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Biochemical Evaluation of Some Fruit Characteristics of Blueberry Progenies Obtained from ‘Simultan × Duke’

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Cite This: *ACS Omega* 2023, 8, 18603−18616
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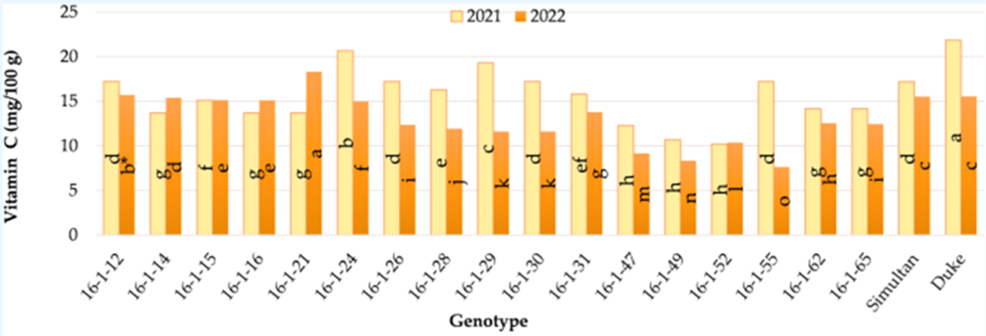


*Duncan`s Multiple Range Test, $P < 0.05$

ABSTRACT: The popularity of *Vaccinium corymbosum* blueberry cv. has increased over time because its fruits are highly valued for their taste, aroma, and multiple ways of use. A field trial with two genotypes and their hybrids was organized during 2021−2022 at the Research Institute for Fruit Growing Pitesti-Maracineni, Romania. This paper proposes a comparative analysis of the quality of berries in 17 hybrids of the ‘Simultan’ and ‘Duke’ cultivars, selected by the size and the soluble solid content, in agreement with the objectives of the blueberry breeding program. The genotype influence on berry weight, total soluble solids, pH, vitamin C, total polyphenols, total flavonoids, total anthocyanins, lycopene, β-carotene, and antioxidant activity was determined considering the climatic factors. The results showed that fruit weight varied between 1.22 and 2.47 g, total soluble solids reached a maximum of 19.22 °Brix, and the pH oscillated between 3.14 and 3.89. Vitamin C content varied from 9.52 to 18.69 mg in 100 g fresh weight, with an average of 14.35 mg/100 g. Total polyphenol, flavonoid, and anthocyanin contents averaged 709.92 mg gallic acid equivalent in 100 g fresh weight, 165.48 mg catechin equivalent in 100 g fresh weight, and 81.88 mg cyanidin-3-*O*-glucoside equivalent in 100 g fresh weight, respectively. Results show that the strategy of growers to produce blueberries with a large diameter, visually attractive for traders and consumers, is not sufficient for repeat sales. Our study proves that large fruits do not have the highest content of bioactive compounds. Smaller berries had higher polyphenol, lycopene, and β-carotene contents. It is recommended that the selection of the hybrid in the breeding program also takes into account the content of bioactive compounds.

INTRODUCTION

The cultivated blueberry (*Vaccinium corymbosum*) is a tetraploid species native to North America.[1] The highbush blueberry is primarily characterized by its fruits, which are 2−4 times larger than those of the blueberry wild, and their content in nutrients exceeds that of black blueberry (*Vaccinium fuscatum*) or wild blueberry (*Vaccinium myrtillus*) from the spontaneous flora.[2]

Fresh blueberries contain ∼84% water, ∼9.7% carbohydrates, ∼0.6% proteins, and ∼0.4% fat. The dietary fibers represent ∼3.5% of fruit weight, and a portion of 100 g fresh blueberries provides ∼192 kJ and, also, ∼10 mg of vitamin C.[3] Blueberries are an excellent source of bioactive compounds, such as polyphenols, mainly flavonoids, procyanidins, flavonols, phenolic acids, and derivatives of stilbenes.[4−6] The main anthocyanins from the blueberry fruits are malvidin, delphinidin, petunidin, cyanidin, and peonidin, with the sugar moieties of glucose, galactose, and arabinose.[7] The anti-inflammatory and anticarcinogenic properties as well as the cardiovascular protective effects of blueberries[8,9] have been proven in multiple studies.

The antioxidant compounds present in blueberries seem to diminish the risk of coronary diseases and prevent the oxidation of cholesterol, thus lowering the risk of atherosclerosis with the possibility of averting neurodegenerative disorders.[10] The hypoglycemic and hypolipidemic effects of

Received: January 23, 2023
Accepted: April 24, 2023
Published: May 15, 2023

https://doi.org/10.1021/acsomega.3c00466
ACS Omega 2023, 8, 18603−18616

blueberry[11−13] have also been detected, which highlighted their potential to prevent (pre)diabetes.[14,15]

The highbush blueberry requires special conditions to grow and produce fruit, such as low soil pH (4.8−5.5) and good water drainage. Therefore, frequently, the substrate on which blueberries are grown is represented by a mixture of soil with peat, coniferous litter, manure, and coniferous sawdust, while sulfur applications help to correct the pH.[16]

The fruit of highbush blueberry is a spherical-turned or spherical berry, colored light blue to dark blue with an intense cerum layer, and can be consumed fresh, frozen, or dried. They are also used as raw materials for the food industry, in different processed forms, such as juices, syrups, jams, jellies, wine analogues, liqueurs, or food supplements, which are highly appreciated by consumers.[17−19] Taking into account the fact that during the processing of the above-mentioned products, the levels of polyphenolic compounds are diminished, the consumption of fresh fruits is much more beneficial, but the processed products represent important sources of phenolic compounds throughout the whole year.[4,6]

