

**VARIABILITY OF THE STRUCTURE OF THE BIOCHEMICAL COMPOSITION OF
THE FRUITS OF THE HIGHBUSH BLUEBERRY
AUGSTKRŪMU MELLEŅU OGU BIOĶĪMISKĀ SASTĀVA MAINĪGUMS**

**Rupasova Zh.¹, Pavlovskij N.¹, Kurlovich T.¹, Pyatnitsa F.¹, Yakovlev A.¹, Volotovich A.²,
Pinchukova Yu.³**

¹Central Botanical Garden of the NAS of Belarus,
220012 Minsk str. Surganova, 2v, Belarus, e-mail: rupasova@basnet.by

²Polesky State University, e-mail: volant777@tut.by

³Mogilev State University of food technologies, e-mail: mti@mogilev.by

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.

References

1. GOST 8756.2-82, (1982) Methods of definition of solids. M: Publishing house of standards, 5 p. (In Russian).
2. The State pharmacopoeia of the USSR (1987) *Vol.1. The general methods of the analysis*, M: Medicine, pp.286-287. (In Russian).
3. Ermakov A.I., et al. (1987) *Methods of biochemical research of plants*. M, VO Agropromizdat., 430 p. (In Russian).
4. Zavadsckaja I.G., Gorbacheva G. I and Mamushina N.S. (1962) Quantitative definition of carbohydrates by resorcinol and aniline-phthalate methods by means of a paper chromatography. In: Kretovich V.L. (eds) *The Technique of a quantitative paper chromatography of sugars, organic acids and amino acids at plants*, Moscow-Leningrad, Publishing house of AS of the USSR., pp. 17-26. (In Russian).
5. Zaprometov M. N. (1964) *Biochemistry of catechines*, Moscow, Science, 325 p. (In Russian).
6. Kalebin M. I, and Kolesnik A.A. (1949) Research of fresh fruits, vegetables and products of their processing. In: Tserevitinov F.V. (etc) *Research of foodstuff*, Moscow, Gostorgizdat, pp. 218-245. (In Russian).
7. Mzhavanadze V.V., Targamadze I.L., and Dranik L.I. (1971) Quantitative definition of chlorogenic acid in leaves of a bilberry Caucasian *V. arctostaphylos* L.) *Proc. of AS of GSSR*, Vol. 63, N 1, pp. 205-210. (In Russian).
8. Sapunov V.A., and Fedunjak I.I. (1958) *Methods of an estimation of forages and the zootechnical analysis*. Minsk, 190 p. (In Russian).
9. Sarapuu L., and Mijdla H. (1971) The phenolic constituents of an apple-tree. *Uch. Vest Tart. SU*, N 256, pp. 111-113.
10. Sennov S.N., and Kovjazin V.F. (1990) *Forestry: the Manual*, Leningrad, LTA, 91 p. (In Russian).
11. Simonjan A.V., Shinkarenko A.L., and Oganesyanyan E.T. (1972) Quantitative definition of triterpenes in plants of Thymus sort. *Chemistry of natural compounds*, N 3, pp.293-295. (In Russian).
12. Skorikova J. G, and Shaftan E.A. (1968) Technique of anthocyanins definition in fruits and berries. In: Kefeli V.I. (etc) *Proc. 3 Un. Seminar on biologically active (medical) substances of fruits and berries*. Sverdlovsk, pp. 451-461. (In Russian).

13. Tanchev S.S. (1980) *Anthocyanins in fruits and vegetables*. Moscow, Food ind., 304 p. (In Russian).
14. Fomenko K.P., and Nesterov N.N. (1971) Technique of definition of nitrogen, phosphorus and potassium in plants from one assay. *Chemistry in agriculture*, N 10, pp. 72-74. (In Russian).
15. Shnajdman L.O., and Afanasjeva V.S. (1965) A definition technique of anthocyanic substances. In: Kretovich V.L. (eds) *Proc. of 9th Mendeleevsky Congress on Gen. and Applied Chemistry*, Moscow, pp. 79-80. (In Russian).
16. Swain T., and Hillis W., (1959) The phenolic constituents of *Prunus Domenstica*. 1. The quantitative analysis of phenolic constituents. *J.Sci. Food Agric.* Vol. 10, N 1, pp. 63-68.