including International Crop Descriptors, environmental biological control (monitoring), and geographic information systems (GIS-ArcView), were utilized for this research.

The separation and identification of biologically active substances were carried out using HPLC-UV, RI, ultra-high-performance liquid chromatography UPLC-PDA, MS methods, including, qualitative and quantitative determination of carbohydrates (Chromatographic method). Research was conducted with a high-pressure liquid chromatograph of the company Waters HPLC system equipped with a model 525 pump by refractometric detector (2414 Refractive Index Detector), it has been used Carbohydrate column, mobile phase – 75% acetonitrile. Pectin substances were determined by spectral method using carbazole.

Total content of phenolic compounds was assessed using the Folin-Ciocalteu method. Extraction of the samples was conducted using 80% ethanol; 0.5 or 1.0 mL of the extract was transferred into 25 mL volumetric flask and 5.0 mL of distilled water was added along with 1.0 mL of Folin-Ciocalteu reagent. After 8 min at 25^0C, 10.0 mL of 7% Na_2CO_3 was added, the flask was then filled to the mark with water and was left at room temperature (18^0C) for 2 hours Absorbance was measured at 750 nm. Reagent (1 mL) was used as a control. The values were calculated using the calibration curve of gallic acid. The following equation was used for the determination of phenols, $X = (D\ K\ V\ F) \times 1000/m$, where X is phenolic content (mg/kg), D – optical density, K – coefficient, F – dilution factor, V – volume of extract in mL, m – mass of the raw material used for extraction (g). All analyses were performed in triplicates and results were expressed as mean value.

The catechin content was determined using the Swain and Hill method. 1 mL aliquot of each sample pipetted into 3 mL of 1% vanillin reagent (1 g vanillin in 70% sulfuric acid solution in distilled water). Ethanol (1 mL) was used as a control. After 15 min, the solution became red, and then after 15 min, the absorption at 750 nm was determined. All analyses were performed in triplicate and results were expressed as mean value.

Vitamin C was estimated according to ISO-6557-1984 standard for “Fruits, vegetables and derived products”. The titrimetric method was employed, which involves the extraction of vitamin C with acid and titrating it with 2,6-diclophenol indophenol until a light pink color appears. Vitamin C was also determined using the HPLC-UV method;

Qualitative and quantitative determination of organic acids was carried out using HPLC (Waters (USA; pump a model 525); detection was carried out in the ultraviolet region of the spectrum at 214 nm (Waters 2489 UV/Visible). A chromatographic column with RSpak KC811 (Shodex), where the mobile phase is 0.1% orthophosphoric acid was used. Before injection, the test sample was filtered through Waters 0.45 μm pore size membrane filter.

Results and Discussion

It should be noted that there were no climatic anomalies during the research years (2019-2023), and therefore, the ontogenesis process was carried out according to physiological phases and stages characteristic of research plant. The amount of biologically active substances was accumulated in a strictly defined quantitative and qualitative amount [5].

Wild, cultivated and *in vitro* propagated blackberry fruits were collected from the different soil and climate conditions. The technical indicators were determined by mass, volume, taste, color, longitudinal and transverse section, and their chemical composition was studied. It should be noted, that blackberry`s main products of interest to consumers are fruits in raw or frozen forms. Blackberry fruits are rich in useful substances and perishable at the same time, so it is necessary to store them in such a way as to preserve biologically active substances in them as much as possible [6].

The quality characteristics of blackberry fruit are significantly determined by qualitative and quantitative content of carbohydrates and their variation during the ripening process. In particular, they affect the formation of taste, color, smell, size, total acidity and microbiological stability, as well as participate in the metabolic processes during the fruit ripening [7,8].

Wild blackberry fruits contain total sugars up to 5.17%, and reduced – up to 4.37%, total sugars in cultivated blackberries are 4.56%, reduced sugars – up to 3.86%, total sugars in the fruits of plants propagated by *in vitro* technology are 5 .96%, and reduced sugars up to 5.25%. Fruits of plants propagated by *in vitro* technology are not inferior in terms of quality to the fruits of wild or cultivated plants. The sugar content in the mentioned fruits means that blackberries are low-calorie and dietary product. For the qualitative and quantitative determination of carbohydrates, we used the chromatographic method of the study (MSC). The research was conducted using Waters HPLC system, equipped with a model 525 pump by a refractometric detector (2414 Refractive Index Detector). Carbohydrate column was used, the mobile phase was 75% acetonitrile. Blackberry fruit juice and aqueous fruit extracts were used at all stages of maturity (Table).

Qualitative and quantitative determination of organic acids was carried out in the ultraviolet area of the spectrum – at 214 nm. In *in vitro*, cultured and wild blackberry fruits, acetic, malic, ascorbic and citric acids were identified as the main acids.

By the generalizing experimental results, it was determined that the amount of pectin substances in wild blackberries prevails. In addition, it was observed that the amount of these valuable and important substances in blackberry fruits propagated via *in vitro* technology and cultivated blackberry fruits are almost identical and quite high, which determines the commodity value of this product [9].

According to the research results, the total content of wild blackberries of anthocyanins is 53.0%, *in vitro* blackberries – 50.8% and cultivated blackberries – 57.9%. It should be noted that the maximum anthocyanin content in blackberries in the period of full ripening, corresponds to the percentage of total flavonoids [10,11].

Despite relatively high anthocyanin content in cultivated blackberries, fruits of blackberries propagated by *in vitro* technology contain a satisfactory amount of anthocyanins. Since seedlings obtained by the mentioned technology are virus-free, is a precondition for their high quality yield.