The highbush blueberry breeding program started in 1983 and there were obtained varieties with increased adaptability to the edapho-climatic conditions in the Southern Subcarpathian area, where soils with lower acidity prevail compared to the optimal requirements of the species.[20] Over the years, the main indicators taken into account for the evaluation of the degree of adaptation of the blueberry in different ecological conditions have been the growth speed of the bush, fruiting potential,[21] fruit quality, ripening period,[22−25] and the content of compounds with antioxidant action.[26] Thus, in the beginning, the emphasis was laid on the commercial aspect quantified by the size, color, taste, aroma, and the content of the main biochemical components.[23,24] Second, the emphasis was laid on the prolonged shelf-life of blueberries, quantified by the berry firmness and their high resistance to the action of mechanical factors during the technological flow (picking, handling, transport, packaging, and storage).[25,26] Third, the selection criterion was represented by the content of bioactive compounds with antioxidant action, such as vitamin C, polyphenols, and anthocyanins.[26]

Until now, through the highbush blueberry breeding activity, at the Research Institute for Fruit Growing Pitesti-Maracineni, some of these objectives have been achieved and valuable cultivars have been homologated: 'Azur' (1998), 'Safir' (1998), 'Augusta' (1999), 'Delicia' (2001), 'Simultan' (2001), 'Lax' (2002), and 'Pastel' (2019). As the next step, these cultivars were tested in different pedoclimatic conditions in the country and abroad and were also included in new breeding programs.

'Duke' and 'Simultan' cultivars are considered the best early-season cultivars available. The size and quality of the berries are very good, but late harvesting can negatively influence their taste and aroma.

To create genotypes/cultivars with a high level of bioactive compounds, visually attractive for traders and consumers, and productive in the edapho-climatic conditions from Romania, one has to select the appropriate parental forms. If we are aware of the correlations between the biochemical characteristics and the physical ones, this will allow us to select the parent pairs within the enhancement process aiming to obtain new varieties with a high content of bioactive compounds, which will be more beneficial for consumers.

The first trigger on which consumers' attention is focused is the fruit's appearance (size, shape, color). However, the repurchase decision of the fruits is frequently based on the taste experience, acquired previously. For this reason, the content of blueberries in organic acids, sugars, and tannins becomes of similar or even greater importance to the fruit size. Last but not least, for the fruits used to obtain food supplements, the purchase criterion is the content of bioactive compounds. In light of these considerations, the aim of breeding programs is not only to obtain large-sized fruits but also tasty fruits with the highest possible level of bioactive compounds. This paper proposes a comparative analysis of the berries quality in 17 hybrids of the 'Simultan' and 'Duke' cultivars, selected by the size and the soluble solid content, in agreement with the objectives of the blueberry breeding program. The genotype influence on berry weight, total soluble solids (TSS), pH, vitamin C, total polyphenols, total flavonoids, total anthocyanin, lycopene, β-carotene, and antioxidant activity (AA) was determined considering the plant age and climatic factors.

■ MATERIALS AND METHODS

Chemicals and Reagents. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,6-dichloroindophenol (DCPIP), sodium hydroxide, sodium carbonate, sodium bicarbonate, sodium nitrite, disodium phosphate, aluminum chloride, methanol, acetone, *n*-hexane, ethanol, citrate/acetate buffer, gallic acid, catechin, vitamin C, cyanidin-3-*O*-glucoside, metaphosphoric acid, acetic acid, hydrochloric acid, citric acid, and Folin−Ciocalteu reagent were obtained from Merck, Darmstadt, Germany.

Plant Material. The fruits of two commercial cultivars of highbush blueberry, namely, 'Simultan' obtained from open pollination of 'Spartan' cv. and 'Duke' obtained from breeding following cross combination ('Ivanhoe × Earliblue') × 192-8 (E-30 × E × 11) and 17 selected progeny hybrids (16-1-12, 16-1-14, 16-1-15, 16-1-16, 16-1-21, 16-1-24, 16-1-26, 16-1-28, 16-1-29, 16-1-30, 16-1-31, 16-1-47, 16-1-49, 16-1-52, 16-1-55, 16-1-62, and 16-1-65) deriving from the two above-mentioned cultivars represent the plant material debated upon in this study. The selection of these hybrids was performed according to the main breeding objectives: fruit weight and soluble solid content.

The experiment was set up at the Research Institute for Fruit Growing Pitesti-Maracineni, Arges, Romania, within the Genetic and Breeding Department in 2019, in an experimental seedling plot. The experimental plot was organized in an open field according to a randomized design with three repetition plots. Selected hybrids and genitors were planted at a distance of 3 m × 1 m on a mixture of soil and peat (30 t/ha). The soil contains clay (17.6%), organic matter (1.84%), and pH (5.8). The experimental lot was irrigated by sprinklers, and Cropmax (0.5 L/ha) was used for foliar fertilization.

The blueberries were harvested at the full maturity stage (visually appreciated by the specific integral coloring of berries) between 15 June and 7 July (2021) and 10 and 30 June (2022) and analyzed immediately after the last harvest.

