

References

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VARIABILITY OF THE STRUCTURE OF THE BIOCHEMICAL COMPOSITION OF THE FRUITS OF THE Highbush BLUEBERRY AUGSTKRĪMU MELLEŅU OGU BIOĶĪMISKĀ SASTĀVA MAINĪGUMS

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Abstract

In this article the values of the coefficients of variation of 30 indicators of the biochemical composition of the fruits of the blueberry in a three-year cycle of supervision are presented. Signs

with resistance against the complex influence of meteorological factors based on the level of the variability of traits are designated. On the basis of the comparative analysis of the averaged variation coefficients calculated for a varietal row of *Vaccinium corymbosum* L. during a long-term cycle of observation it has been established that the parameters of accumulation of solids, fructose, total soluble sugars, flavonols, total bioflavonols, benzoic acid, nitrogen, potassium, calcium and magnesium in fruits can be characterized by the smallest degree of genotypic variability and, consequently, of intervarietal distinctions, whereas the content of free organic acids, anthocyanins proper and the values of a sugar-acid index in fruits are characterized by the highest degree of genotypic variability.

Kopsavilkums

Šajā rakstā atspoguļoti 30 melleņu ogu bioķīmiskā sastāva indikatoru variācijas koeficienti trīs gadu izmēģinājumu ciklā. Salīdzinošai analīzei ir aprēķināts vidējais variācijas koeficients *Vaccinium corymbosum* L. šķirņu rindai ilgtermiņā ir novērots, ka sausnes, fruktozes, kopējo šķīstošo cukuru, flavonolu, kopējo bioflavonolu, benzoskābes, slāpekļa, kālija, kalcija un magnija uzkrāšanās ogās var raksturot ar mazāku genotipisko mainību un, tātad arī mazākām atšķirībām starp šķirnēm. Turpretim brīvo organisko skābju saturu, antociānu saturu un cukuru-skābju attiecības indeksu raksturo augstāka genotipiskā mainība.

Key words: *Vaccinium corymbosum* L., cultivars, biochemical composition, fruits

Introduction

One major aspect of the introductory research examining berry plant cultivars is an integrated assessment of the biochemical structure of fruits which can prove that the latter contain a wide spectrum of wholesome substances. At the same time it is well-known that the quantity of these substances in a fruit is cultivar dependant. In recent years the collection stock of the Central Botanical Garden of Belarus' NAS has been replenished with some new taxons of *Vaccinium corymbosum* L. that have different ripening times under the weather conditions in Belarus. Among them are such early-ripening varieties as Bluetta, Northblue, Weymouth, Duke, Reka, Earliblue, Spartan, Puru, Nui; such mid-ripening varieties as Bluecrop, Northland, Patriot, Toro, Jersey and such late-ripening varieties as Elizabeth and Coville. This replenishment gives additional opportunities to expand the assortment of the varieties offered for regionalization and selection which is done by choosing the most promising ones according not only to their crop and bioproductional characteristics, but also to the nutritive and vitamin value of berries that depends on their biochemical composition.

At the same time it seems fair to suppose that there exist some distinctions among the cultivars according to the genotypic variability of the parameters that influence the accumulation of certain compounds in fruits. The distinctions can indirectly specify the degree of the genetic determinancy of the parameters and make it possible to bring out the characteristics that are more or less stable when breeding new cultivars.

Materials and methods

In 2006 – 2008 research was conducted into the biochemical composition of the above-listed cultivars of *Vaccinium corymbosum* L. examining a wide spectrum of indicators relating to different classes of active substances. Fresh averaged samples of vegetable material were taken to determine the content of: solid matter – in accordance with GOST (State Standard) 8756.2 – 82 (1982); ascorbic acid (vitamin C) – using the standard indophenol method (Ermakov, etc., 1987); titratable acids (total acidity) – using the volumetric method (Ermakov, etc., 1987). Dried at 65 degrees Celsius averaged samples of fruits were used to determine the content of such chemical elements as nitrogen, phosphorus, potassium using the method of K.P. Fomenko and N.N. Nesterov (1971); calcium and magnesium – using the complexometric method (Ermakov, etc., 1987); glucose, fructose, sucrose – using the resorcinol and aniline-phthalate methods of paper chromatography of I.G. Zavadsky and others (1962); pectins (water-soluble pectin and protopectin) – using the carbazolic method (Ermakov, etc., 1987); the sums of anthocyanic pigments – using the method of T. Swain, W. E. Hillis (1952) constructing a calibration curve on cyaniding crystal

