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ASSESSMENT OF FRUIT QUALITY AND BIOCHEMICAL COMPOUNDS OF SOME BLUEBERRY HYBRIDS

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Abstract

Vaccinium corymbosum fruits are used as raw material for the food industry. It is known that the highbush berries, compared to lowbush blueberries, are richer in dry matter and total sugar content. During 2021-2022, at the Researsch Institute for Fruit Growing Pitești-Mărăcineni, Romania was organized a field trial with two genotypes and their progenies. Data were collected for: average fruit weight,size index, firmness, ph,total soluble solid, total sugar content, vitamin C, total polyphenol, flavonoid, antocyanin, lycopene and β-carotene content. The aim of this study was to compare the analysis of the fruit quality in hybrids of 'Simultan' and 'Northblue' cutivars. The results showed significant differences between progenies. The fruit weight varied between 0.82 and 2.05 g, the firmness oscillated between 8.13 and 28.27 N, total soluble solids reached a maximum of 17.43° BRIX, size index varied between 8.13 to 28.27, vitamin C content varied from 3.48 to 13,49 mg/100 g. Total polyphenol, lycopene and β-carotene content averaged 1296.66 mg GAE/100 G, 0.10 mg and 0.42 mg. For the blueberry breeding programme, biochemical evaluation of hybrids characteristics is an important objective.

Key words: *hybrids, biochemical compounds, highbush blueberry.*

INTRODUCTION

In the last years, the recent global interest in the consumption of berries with high levels of functional properties and nutraceuticals compounds is gaining momentum (Zimmer et al., 2014). The highbush blueberry (*Vaccinium corymbosum)* is original to North America and their content in nutrients exceeds that of lowbush blueberry or rabbiteye blueberry. (Korkak, 1988; Finn et al., 1993).

The blueberries are one of the most important functional and nutraceutical foods in our diets (Kahkonen et al., 2001) and it is were consumed worldwide. It is known that blueberries (*Vaccinium corymbosum)* are an execellent source of bioactive compounds, such as polyphenols, flavonoids, tannins, anthocyanins

In Romania, highbush blueberry was introduced for the first time in 1968 at the Research Institute for Fruit Growing Pitesti-Maracineni (Botez et al., 1984). For optimal growth and productivity high bush blueberry needs well drained acid soils (pH $_{H2O}$ 4.8-5.5) with the pH adjustment and additional organic matter before planting is necessary .The blueberry breeding program started in 1983 and were obtained varieties for adaptation in different ecological condition, prolonged shelf-life of blueberries, quantified by the berries firmness, technological flow and the content of bioactive compounds.

The highbush breeding activity at the Research Institute fot Fruit Growing Pitesti-Maracineni, achieved 9 cultivars: 'Azur', 'Safir', 'Augusta', 'Delicia', 'Simultan', 'Lax', 'Pastel', 'Prod' and 'Vital'.

'Simultan' and 'Northblue' cv. were selected for breeding program due to earliness and the special qualities of the fruits.

In the light of this, the purpose of our study was the analysis of the fruit quality (berry weight, firmness, size index, total soluble solids) and biochemical characteristics (total sugar contents, vitamin C, total polyphenol, flavonoid, antocyanin, lycopene and β-carotene content) of blueberry progenies obtained from ‘Simultan’ × ‘Northblue’ cv. for new cultivars with more aroma, size and taste.

MATERIALS AND METHODS

Chemicals and Reagents

Folin-Ciocalteu’s phenol reagent, 2,4,6-Tripyridyl-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium hydroxide, sodium carbonate, sodium bicarbonate, sodium nitrite, aluminium chloride, methanol, acetone, n-hexane, ethanol, citrate/acetate buffer, glucose, galli acid, catechin, vitamin C, metaphosphoric acid, acetic acid, hydrochloric acid, concentrated sulphuric acid were purchased the reagents used in the study were obtained from Merck, Darmstadt, Germany

Plant material

The biological material was represented by 20 promising blueberry progenies, tested in an experimental seedling plot with their parental forms as control. A number of 22 genotypes were studied (2 cultivars and 20 progenies).