A WatchDog 900 ET weather station, located in the vicinity of the experimental lot, was used to record the evolution of climatic factors. The Pitesti-Arges area has a continental humid climate, Cfbx category.[27] The average multiannual temperature of the area (in the last 53 years) is 10 °C, and the annual amount of precipitation averages 678.1 mm. Taking into account the fact that blueberry bud-breaking occurred starting from the second half of March and the last harvest was made in the first week of July, Table 1 presents the meteorological

Table 1. Meteorological Parameters (Mean Temperature, Maxim and Minim Temperature Averages, Daily Thermal Amplitude, Sunshine Hours, Air Relative Humidity, and Rainfall) during March−July in 2021 and 2022, and Their Multiannual Values (1969−2021)

meteorological parameters		interval	March	April	May	June	July	average (temperatures, air relative humidity)/ sum (sunshine, rainfall)
air temperature (°C)	monthly average	2021	4.12	8.59	15.60	19.32	23.48	14.22
		2022	3.58	10.12	16.45	21.11	22.85	14.82
		1969−1921	4.86	10.39	15.35	18.94	20.71	14.05
	maximum temperature average	2021	10.50	15.04	22.25	26.55	31.05	21.08
		2022	10.26	17.42	24.37	29.18	31.52	22.55
		1969−1921	11.03	16.95	22.17	25.79	27.97	20.78
	minimum temperature average	2021	−1.35	2.57	9.03	13.40	16.38	8.01
		2022	−2.55	3.23	8.72	13.64	15.42	7.69
		1969−1921	−0.10	4.55	9.30	12.80	14.32	8.17
	daily thermal amplitude	2021	11.85	12.48	13.22	13.15	14.67	13.07
		2022	12.82	14.19	15.65	15.54	16.10	14.86
		1969−1921	11.12	12.40	12.87	12.99	13.65	12.61
sunshine hours (monthly sum, hours)		2021	160.30	176.80	266.23	259.91	288.16	1151.40
		2022	185.44	215.30	286.00	286.30	289.70	1262.74
		1969−1921	160.16	193.89	246.15	275.99	304.17	1180.36
air relative humidity (%)		2021	64.58	64.77	65.13	73.30	61.42	65.84
		2022	65.90	74.69	72.91	75.22	70.34	71.81
		1969−1921	71.39	68.75	71.89	72.64	70.82	71.10
rainfall (monthly sum, mm)		2021	66.80	38.40	65.40	104.00	33.50	308.10
		2022	19.40	88.00	72.60	25.60	25.30	230.90
		1969−1921	37.74	55.02	81.77	100.63	80.50	355.66

parameters from March to July in the years 2021 and 2022, along with the average values of the 1969−2021 period.

In March (both 2021 and 2022), average minimum temperatures dropped under the multiannual value, and the last frost occurred in March−April (2021 and 2022, data not presented). During the growing season, until harvest, the average temperature, the average maximum, and the thermal amplitude exceeded the multiannual averages, while the level of precipitation was lower. In particular, July 2021, June, and July 2022 were warmer and drier than normal.

Determination of Average Weight. By weighing a sample of 50 fruits for each genotype (15 plants/genotype), the average weight of fruits was determined and the results were expressed in g/fruit.

Determination of Total Soluble Solid Content. Soluble solids were determined using a Kruss DR201-95 refractometer and the results were reported as °Brix at 20 °C.

Determination of pH. pH values were measured in freshly extracted blueberry juice at 20 °C, using a Consort C-561 multimeter.

Determination of Total Polyphenol Content (TPC). The total polyphenol content (TPC) was determined according to the methodology suggested by Matić et al.[28] By the reaction of polyphenols with phosphotungstic acid, in an alkaline medium, a blue-colored compound is formed. This newly formed compound has maximum absorption at 760 nm. The results were expressed as mg gallic acid equivalent (GAE)/100 g fresh weight (FW).

Determination of Total Flavonoid Content (TFC). The total flavonoid content (TFC) was determined according to the methodology suggested by Tudor-Radu et al.[29] By the reaction of flavonoids with aluminum chloride, a yellow-orange-colored compound is formed. This newly formed compound has maximum absorption at 510 nm. The results were expressed as mg catechin equivalent (CE)/100 g fresh weight (FW).

Determination of Vitamin C Content. According to the colorimetric method and the methodology suggested by Omaye et al.,[30] the vitamin C content was determined by using 2,6-dichloroindophenol (DCPIP) at pH 3−4.5. Vitamin C reduces the DCPIP indicator to a colorless solution, causing a decrease in the absorption of an indicator at 520 nm. The results were expressed in mg vitamin C/100 g fresh weight (FW).

Determination of Total Anthocyanin Content (TAC). Total anthocyanin content (TAC) was determined by the pH differential method suggested by Di Stefano and Cravero.[31] The method determines the total monomeric anthocyanin content because the anthocyanin chromophore undergoes a reversible structural transformation as a function of the pH. The absorbance at 520 nm was measured after 30 min from the preparation of the blueberry extract samples in pH 0.6 (2% hydrochloric acid) and pH 3.5 (a phosphate buffer, containing 0.1 M citric acid and 0.2 M disodium phosphate) buffer. The results were expressed as cyanidin-3-*O*-glucoside equivalent (C3-GE)/100 g fresh weight (FW).

Determination of Lycopene and β-Carotene Levels. The lycopene and β-carotene content was determined according to the methodology proposed by Tudor-Radu et al.,[29] by the carotenoid extraction in a mixture of hexane/ethanol/acetone. The results were expressed in mg lycopene or β-carotene in 100 g fresh weight (FW), using molar extinction coefficients of both compounds at 470 and 503 nm.[32]

Determination of Antioxidant Activity. Total antioxidant activity was evaluated according to the radical scavenging capacity of DPPH free radicals based on the methodology suggested by Moon and Shibamoto[33] with some modifications. A solution of DPPH in methanol (0.116 mM) was prepared and 2.97 mL of DPPH solution was mixed with 0.03 mL of