obtained from fruits of black chokeberry and cleared using the technique of J.G. Skorikova and E.A. Shaftan (1968) and employing S.S. Tanchev's formula evaluation (1980); anthocyanins – using the method of L.O. Shnaidman and V.S. Afanasyeva (1965); the flavonol sums – according to the method of L.Sarapuu and H.Miydla (1971); the catechine sums – according to the photometric method with the use of a vanillin reagent (Zaprometov, 1964); phenol-carboxylic acids (in terms of chlorogenic acid) – according to the method of descending chromatography on paper (Mzhavanadze, etc., 1971); tannins – according to the titrimetric method of Levental (State Pharmacopoeia of the USSR, 1987); lignins – using the modified method of Klason (Ermakov, etc., 1987); benzoic acid – using the method of M.I. Kalebin and A.A. Kolesnik (1949); fatty oils – using the method of V.A. Sapunov and I.I. Fedunyak (1958); triterpenic acids (in terms of ursolic acid) – using the method of A.V. Simonyan and others (1972). All analytic determinations have been carried out in triplicate. The data has been statistically processed using the computer program Excel.

When estimating the genotypic variability of the parameters that influence the accumulation of the above-mentioned compounds we were guided by the coefficients of variation (V) of the characteristics under study within the range of *Vaccinium corymbosum* L. taxons that were examined during a long-term cycle of observation. The values of variation coefficients indicate that they depend on a cultivar, i.e. the higher the variation coefficient is, the stronger this dependence is and, therefore, the level of genetic determinacy of the characteristic goes down, and vice versa.

According to S.N. Sennov and V.F. Kovyazina (1990), series variability for biological objects is regarded low if it is equal to 11-30 % and is considered high if it exceeds 31 %. When examining the data presented in this paper, we should take into account the active reaction of an alien crop to the breeding process. This reaction allows the crop to resist the breeding process to a certain extent as well as to regulate the biochemical composition of generative organs within genetically determined variation ranges of each characteristic. It allows one to narrow down the above mentioned limits of small series variability for the indicators under study to 15 %. Accordingly, its average range will be characterized by the level of variability within 16 – 30 %, while the maximum one is over 31 %.

Results and Discussion

The biochemical screening of the three groups of *Vaccinium corymbosum* L. cultivars characterized by different terms of ripening made it possible to identify the taxons with the highest and the lowest content of wholesome substances in fruits. These substances refer to different classes of chemical compounds. At the same time, there have been discovered some essential intervarietal distinctions concerning the accumulation of certain compounds in the dry weight of fruits, which is confirmed by the wide range of changes of their quantitative characteristics that during a three-year cycle of observations made up (Table 1): for titratable acids – 1.5 – 11.1 %; for ascorbic acid – 296-941.0 mg% (mg of ascorbic acid in 100 g of dry weight of sample); phenol-carboxylic acids – 543-1189 mg%; benzoic acid – 0.82 – 1.59 %; soluble sugars – 10.9 – 29.2 %, including glucose – 3.2 – 7.1 %, fructose – 5.2 – 19.3 %; sucrose – 0.4 – 4.5 %; pectins – 3.6 – 7.9 %, including hydropectin – 1.3 – 3.2 %, protopectin – 1.8 – 5.2 %; for the total content of bioflavonols – 1935 – 3110 mg %, including anthocyanic pigments – 9 – 58 mg %, catechines – 383 – 1404 mg %, flavonols – 1471 – 2251 mg %; tannins – 0.9 – 2.5 %; fat oils – 1.6 – 5.5 %; major mineral elements: N – 0.57 – 1.34 %, P – 0.10 – 0.24 %, K – 0.44 – 0.99 %, Ca – 0.26 – 0.45 %, Mg – 0.07 – 0.12 %. The content of solid in the fresh weight of fruits changed from 10.9 % to 17.6 %.

The analysis of the information presented in the table has revealed among the given taxons of *Vaccinium corymbosum* L quite a wide range of coefficients showing variation in the quantitative indicators of the biochemical composition of fruits both during individual years and during the whole period of observation. That indicated different levels of their dependence on the genotype of a plant. This fact enabled us to identify the characteristics possessing the highest and the lowest degree of varietal distinctions and to order them according to the decrease in genetic determinacy. The majority of the parameters showing accumulation of reactants in blueberries within the period of observations were characterized mainly by an average variability (V = 15 – 30 %). A considerable part of the indicators were notable for a low (V <15 %) level of variability in a varietal series, testifying to their weak dependence on genotype, and only some of the

characteristics under study have shown a high level ($V > 30\%$) of variability indicating the highest degree of varietal distinctions.

Table 1. Averaged quantity indicators of the biochemical structure of fruits (dry weight) and variation coefficients in a long-term cycle of supervision for a varietal row of *Vaccinium corymbosum* L.