The fruits of two cultivars of highbush, namely ‘Simultan’ obtained from open pollination of ’Spartan’ cv. and ‘Northblue’ cv. was selected in 1973 from a cross of G × Ashworth (*Vaccinium corymbosum)* × US3 - paternal selection, (*Vaccinium angustifolium).*

The experiment was set up at the Research Institute Growing Pitesti, within the Genetic and Breeding Department in 2019. The genotypes were planted at a distance of 3 m × 1 m on a mixture of soil and peat. The samples were harvested and immediately analyzed.

Determination of average weight and chemical analysis

Following quality parameters and biochemical analyzes were determined: berry weight, size index, firmness, pH, total soluble solid,total polyphenols, total flavonoids, antocyanin, vitamin C content, total sugar content and lycopene and β-carotene levels.

The average fruit weight was determined by weighing of 50 fruits for each variety using HL-400 digital. The index size of the fruit was calculated by formula: (height + large diameter + small diameter)/3, (Botu et al., 1997). The fruit firmness was determined for each sample with a penetrometer Bareiss HPE II Fff nondestructive test, with a measuring surface of 0.25 cm². pH were determined in fruit flesh juice using a digital pH-metter.

The soluble solids (TSS) content was determined as °Brix (°Bx) with digital refractometer (Kern, ORF 45BM, Germany).

Determination of total polyphenols content (TPC) was determined according to the methodology suggested by Matić et al., 2017.

The results were expressed as mg gallic acid equivalent (GAE)/100 g dry weight of vegetal material.

The total flavonoids content (TFC) was determined according to the methodology suggested by Tudor-Radu et al., 2016. The results were expressed as mg catechin equivalent (CE)/100 g dry weight of vegetal material.

The level of total anthocyanins pigments (TAC) was determined according to the methodology suggested by Di Stefano and Cravero, 1989. The results were expressed as cyaniding-3glucoside mg/100 g dry weight of vegetal material (C 3 - G mg 100 g).

The total sugar content was determined using the colorimetric method and the methodology suggested by Dubois et al., 1956.

The results were expressed as g glucose/100 g dry weight of vegetal material.

Vitamin C content was estimated according to the colorimetric method and the methodology suggested by Omaye et al., the vitamin C content was determined by using 2,6-dichloroindophenol (DCPIP), at pH 3-4.5.The results were expressed in mg vitamin C/100 g dry weight of vegetal material.

The lycopene and β-carotene content were determined according to the methodology proposed by Tudor-Radu et al., by the carotenoids extraction in a mixture of hexane: ethanol: acetone. The results were expressed in mg lycopene/100 g dry weight of vegetal material and mg β-carotene/100 g dry weight of vegetal material.

Statistical analysis was performed using IBM SPSS 14 program (SPSS Inc., Chicago, IL, USA). All results were statistically evaluated by analysis of variance (ANOVA). Differences between cultivars were highlighted through Duncan’s multiple test range ($p< 0.005$). Graphical representations were performed with Microsoft Office Word 2007.

RESULTS AND DISCUSSIONS

On the ensemble of the 22 genotypes from the experiment, tables 1-3 reflect close biochemical traits of the berries

As presented in Table 1, the average berry weight oscillated between 0.82 g for 16-2-1 hybrid and 2.05 g for 16-2-26 hybrid. The control ranged between 1.93 g 'Simultan' cv. and 2.04 g 'Northblue' cv.

In the case of the size index, the hybrid 16-2-1 had the highest average compared to the other genotypes studied (Table 1).

Analyzing the average values of the all three years of the study, in the case of firmness, the the highest value was recorded by 'Simultan' cv. 30.22 N, followed by the hybrid 16-2-13 with the value 28.27 N.

The pH (table 1) recorded normal values for the blueberry berries, oscillated between 3.08 for the hybrid 16-2-6 and 3.99 for the hybrid 16-2-17.

Total soluble solids (TSS) content reached a maximum of 17.63°Bx for the control 'Simultan' cv., and the hybrid 16-2-1 reached of 17.63°Bx. Similar data of the soluble solids content of the harvested fruits was in Guasca, Colombia was in a range of 12.4 to 14.5 °Brix (Cortes et al., 2016).

The results reported that the total phenolic content of genitors varied from 505.66 mg GAE/100 g for 'Northblue' cv. to 668.44 mg GAE/100 g 'Simultan' cv., while their progenies ranged from 225 to 1,296.67 mg GAE/100 g. Similar results, 455.01 mg GAE/100 g for 'Duke' cv. and 474.184 mg GAE/100 g for 'Hannah's choice'cv. reported by Ciucu et al., 2021.