Table 2. Variations in the Average Berry Weight, Total Soluble Solids (TSS), and pH in Blueberry Fruits of 'Simultan' and 'Duke' Cultivars and Their Progeny Hybrids

year	genotype	berry weight (g)	TSS (°Brix)	pH
2021	average	2.26 ± 0.58^{a}	12.35 ± 4.81^{b}	3.47 ± 0.40^{a}
2022	average	1.45 ± 0.48^{b}	14.85 ± 2.72^{a}	3.32 ± 0.37^{b}
2021 + 2022	16-1-12	2.16 ± 0.46^{abc} [a,b]	13.47 ± 2.12^{b}	3.46 ± 0.45^{bcd}
	16-1-14	2.46 ± 0.13^{ab}	12.77 ± 1.48^{b}	3.86 ± 0.26^{a}
	16-1-15	2.38 ± 0.32^{ab}	12.25 ± 2.97^{b}	3.16 ± 0.21^{d}
	16-1-16	1.93 ± 0.49^{abcd}	13.17 ± 2.03^{b}	3.89 ± 0.29^{a}
	16-1-21	1.76 ± 0.74^{abcd}	13.82 ± 3.22^{b}	3.22 ± 0.31^{d}
	16-1-24	1.35 ± 0.78^{cd}	12.45 ± 1.97^{b}	3.35 ± 0.49^{cd}
	16-1-26	1.44 ± 0.69^{cd}	13.72 ± 3.19^{b}	3.14 ± 0.38^{d}
	16-1-28	1.60 ± 0.37^{bcd}	11.30 ± 1.86^{b}	3.37 ± 0.45^{cd}
	16-1-29	1.73 ± 0.74^{abcd}	15.02 ± 3.45^{ab}	3.18 ± 0.31^{d}
	16-1-30	1.33 ± 0.54^{cd}	19.22 ± 8.34^{a}	3.30 ± 0.50^{cd}
	16-1-31	1.41 ± 0.51^{cd}	19.13 ± 8.66^{a}	3.30 ± 0.44^{cd}
	16-1-47	2.47 ± 0.83^{a}	13.43 ± 1.88^{b}	3.17 ± 0.27^{d}
	16-1-49	2.07 ± 0.80^{abcd}	11.07 ± 0.15^{b}	3.45 ± 0.14^{bcd}
	16-1-52	1.86 ± 1.13^{abcd}	12.45 ± 6.00^{b}	3.37 ± 0.27^{cd}
	16-1-55	2.33 ± 0.75^{ab}	11.58 ± 2.22^{b}	3.23 ± 0.10^{d}
	16-1-62	1.22 ± 0.33^{d}	14.42 ± 4.92^{ab}	3.62 ± 0.31^{abc}
	16-1-65	1.89 ± 0.75^{abcd}	11.93 ± 4.58^{b}	3.36 ± 0.24^{cd}
	'Simultan'	1.93 ± 0.70^{abcd}	11.92 ± 3.41^{b}	3.33 ± 0.38^{cd}
	'Duke'	1.96 ± 0.37^{abcd}	15.32 ± 1.05^{b}	3.74 ± 0.53^{ab}
	mean	1.85	13.67	3.39
	std. deviation	0.68	4.12	0.39
	range	3.05	27.40	2.04
	minimum	0.60	7.80	2.76
	maximum	3.65	35.20	4.80
genotype influence sig. (*P*)		0.002	0.010	0.000
year influence sig. (*P*)		0.000	0.000	0.002
genotype × year influence sig. (*P*)		0.000	0.569	0.000

[a] Means of data collected in 2 years and standard deviation (2021−2022) are presented. [b] Means with the same letter are not significantly different at the 5% level.

methanolic extract of blueberry. The mixture was gently homogenized and kept to stand at room temperature for 30 min. Then, the absorbance of the mixture was spectrophotometrically measured at 517 nm.

Statistical Analysis. All analyses were performed in triplicate and data were reported as mean ± standard deviation (SD). Excel 2021 (XLSTAT) was used for data statistical analysis. One-way analysis of variance (ANOVA) and two-way ANOVA and Duncan's multiple range tests were performed.

■ RESULTS AND DISCUSSION

Tables 2−4 show the values for the average berry weight, total soluble solids, pH, polyphenols, flavonoids, anthocyanins, vitamin C, lycopene, β-carotene, and antioxidant activity with the indication of the values of minimum, maximum, mean, and standard deviation for the berries of the 'Simultan' and 'Duke' genitors and the hybrids of the 'Simultan' and 'Duke' cultivars.

A set of criteria was suggested as a minimum quality standard for fruits, such as pH between 2.25 and 4.25, acidity, expressed by citric acid, from 0.3 to 1.3%, over 10% soluble solids, and sugar-to-acid ratios from 10 to 33.[34] The fruits of any cultivar with a lower sugar-to-acid ratio (i.e., more acidic fruits) tend to maintain their integrity for a longer time.

Berry weight is one of the representative quality parameters for the commercial blueberry market, since the cultivars with good production and larger-sized fruits positively influence buyers' decisions.

As presented in Table 2, blueberry hybrid progeny quality indicators were significantly genotype-dependent. A significant variation between 2020 and 2022 was also noted, while, except for total soluble solids (TSS), the genotype × year effect showed that the genetic influence is still variable, depending on the environmental factors (and the age of the plants).

Berry weight for both years (2021 and 2022) has oscillated between 1.22 g for the 16-1-62 hybrid and 2.47 g for the 16-1-47 hybrid with a mean value of 1.85. Similar results were reported by Ancu et al.[35] who found values between 1.24 and 2.15 g for the fruits of seven Romanian blueberry varieties ('Simultan', 'Delicia', 'Lax', 'Compact', 'Augusta', 'Azur', and 'Blueray'). The average berry weight grown in Bosnia ('Earliblue','Bluegold','Bluecrop', and 'Goldtraube') ranged from 1.12 to 2.11 g,[36] in Korea (45 highbush blueberries cultivars) from 1.6 to 2 g,[37] and in Serbia ('Bluecrop', 'Jersey', and 'Earliblue', from two different locations) from 1.47 to 1.83 g.[38]

Soluble solid content is a measure of sweetness.[39] The higher the soluble solid content, the more convenient and desirable it is to process the blueberry fruits.[40] The total soluble solid content oscillated from 11.07 °Brix (16-1-49 hybrid) to 19.22 °Brix (16-1-30 hybrid) with a mean value of 13.67 °Brix. Similar results were reported by Ancu et al.[35] who obtained values between 12.51 and 16.09 °Brix for the fruits of seven Romanian blueberry varieties. The total soluble solid content in blueberry fruits from 45 commercial cultivars (39