Index	2006		2007		2008		Period average V, %
	mean	V, %	mean	V, %	mean	V, %	
Soluble solids, % of fresh fruit	13.9	12.8	13.9	10.1	14.1	12.1	11.7
Free organic acids, %	5.7	36.4	3.8	45.7	6.7	39.1	40.4
Ascorbic acid, mg %	601.2	20.3	426.6	26.3	604.8	30.3	25.6
Glucose, %	5.34	16.4	4.49	15.8	4.96	27.5	19.9
Fructose, %	18.74	2.9	14.22	3.9	7.26	22.3	9.7
Sucrose, %	3.19	19.8	2.08	20.2	0.56	22.1	20.7
Sum of soluble sugars, %	27.25	4.1	20.79	5.1	12.79	6.8	5.3
Fructose/ Glucose	3.6	19.2	3.2	16.5	1.7	46.0	27.2
Monose/Disaccharide	7.9	19.4	9.4	19.8	22.7	21.5	20.2
Sugar-acid index	5.4	31.2	6.5	39.5	2.5	75.9	48.9
Hydropectin, %	1.98	24.3	2.18	23.8	2.37	18.0	22.0
Protopectin, %	3.45	22.4	2.60	19.8	3.35	21.0	21.1
Sum of pectins, %	5.43	16.5	4.77	19.2	5.71	17.8	17.8
Protopectin/Hydropectin	1.8	33.8	1.2	22.9	1.4	17.1	24.6
Anthocyanins, mg % *	7.6	45.1	2.0	91.5	17.1	35.5	57.4
Leucoanthocyanins, mg%	17.4	23.2	12.1	20.6	24.1	29.1	24.3
Sum of anthocyanic pigments, mg %	25.0	19.7	14.1	21.5	41.2	27.1	22.8
Catechines, mg %	984.3	20.2	923.4	27.6	570.1	15.4	21.1
Flavonols, mg %	1766.6	5.5	1626.0	11.0	1890.6	12.2	9.6
Flavonols/Catechines	1.9	27.1	1.9	36.3	3.4	20.9	28.1
Sum of bioflavonols, mg %	2776.0	6.9	2563.5	11.7	2501.8	9.2	9.3
Phenol-carboxylic acids, mg %	781.4	18.0	800.3	24.4	787.3	24.3	22.2
Benzoic acid, %	1.15	6.1	1.11	18.2	1.18	14.3	12.9
Tannins, %	1.21	19.5	1.72	23.2	1.83	16.3	19.7
Fat oils, %	3.61	29.9	3.17	13.0	3.25	21.9	21.6
Nitrogen, %	0.76	15.1	0.91	16.2	1.10	10.3	13.9
Phosphorus, %	0.17	24.0	0.14	11.5	0.14	13.0	16.2
Potassium, %	0.53	10.0	0.57	8.7	0.76	11.3	10.0
Calcium, %	0.31	10.0	0.42	4.9	0.40	4.9	6.6
Magnesium, %	0.09	11.9	0.08	8.8	0.11	6.8	9.2

* mg% - mg of substance in 100 g of dry weight of sample.

The variability level of a particular characteristic only in some cases remained stable throughout the whole 3-year period of observation. For example, such stability of a low variability level was typical for the parameters of the accumulation of solid, flavonols, potassium, calcium, magnesium, the total amount of sugars and the sum of bioflavonols in fruits whereas the parameters of the accumulation of fructose, benzoic acid, fat oils, nitrogen and phosphorus in fruits were notable for low variability only during one or two seasons. Similarly, the high level of genotypic variability during the whole period of observation was typical for the parameters of the accumulation of free organic acids, anthocyanins and the values of a sugar-acid index whereas for the ascorbic acid content it has been notable only in 2008. Moreover, even within a particular area of variability of the characteristics analyzed there have been identified some essential interseasonal distinctions. This fact unequivocally indicates a dependence of the genotypic variability of quantity indicators of biochemical composition of *Vaccinium corymbosum* L. fruits on the hydrothermal mode of its formation period.

In our opinion, the best estimate about the degree of variability of the indicators in the varietal row can be given by the averaged values of variation coefficients calculated within a 3-year period of observation. In this case the characteristics under study can be provisionally divided into 3 groups in an increasing order of genotypic variability, and, hence, intensification of varietal distinctions:

1 – with small variability ($V = 5.3 - 13.9 \%$) – the content of solid, fructose, total of soluble sugars, flavonols, total bioflavonols, benzoic acid, nitrogen, potassium, calcium and magnesium in fruits;

2 – with average variability ($V = 16.2 - 25.6 \%$) – the content of phosphorus, ascorbic acid, glucose, sucrose, hydropectin, protopectin, leucoanthocyanins, catechines, phenol-carboxylic acids, tannins and fat oils in fruits;

3 – with high variability ($V = 40.4 - 57.4 \%$) – the content of free organic acids, anthocyanins proper and values of a sugar-acid index in fruits.