All raw blackberry samples are characterized by a high content of total phenols. The cultivated blackberry (256 m asl) contained 128.7 mg/g, while the wild blackberry at the same altitude has 104.4 mg/g, Karaka Black variety (80 m above sea level) – 101.3 mg/g, Asterina (80 m above sea level) – 100.9 mg/g, wild blackberry (740 m above sea leve) – 94.5 mg/g, wild blackberry (830 m above sea level) – 104.6 mg/g, and blackberry propagated by *in vitro* technology – 110.9 mg/g.

Table. ***In vitro*, wild and cultivated blackberry fruit carbohydrate chromatogram.**

N	*In vitro* blackberry unripe fruit	Retention time	Area	Area, %
1	Fructose	3.777	153074	40.05
2	Glucose	4.130	153926	41.67
3	Sucrose	6.578	8303	2.25
4	Maltose	7.627	33099	8.96
N	***In vitro* blackberry ripe fruit**	**Retention time**	**Area**	**Area, %**
1	Fructose	3.785	393467	43.10
2	Glucose	4.132	532410	54.19
3	Sucrose	6.507	11160	1.14
4	Maltose	7.627	45433	4.62
N	**Unripe fruit of cultivated blackberry**	**Retention time**	**Area**	**Area, %**
1	Fructose	3.778	465201	38.72
2	Glucose	4.130	651341	54.21
3	Sucrose	6.57	11160	1.14
4	Maltose	7.625	85051	7.08
N	**Ripe fruit of cultivated blackberry**	**Retention time**	**Area**	**Area, %**
1	Fructose	3.780	811773	39.66
2	Glucose	4.135	1162625	56.37
3	Sucrose	6.578	70844	3.37
4	Maltose	7.627	54374	7.59

Among the total phenolic compounds, the dominant substance is anthocyanin pigments and among anthocyanins – petunidin, delphinidin and malvinidin galactosides. The content of total phenolic compounds in the fruit depends on the stage of maturity and processing conditions. The anthocyanins content can lead to the creation of the most valuable natural food colorings, which are in high demand [12,13].

The total flavonoids content in the cultivated frozen (-45^{0}C) blackberry fruits is 87.3 mg/g, in wild blackberries – 77.6 mg/g, and in blackberries propagated via *in vitro* technology – 61.7 mg/g. Based on our experiments, it has been determined that the content and productivity of anthocyanins, total flavonoids and total phenols in the fruits of cultivated plants are higher, and the qualitative indicators and productivity of fruits of plants propagated by *in vitro* technology are not inferior to those obtained from wild and cultivated plants [14].

In the determination of vitamin C in frozen (-45^{0}C) blackberry fruits, blackberries propagated by *in vitro* technology were distinguished by their high content – 36.2%, wild blackberries by 26.0%, and cultivated blackberries by 25%.

Conclusion

Creation of industrial blackberry plantations, including production of planting material propagated by *in vitro* technology, i.e., production of planting material purified from viruses, will contribute to the preservation of Georgia`s phytogene fund, especially in mid highlands (256 m asl), where qualitative indicators and blackberry yield are high.

Based on our experiments, it has been determined that the content of biologicaly active substances is higher in mid-highlands, and raw materials and products of plants propagated by *in vitro* technology are not inferior to products obtained from wild and plants propagated by conventional technology.

მემცენარეობა

მაყვლის (*Rubus fruticosus*) ბიორესურსები და ხარისხობრივი მაჩვენებლები საქართველოში

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კენკროვანი კულტურები, მათ შორის, მაყვალი (*Rubus fruticosus*) საქართველოში უძველესი დროიდან ძირითადად ველურ ჰაბიტატებში გვხვდება, არსებობს მათი ნაყოფის შეგროვებისა და გამოყენების მდიდარი ეთნობოტანიკური ტრადიცია. თუმცა, ამჟერად, უპირატესობა ენიჭება ჯიშებს, რომელთა მოყვანა და მოვლა თანამედროვე ტექნოლოგიებით უფრო მოსახერხებელია, რადგან ისინი ხასიათდება ნედლეულის მაღალი პროდუქტიულობით და აქვს დიდი მოთხოვნა როგორც ადგილობრივ, ისე მსოფლიო ბაზარზე. მაყვლის სამრეწველო პლანტაციების შექმნა ხელს შეუწყობს ქვეყნის ბიორესურსების კონსერვაციას, განსაკუთრებით მაღალმთიანეთში, სადაც მაღალია მაყვლის მოსავლიანობა და ხარისხის მაჩვენებლები. საჭირო ხდება სანერგე მასალის წარმოების ტექნოლოგიების დახვეწა, მათ შორის, *in vitro* მეთოდით. ამ ტექნოლოგიით გამრავლებული მცენარეებიდან მიღებული ნედლეულის ხარისხობრივმა მაჩვენებლებმა, თავის მხრივ, განსაზღვრა ჩვენი კვლევის მიმართულება. ექსპერიმენტული კვლევების საფუძველზე დადგინდა, რომ ბიოლოგიურად აქტიური ნივთიერებების შემცველობა უფრო მაღალია მთის წინა ზონაში, მაგრამ არ ჩამოუვარდება *in vitro* ტექნოლოგიით გამრავლებულ მცენარეებში არსებულ შემცველობას, ხოლო, ზოგიერთ შემთხვევაში აღნიშნული ნივთიერებების შემცველობა სჭარბობს ველურ ფორმებში არსებულს.

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