Table 3. Variations in the Total Content of Polyphenols (TPC), Flavonoids (TFC), Anthocyanins (TAC), and Vitamin C in Blueberry Fruits of 'Simultan' and 'Duke' Cultivars and Their Progeny Hybrids

year	genotype	TPC (mg GAE/100 g FW)	TFC (mg CE/100 g FW)	TAC (mg C3-GE/100 g FW)	vitamin C (mg/100 g FW)
2021	average	378.13 ± 168.08^{b}	141.31 ± 52.94^{b}	76.61 ± 60.75^{b}	15.67 ± 3.04^{a}
2022	average	1041.72 ± 569.08^{a}	189.66 ± 68.75^{a}	87.13 ± 42.22^{a}	13.03 ± 2.79^{b}
2021 + 2022	16-1-12	1113.76 ± 0.87^{ab} [a,b]	195.15 ± 20.78^{c}	136.00 ± 39.81^{def}	16.45 ± 0.87^{c}
	16-1-14	866.15 ± 0.94^{abcd}	131.32 ± 5.63^{i}	66.94 ± 33.87^{c}	14.55 ± 0.94^{g}
	16-1-15	1186.09 ± 0.25^{a}	217.94 ± 42.78^{a}	106.00 ± 25.28^{b}	15.11 ± 0.25^{ef}
	16-1-16	663.14 ± 0.80^{abcd}	199.07 ± 66.29^{bc}	112.68 ± 10.34^{ab}	14.41 ± 0.80^{gh}
	16-1-21	1032.49 ± 2.53^{abc}	144.03 ± 23.03^{fg}	82.55 ± 24.68^{c}	16.01 ± 2.53^{d}
	16-1-24	1133.95 ± 3.13^{ab}	144.57 ± 29.64^{fg}	65.48 ± 14.90^{c}	17.84 ± 3.13^{b}
	16-1-26	1153.99 ± 2.66^{ab}	206.19 ± 102.94^{b}	135.43 ± 77.75^{a}	14.79 ± 2.66^{fg}
	16-1-28	366.37 ± 2.38^{cd}	221.86 ± 145.24^{a}	68.9 ± 40.02^{c}	14.11 ± 2.38^{h}
	16-1-29	721.70 ± 4.20^{abcd}	116.45 ± 8.15^{j}	63.97 ± 19.03^{c}	15.47 ± 4.20^{e}
	16-1-30	942.14 ± 3.06^{abcd}	142.23 ± 22.21^{gh}	66.68 ± 28.88^{c}	14.42 ± 3.06^{gh}
	16-1-31	402.35 ± 1.19^{cd}	199.44 ± 19.38^{bc}	132.98 ± 64.68^{a}	14.80 ± 1.13^{fg}
	16-1-47	387.96 ± 1.75^{cd}	106.23 ± 19.25^{k}	116.67 ± 117.83^{ab}	10.74 ± 1.75^{k}
	16-1-49	722.33 ± 0.57^{abcd}	150.95 ± 93.23^{f}	62.67 ± 4.03^{c}	9.52 ± 1.30^{m}
	16-1-52	427.70 ± 0.27^{cd}	178.38 ± 94.42^{d}	104.17 ± 35.68^{b}	10.30 ± 0.27^{l}
	16-1-55	487.03 ± 5.25^{bcd}	140.90 ± 85.22^{gh}	75.83 ± 40.39^{c}	12.42 ± 5.25^{j}
	16-1-62	290.54 ± 0.92^{d}	176.45 ± 44.01^{d}	29.14 ± 24.04^{d}	13.38 ± 0.91^{i}
	16-1-65	592.24 ± 0.98^{abcd}	159.13 ± 45.30^{e}	73.43 ± 27.43^{c}	13.32 ± 0.98^{i}
	'Simultan'	396.51 ± 2.21^{cd}	178.69 ± 27.24^{d}	29.00 ± 1.27^{d}	16.35 ± 1.05^{cd}
	'Duke'	689.31 ± 0.65^{abcd}	135.17 ± 33.93^{hi}	27.11 ± 12.26^{d}	18.69 ± 3.50^{a}
	mean	709.92	165.48	81.88	14.35
	std. deviation	534.38	65.73	52.34	3.20
	range	2073.34	296.54	281.04	14.69
	minimum	99.66	58.45	14.96	7.63
	maximum	2173.00	354.99	296.00	22.32
genotype influence sig. (P)		0.000	0.000	0.000	0.000
year influence sig. (P)		0.000	0.000	0.004	0.000
genotype × year influence sig. (P)		0.000	0.000	0.000	0.000

[a] Means of data collected in 2 years and standard deviation (2021–2022) are presented. [b] Means with the same letter are not significantly different at the 5% level.

northern highbush and 6 half highbush blueberries) grown in Suwon, Korea ranged from 8.3 to 14.3 °Brix.[37] On average, the soluble solid content of the harvested fruits in Guasca, Colombia, from the cultivars 'Biloxi' and 'Sharpblue', was in a range of 12.4–14.5 °Brix.[39]

The pH of blueberries had a mean value of 3.39 and increased from 3.14 (16-1-26 hybrid) to 3.89 (16-1-16 hybrid). Similar data were reported by Aliman et al.[36] who found values for pH of 3.2–3.6 for the highbush blueberry and wild bilberry fruit grown in central Bosnia and Zorenc et al.[38] who found values for pH of 2.76–3.89 for the highbush blueberry fruits of three traditionally cultivated cultivars in Slovenia, 'Bluecrop', 'Earliblue', and 'Jersey'.