Conclusion

The biochemical screening of the fruits of 16 representatives within three groups of introduced blueberry cultivars with different terms of ripening (early-ripening, mid-ripening and late-ripening) according to 30 indicators specifying the content of some organic acids, carbohydrates, bioflavonols, terpenoids and chemical elements under vegetative seasons of 2006 – 2008 made it possible to reveal the essential intervarietal distinctions in the accumulation of certain compounds in the dry weight of fruits, confirmed by a wide range of changes of their quantitative characteristics. On the basis of the comparative analysis of the averaged variation coefficients calculated for a varietal row of *Vaccinium corymbosum* L. during a long-term cycle of observation it has been established that the parameters of accumulation of solids, fructose, total soluble sugars, flavonols, total bioflavonols, benzoic acid, nitrogen, potassium, calcium and magnesium in fruits can be characterized by the smallest degree of genotypic variability and, consequently, of intervarietal distinctions, whereas the content of free organic acids, anthocyanins proper and the values of a sugar-acid index in fruits are characterized by the highest degree of genotypic variability.

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MICROPROPAGATION OF HIGHBUSH BLUEBERRY CULTIVARS AUGSTKRŪMU MELLEŅU ŠĶIRŅU MIKROPAVAIROŠANA

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Abstract

The aim of this study was to determine an efficient micropropagation system for the highbush blueberry cultivars ‘Spartan’, ‘Bluecrop’ and ‘Berkeley’. The shoot tips of the selected three genotypes were successfully established *in vitro* using mercuric chloride in a concentration of 0.15 % as a sterilization solution. Anderson’s rhododendron medium (AN), half-strength Murashige and Skoog medium (half-MS) and McCown woody plant medium (WPM) containing cytokinin zeatin in concentrations 0.5, 1 or 2 mg l⁻¹ were tested. Multiplication rates varied depending on the cultivar, medium and concentration of the zeatin. The highest multiplication 4.8 ± 0.2 was noted for ‘Berkeley’ on WPM medium with zeatin (2 mg l⁻¹). Out of three media tested, the WPM medium was found to be more effective than the AN medium and half-MS medium for shoot multiplication. The *in vitro* rooting on the WPM medium is also reported.

Kopsavilkums

Pētījuma mērķis bija noteikt augstkrūmu melleņu šķirņu ‘Spartan’, ‘Bluecrop’ un ‘Berkeley’ efektīvāko mikropavairošanas sistēmu. Izvēlēto trīs genotipu dzinumu gali tika veiksmīgi ievietoti *in vitro* izmantojot 0.15% koncentrācijas dzīvsudraba hlorīdu kā sterilizācijas šķīdumu. Tika pārbaudīta Andersona rododendra barotne (AN), pus-stiprā Murashige un Skoog barotne (pus-MS) un McCown koksnaino augu barotne (WPM), kas satur citokinīna zeatīnu 0.5, 1 vai 2 mg l⁻¹ koncentrācijā. Pavairošanas ātrums bija atkarīgs no šķirnes, barotnes un zeatīna koncentrācijas. Augstākais pavairošanas koeficients 4.8 ± 0.2 bija vērojams šķirnei ‘Berkeley’ WPM barotnē ar zeatīnu (2 mg l⁻¹). No trim pārbaudītājām barotnēm dzinumu pavairošanai WPM barotne bija efektīvāka par AN barotni un pus-MS barotni. WPM barotnē tika novērota arī dzinumu *in vitro* apsākšanās.

Key words: explant, *in vitro*, zeatin, multiplication, rooting

Introduction

The highbush blueberry (*Vaccinium corymbosum* L.), native to North America, is a commercially important fruit crop (Zmarlicki, 2006). Although plants of the *Vaccinium* genus have not been cultivated on a large scale in the Czech Republic, there is potential for commercial highbush blueberry production in some mountain regions. A number of research programs continue to find suitable high yielding highbush blueberry cultivars with superior berry qualities for commercial growing in the rural countryside of the Czech Republic (Paprstein *et al.*, 2006; Paprstein and Ludvikova, 2006). In recent years, the blueberry has received considerable attention for its nutritional quality and health benefits (Howell, 2009).

If suitable cultivars are to receive wide distribution rapid propagation techniques will be essential. Highbush blueberry can be propagated vegetatively by multiple-node softwood or hardwood cuttings. This method, although generally successful, is slow and labor intensive. Success with