Total flavonoid content in blueberry fruits of 'Simultan' and 'Northblue'cultivars and their progenies hybrids varied between 87.00 and 221.00 mg/100 g, having a mean value of 188.87 mg/100 g. It could be observed that most of the 'Simultan x Northblue' hybrids had flavonoid contents higher than their genitors (195.04 mg/100 g, for 'Simultan' and 211.39 mg CE/100 g for 'Northblue') and 16-2-17 hybrid was remarked (221.00 mg CE/100 g), followed by 16-2-24 (203.67 mg CE/100 g).

The total sugar content is an important biochemical marker used to determine the quality of sweetness berries (Okan et al., 2019) and sugars determine the organoleptic traits (Li et al., 2020). TSC (Table. 2) of the fruits varies between 4.76 g glucose/100 g for 16-2-3 hybrid and 12.67 g glucose/100 g for 16-2-14 hybrid. While the genitors registered the values 7.40 for 'Simultan' cv. and 8.09 for 'Northblue' cv.

Table 1. The average values of berry weight, size index, firmness, °Brix and pH at 22 blueberry genotypes

Genotypes	Average berry weight (g)	Size index	Firmness (N)	TSS (°Brix)	pH
16-2-1	0.82±0.24[g*]	0.85±0.12[abc]	21.73±4.02[abcd]	17.43±0.68[a]	3.58±0.07[abcd]
16-2-2	1.38±0.35[de]	0.75±0.02[bcd]	17.3±1.31[bcd]	14.2±0.26[cde]	3.67±0.1[abcd]
16-2-3	1.65±0.13[bc]	0.73±0.07[d]	27.5±8.36[a]	14.2±2.15[cde]	3.83±0.41[ab]
16-2-4	1.50±0.1[cde]	0.85±0.12[ab]	24±5.63[abc]	16.57±2.14[ab]	3.24±0.09[de]
16-2-6	1.05±0.13[fg]	0.73±0.04[cd]	20.73±915[abcd]	14.1±1.35[cde]	3.08±0.08[e]
16-2-8	1.49±0.1[cde]	0.71±0.03[d]	26.35±3.2[ab]	13.42±0.28[e]	3.25±0.07[cde]
16-2-9	1.02±0.03[fg]	0.77±0.08[abcd]	15.9±0.56[cde]	17.37±0.67[a]	3.30±0.08[cde]
16-2-10	1.80±0.12[ab]	0.88±0.07[a]	19.23±3.09[abcd]	14.8±1.35[bcde]	3.37±0.22[cde]
16-2-11	1.53±0.35[cd]	0.71±0.03[d]	24.67±5.59[abc]	14.44±0.86[bcde]	3.09±0.04[e]
16-2-12	1.25±0.02[ef]	0.73±0.04[cd]	24.07±2.71[abc]	14.1±0.30[cde]	3.27±0.09[cde]
16-2-13	1.44±0.03[cde]	0.73±0.09[d]	28.27±1.12[a]	14.67±0.45[bcde]	3.26±0.13[cde]
16-2-14	1.82±0.13[ab]	0.73±0.07[d]	16.23±1.72[cde]	16.1±2.11[abcd]	3.25±0.32[cde]
16-2-15	1.88±0.06[ab]	0.71±0.03[d]	16.9±3.42[cde]	14.38±1.53[bcde]	3.39±0.33[cde]
16-2-16	1.66±0.06[bc]	0.73±0.05[cd]	21.19±5.73[abcd]	14.39±0.93[bcde]	3.57±0.21[bcd]
16-2-17	1.37±0.03[de]	0.78±0.04[abcd]	17.27±4.13[bcd]	17.13±1.03[a]	3.99±0.08[a]
16-2-22	1.38±0.02[de]	0.77±0.07[bcd]	24.4±8.61[abc]	16.2±1.04[abc]	3.82±0.37[ab]
16-2-24	1.68±0.02[bc]	0.75±0.02[bcd]	18.23±6.16	14.43±0.9[bcde]	3.63±0.19[abcd]
16-2-25	1.81±0.02[ab]	0.76±0.03[bcd]	8.13±1.51[e]	12.87±1.69[e]	3.59±0.53[abcd]
16-2-26	2.05±0.05[a]	0.77±0.02[abcd]	16.7±0.62	13.8±0.69[de]	3.68±0.07[abc]
16-2-31	1.33±0.1[de]	0.76±0.04[bcd]	13.17±1.5	14.4±0.46[bcde]	3.40±0.11[bcde]
Simultan	1.93±0.17[de]	0.83±0.06[abc]	30.22±7.58[a]	17.63±6.56[a]	3.47±0.43[bcde]
Northblue	2.04±0.29[a]	0.78±0.04[abcd]	15.33±5.38[cde]	13.14±2.76[e]	3.23±0.32[de]