A significant influence of genotype was registered for berry weight, total soluble solids, and pH ($P = 0.000$–0.010).

As presented in Tables 3 and4, total phenolic, flavonoid, anthocyanin, vitamin C, lycopene, and β-carotene content and the antioxidant activity of the discussed genitors and hybrids varied significantly under the genotype influence ($P = 0.000$).

The total phenolic content represents a marker of antioxidant capacity and it is generally used as an antioxidant activity test. Phenolic compounds are known to inhibit free radicals and prevent the deformation of DNA.[40,41] Total phenolic content in blueberry fruits depends on the cultivar,[5] the growing conditions,[42,43] and the degree of maturity at the harvest of berries.[44]

The total phenolic content of the blueberry fruits of 'Simultan' and 'Duke' cultivars and their progeny hybrids recorded an average value of 709.92 mg GAE/100 g FW and oscillated between 290.54 mg GAE/100 g FW (hybrid 16-1-62) and 1186.09 mg GAE/100 g FW (16-1-15 hybrid). Most of the analyzed samples (including genitors—'Duke', with 689.31 mg/100 g FW, and 'Simultan', with 396.51 mg GAE/100 g FW) presented a phenolic content under average, but 5 out of the 19 genotypes had total phenolic content higher than 1000 mg GAE/100 g FW.

The total phenolic content in blueberry fruits from 45 commercial cultivars grown in Suwon, Korea ranged from 170.9 to 385.7 mg GAE/100 g FW.[36] Lee et al.[45] obtained values for the total polyphenol content between 367 and 1286 mg GAE/100 g FW for *Vaccinium membranaceum* species and 677–1054 mg GAE/100 g FW for *Vaccinium ovalifolium* species, native to Pacific Northwest of North America. Prior et al.[3] reported values for the total phenolic content between 181 and 390 mg/100 g FW for *V. corymbosum* L. species ('Bluecrop', 'Jersey', 'Croatan', 'Duke', 'Rancocas', 'Rubel', 'O'Neal', 'Reveille', 'Blue Ridge', 'Cape Fear', 'Pender', and 'Bladen' cvs.). Dragovič-Uzelac et al.[46] reported for the 'Bluecrop' variety a higher amount of polyphenols than 'Duke' (blueberry cultivars grown in Northwest Croatia), while Prior et al.[3] obtained a higher total phenolic content value for the 'Duke' cultivar, grown in Chatsworth, New Jersey, United States. Gündeşli et al.[8] reported values between 158.4

https://doi.org/10.1021/acsomega.3c00466
ACS Omega 2023, 8, 18603–18616

Table 4. Variations in the Total Content of Lycopene and β-Carotene, and Antioxidant Activity (AA) in Blueberry Fruits of 'Simultan' and 'Duke' Cultivars and Their Progeny Hybrids

year	genotype	lycopene (mg/100 g FW)	β-carotene (mg/100 g FW)	AA (mmol Trolox/100 g FW)
2021	average	0.025 ± 0.025^{b}	0.088 ± 0.091^{b}	
2022	average	0.118 ± 0.054^{a}	0.129 ± 0.094^{a}	0.2022 ± 0.0075
2021 + 2022	16-1-12	0.070 ± 0.040^{bcd} [a,b]	0.070 ± 0.040^{def}	0.2039 ± 0.0004^{fg}
	16-1-14	0.070 ± 0.050^{bcd}	0.080 ± 0.050^{cdef}	0.2046 ± 0.0003^{ef}
	16-1-15	0.050 ± 0.010^{cd}	0.020 ± 0.010^{f}	0.1980 ± 0.0005^{klm}
	16-1-16	0.060 ± 0.030^{bcd}	0.060 ± 0.030^{def}	0.2124 ± 0.0003^{b}
	16-1-21	0.090 ± 0.140^{abcd}	0.150 ± 0.140^{bc}	0.2080 ± 0.0004^{d}
	16-1-24	0.060 ± 0.040^{bcd}	0.200 ± 0.040^{b}	0.1973 ± 0.0004^{lm}
	16-1-26	0.040 ± 0.030^{d}	0.120 ± 0.030^{cd}	0.2015 ± 0.0006^{hi}
	16-1-28	0.020 ± 0.030^{d}	0.110 ± 0.030^{cde}	0.2099 ± 0.0004^{c}
	16-1-29	0.030 ± 0.010^{d}	0.030 ± 0.030^{ef}	0.2210 ± 0.0003^{a}
	16-1-30	0.080 ± 0.040^{bcd}	0.100 ± 0.040^{cde}	0.1964 ± 0.0005^{m}
	16-1-31	0.080 ± 0.060^{bcd}	0.120 ± 0.010^{cd}	0.1988 ± 0.0004^{jkl}
	16-1-47	0.070 ± 0.600^{bcd}	0.070 ± 0.060^{def}	0.1997 ± 0.0005^{ijk}
	16-1-49	0.130 ± 0.010^{ab}	0.120 ± 0.010^{cd}	0.1846 ± 0.0005^{n}
	16-1-52	0.130 ± 0.070^{abc}	0.100 ± 0.070^{cdef}	0.2033 ± 0.0006^{fgh}
	16-1-55	0.050 ± 0.010^{cd}	0.030 ± 0.010^{ef}	0.2004 ± 0.0004^{ij}
	16-1-62	0.050 ± 0.040^{cd}	0.060 ± 0.040^{def}	0.2024 ± 0.0009^{gh}
	16-1-65	0.100 ± 0.080^{abcd}	0.080 ± 0.080^{cdef}	0.1973 ± 0.0045^{lm}
	'Simultan'	0.100 ± 0.020^{abcd}	0.210 ± 0.070^{b}	0.2062 ± 0.0003^{de}
	'Duke'	0.160 ± 0.060^{a}	0.380 ± 0.050^{a}	0.1968 ± 0.0002^{lm}
	mean	0.070	0.110	0.2022
	std. deviation	0.060	0.100	0.0075
	range	0.260	0.450	0.0372
	minimum	0.000	0.000	0.1841
	maximum	0.260	0.450	0.2213
genotype influence sig. (P)		0.006	0.000	0.0000
year influence sig. (P)		0.000	0.000	
genotype × year influence sig. (P)		0.000	0.000	