[*]Means with the same letter are not significantly different at 5% level

Table 2. The average values of total content of polyphenols, flavonoids, antocyannins and total sugar content at 22 blueberry genotypes

Genotypes	TPC (mg GAE/ 100 g)	TFC (mg CE/100 g)	TAC (C 3 - G mg/100 g)	TSC (%)
16-2-1	581.33±3.06^{cdef*}	182.67±0.58^{bc}	316.15±89.67^{fgh}	11.56±0.01^{c}
16-2-2	399.67±16.07^{f}	124.33±4.51^{defgh}	222.07±15.84^{gh}	6.38±0.02^{gh}
16-2-3	549.67±15.5^{cdef}	88.67±15.04^{hi}	368.08±13.79^{efg}	4.76±0.05^{j}
16-2-4	615.33±22.37^{cdef}	196.33±0.58^{ab}	274.33±6.66^{gh}	8.61±0.01^{e}
16-2-6	414.33±0.58^{ef}	134±0.41^{defg}	575±10^{bc}	6.49±0.01^{g}
16-2-8	582.33±74.9^{cdef}	139.00±3.21^{de}	280.67±7.09^{fgh}	6.42±0.01^{gh}
16-2-9	389±13.45^{f}	138.00±0.2^{def}	834.00±89.71^{a}	5.59±0.3^{hi}
16-2-10	401±27.4^{f}	108.67±2.52^{efghi}	263.82±10.42^{gh}	5.01±0.01^{j}
16-2-11	464.67±59.08^{def}	195.67±5.69^{ab}	529.02±22.06^{cd}	4.86±0.02^{j}
16-2-12	954.33±103.85^{b}	101.67±3.06^{fghi}	472.97±42.03^{cde}	6.84±0.03^{g}
16-2-13	145.67±4.51^{g}	113.67±3.06^{efghi}	163.83±6.5^{h}	6.77±0.02
16-2-14	657±12,12^{bcd}	87.00±0.21^{i}	348±140.58^{efg}	12.67±0.58^{b}
16-2-15	709.33±22.37^{cd}	136.33±7.57^{def}	474.92±32.09^{cde}	5.9±0.03^{hi}
16-2-16	969±296.08^{b}	151.83±51.05^{cd}	427.56±199.97^{def}	5.4±0.62^{ij}
16-2-17	651±0.1^{bcd}	221.00±0.32^{a}	686.69±0.09^{b}	8.04±0.01^{f}
16-2-22	1296.67±0.58^{a}	118.67±0.58^{defghi}	269.28±0.07^{gh}	7.73±0.01
16-2-24	639±0.1^{bcdef}	203.67±0.58^{ab}	162.96±0.05^{h}	13.28±0.01^{a}
16-2-25	1202.33±0.58^{a}	129±0.9^{defg}	177.22±0.12^{h}	10.05±0.01^{d}
16-2-26	610±0.1^{cdef}	118.00±29.44^{defghi}	355.55±0.05^{efg}	10.29±0.03^{d}
16-2-31	793.67±354.49^{bc}	97.47±15.61^{ghi}	297.94±0.10^{fgh}	10.40±1.03^{d}
Simultan	668.44±109.43^{bcd}	195.04±57.15^{ab}	514.41±236.47^{cd}	7.40±3.04^{f}
Northblue	505.66±181.66^{def}	211.39±79.73^{a}	389.77±222.06^{efg}	8.09±2.96^{f}

[*]Means with the same letter are not significantly different at 5% leve.