[a] Means of data collected in 2 years and standard deviation (2021−2022) are presented. [b] Means with the same letter are not significantly different at the 5% level.

and 2784.45 mg GAE/100 g FW for the total polyphenol content in blueberry fruits, from different countries (Italy, Turkey, United States). Colak et al.[47] reported the total phenolic content ranging from 555 to 638 mg GAE/100 g FW in the wild bilberry population grown in Ardahan province located in eastern Anatolia, Turkey, and 327 mg GAE/100 g FW in the blueberry cultivar 'Bluecrop', that indicate lower values than all wild bilberry accessions. The polyphenol classification proposed by Vasco et al.[48] using low (<1 mg GAE/g), medium (1−5 mg GAE/g), and high (>5 mg GAE/g) values indicates that our blueberry samples are a good source of these compounds.

Flavonoids constitute the largest subgroup of polyphenols. Pietta et al.[49] stated that flavonoids were responsible for antioxidant activity. The total flavonoid content in blueberry fruits of 'Simultan' and 'Duke' cultivars and their progeny hybrids varied between 106.23 and 221.86 mg CE/100 g FW, having a mean value of 165.48 mg CE/100 g FW. It could be observed that most of the 'Simultan × Duke' hybrids had flavonoid contents higher than their genitors (135.17 mg CE/100 g FW for 'Duke' and 178.69 mg CE/100 g FW for 'Simultan') and the 16-1-28 hybrid was remarked (221.86 mg CE/100 g FW), followed by 16-1-15 (217.94 mg CE/100 g FW) and 16-1-26 (206.19 mg CE/100 g FW). Therefore, the 16-1-15 hybrid stood out as having the first higher total polyphenol content and the second higher total flavonoid content. Similar results were reported by Dróżdż et al.,[50] Häkkinen and Törrönen,[51] Koca and Karadeniz.[52] Studies[50−52] illustrate that total flavonoid amounts of the same blueberry cultivar can be different, with the climatic conditions and the cultivation techniques having a great influence.

Anthocyanin content presented an average of 81.88 mg C3-GE/100 g FW and varied significantly under the genotype effect. The highest level was found for the 16-1-12 hybrid, 136.00 mg C3-GE/100 g FW. All progenies had higher anthocyanin content than the genitors (29.00 mg C3-GE/100 g FW—'Simultan' and 27.11 mg C3-GE/100 g FW—'Duke'). The blueberry cultivar 'Bluecrop' grown in Turkey had a total anthocyanin content of 142 mg C3-GE /100 g FW.[53] Similar results were found by Okan et al.,[41] who reported TAC values between 43.03 and 295.06 mg C3-GE/100 g FW for 28 samples of blueberries from the Black Sea region situated in north-eastern Turkey, in 2012−2014 years. In blueberries of three cultivars in Slovenia, 'Bluecrop', 'Earliblue', and 'Jersey', the total anthocyanin content value was in the range of 103.0−241.9 mg/100 g FW and the anthocyanins constituted 35−55% of the total analyzed phenolics.[38]

Vitamin C acts in the human body as an antioxidant by preventing free-radical-induced damage to DNA, quenching oxidants that can lead to the development of cataracts, improving endothelial cell dysfunctions, and decreasing low-density lipoprotein-induced leukocyte adhesion.[54] Blueberries are known among the richest fruits in vitamin C, usually with

Table 5. Intensity of the Correlations between Analyzed Parameters in Blueberry Fruits of 'Simultan' and 'Duke' Cultivars and Their Progeny Hybrids

	TSS	pH	TPC	TFC	TAC	vitamin C	lycopene	β-carotene	antioxidant activity
berry weight (g)	−0.493[c]	0.143	−0.390[c]	−0.362[c]	0.000	0.153	−0.418[c]	−0.298[b]	−0.158
	0.000	0.129	0.000	0.000	0.998	0.104	0.000	0.001	0.241
TSS (°Brix)	1	−0.112	0.215[a]	0.105	0.118	−0.027	0.217[a]	0.191[a]	0.154
		0.235	0.022	0.266	0.210	0.777	0.020	0.042	0.252
pH		1	−0.121	−0.009	−0.109	0.021	−0.082	−0.008	−0.117
			0.199	0.924	0.250	0.827	0.387	0.935	0.387
TPC (mg GAE/100 g)			1	0.288[b]	0.303[b]	−0.044	0.432[c]	0.116	0.022
				0.002	0.001	0.642	0.000	0.218	0.870
TFC (mg CE/100 g)				1	0.450[c]	−0.163	0.133	−0.013	0.073
					0.000	0.083	0.159	0.889	0.587
TAC (mg C3-GE/100 g)					1	−0.193[a]	−0.129	−0.257[b]	0.169
						0.040	0.171	0.006	0.208
vitamin C (mg/100 g)						1	−0.187[a]	0.371[c]	0.288[a]
							0.046	0.000	0.030
lycopene (mg/100 g)							1	0.541[c]	−0.373[b]
								0.000	0.004
β-carotene (mg/100 g)								1	−0.120
									0.375

[a] Correlation is significant at the 0.05 level (two-tailed). [b] Correlation is significant at the 0.01 level (two-tailed). [c] Correlation is significant at the 0.001 level (two-tailed).

values in quite wide intervals, from 4.54 to 100 mg/100 g FW.[47]

Regarding the total vitamin C content in blueberry fruits of 'Simultan' and 'Duke' cultivars and their progeny hybrids, a mean value of 14.35 mg/100 g FW was determined. The vitamin C content oscillated around 9.52 mg/100 g FW (16-1-49 hybrid), while the highest content was obtained for 'Duke' cv. (18.69 mg/100 g FW). In this case, genitors contained higher vitamin C than most of their hybrids.