The biggest level of total anthocyanins pigments (TAC) 834.00 C 3 - G mg 100 g recorded by the hybrid 16-2-9, followed by 16-2-17 (686.69 C 3 - G mg 100 g). The genitors had the lower level for 'Northblue' cv. (389.77 C 3 - G mg 100 g). Regarding the total vitamin C content (Table 3) in blueberry fruits of 'Simultan' and 'Northblue' cultivars and their progenies hybrids, oscillated between 4.76 mg/100 g (16-2-31 hybrid), and 12.67 mg/100 g (16-2-1 hybrid). In this case, genitors contained lower vitamin C compared to most of their hybrids. Carotenoids (Car) are bioactive substances in foods with powerful antioxidant bioactivity. There are abundant data showing theirs preventive effects in humans for a number of diseases (Stahl & Sies, 2003; Lila, 2004; Schmidt, 2004). Carotenoids include diverse compounds such as lycopene, α- and β-carotene. The lycopene level oscillated between 0.01 mg/100 g (16-2-3 hybrid) and 0.37 mg/100 g (16-2-1 hybrid). Also, the β-carotene level varied from 0.02 mg/100 g (16-2-3 hybrid) to 0.42 mg/100 g (16-2-1 hybrid).

Table 3. The average values of total content of Vitamin C, β-carotene and lycopene at 22 blueberry genotypes

Genotypes	Vitamin C (mg/100g)	β-carotene (mg/100g)	Lycopene (mg/100g)
16-2-1	12.47±0.01^{abc}	0.42±0.04^{a}	0.37±0.03^{a}
16-2-2	12.07±0.07^{abcd}	0.07±0.04^{cde}	0.06±0.1^{fg}
16-2-3	7.21±0.16^{abcdefg}	0.02±0.02^{e}	0.01±0.06^{h}
16-2-4	7.42±0.02^{abcdefg}	0.09±0.01^{cde}	0.06±0.07^{fg}
16-2-6	12.90±0.02^{ab}	0.22±0.02^{b}	0.2±0.01^{b}
16-2-8	11.73±0.36^{abcdef}	0.13±0.01^{c}	0.09±0.01^{def}
16-2-9	11.35±0.01^{abcde}	0.07±0.01^{cde}	0.1±0.01^{cd}
16-2-10	10.23±0.13^{abcdef}	0.07±0.01^{cde}	0.06±0.02^{fg}
16-2-11	11.34±0.29 ^{abcdef}	0.1±0.01^{cd}	0.06±0.01^{fg}
16-2-12	13.49±0.51^{a}	0.03±0.01^{de}	0.03±0.02^{fg}
16-2-13	8.86±0.01 ^{abcdefg}	0.07±0.02^{cde}	0.06±0.01^{fg}
16-2-14	3.77±0^{g}	0.2±0.01^{b}	0.12±0.01
16-2-15	7.21±0.11 ^{abcdefg}	0.08±0.01^{cde}	0.06±0.01^{g}
16-2-16	7.42±1.59^{abcdefg}	0.11±0.09^{cd}	0.09±0.03
16-2-17	4.91±0.01^{fg}	0.04±0.01^{de}	0.03±0.01^{h}
16-2-22	5.12±0.02^{efg}	0.13±0.01^{c}	0.07±0.01^{efg}
16-2-24	5.96±0.01^{cdefg}	0.11±0.01^{cd}	0.06±0.01^{fg}
16-2-25	3.89±0.01^{g}	0.06±0.01^{cde}	0.07±0
16-2-26	4.79±0.54^{fg}	0.11±0.12^{cd}	0.08±0.04
16-2-31	3.48±0.16^{g}	0.02±0.03^{e}	0.02±0.01^{h}
Simultan	7.52±7.16^{abcdefg}	0.39±0.06^{a}	0.18±0.08^{b}
Northblue	6.29±6.8^{bcdefg}	0.64±0.45^{cde}	1.34±1.45^{c}

CONCLUSIONS

According to the results presented in our study, the berry weight, morphological and biochemical characteristics of the fruits of these new hybrids obtained by artificial hybridization were closely of the genitors or the progenies even exceeded their parents.

Important indices of biochemical evaluation, can be analyzed in the next years, in order to increase the efficiency of creating new cultivar. Our results will strengthen the breeding process in the Research Institute for Fruit Growing Pitesti which aims to create new valuable varieties of *Vaccinium corymbosum* L. with a significant complex of biologically active compounds, which makes the fruits of this crop a trendy food product

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