The vitamin C content in 10 highbush blueberry cultivars grown in Latvia[55] ('Northland', 'Spartan', 'Barkeley', 'Duke', 'Chippewa', 'Bluecrop', 'Jersey', 'Blueray', 'Chandler', and 'Bluejay') ranged between 6.9 and 11.8 mg/100 g FW, but the fruits of these cultivars were analyzed after freezing. The amount of vitamin C in fruits of three cultivars ('Reca', 'Elizabeth' and 'Bluegold') of the highbush blueberries grown in the Western forest-steppe of Ukraine in 2017−2019 years varied from 15.70 to 20.00 mg/100 g FW, with an average value of 17.30 mg/100 g FW for the 3 years ('Reca'), from 16.70 to 27.00 mg/100 g FW, with an average value of 20.17 mg/100 g FW ('Elizabeth'), and from 19.60 to 22.50 mg/100 g FW, with an average value of 20.90 mg/100 g FW ('Bluegold').[56] The experiment of Ukrainian researchers included cultivars with different ripening times and different countries of origin: 'Reca'—early season, New Zealand; 'Elizabeth' and 'Bluegold'—mid-season, USA. Correia et al.[57] claimed that the different contents of vitamin C in blueberries were a varietal trait that can be adjusted to the conditions of the year, ranging from 6 to 162 mg/100 g.

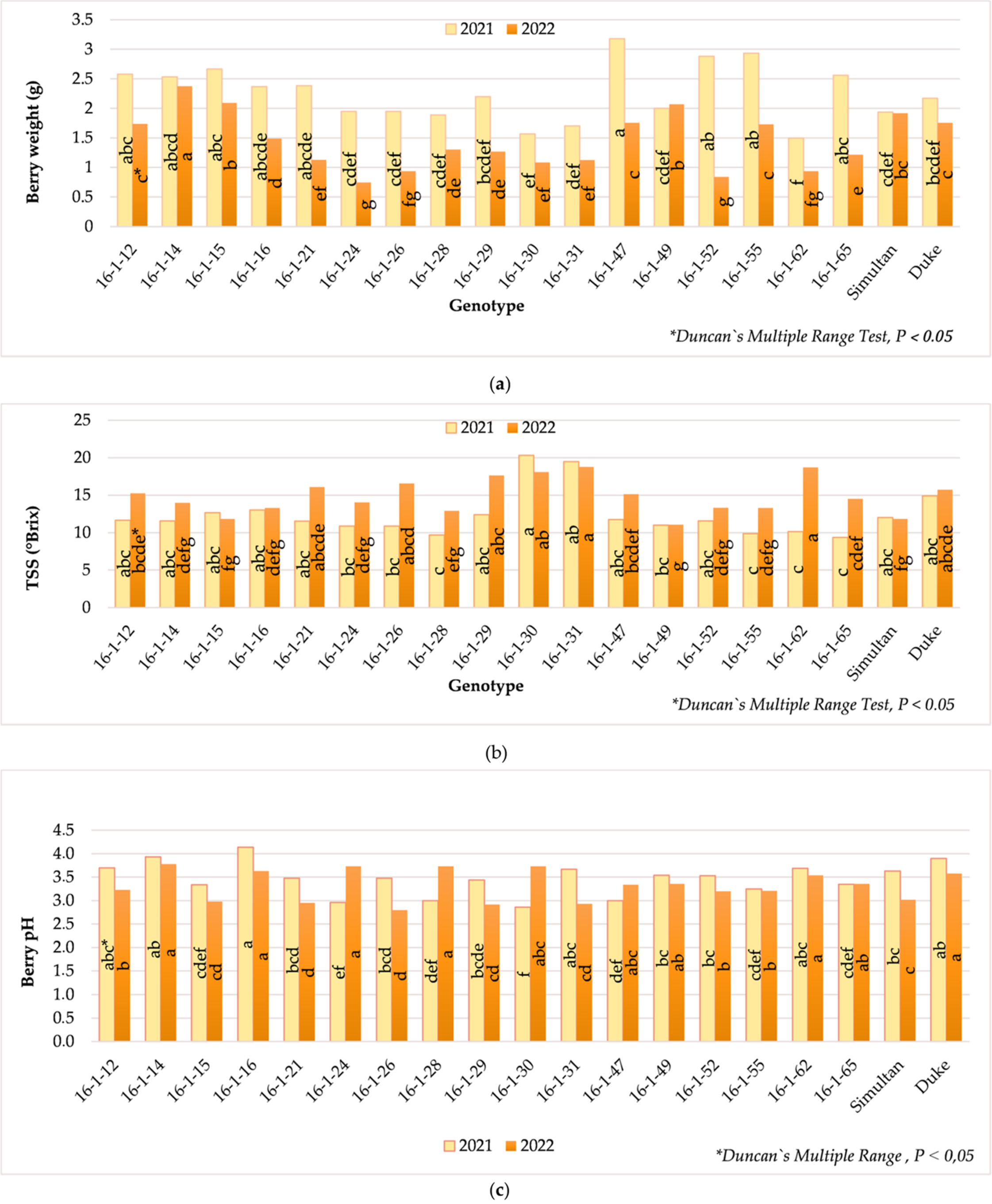
Carotenoids include diverse compounds such as lycopene, α- and β-carotene, lutein, and xanthophylls, and they are found in almost all colored vegetables. Scientific evidence is referring to the fact that lycopene and β-carotene are the primary bioactive components in fruits and vegetables that reduce cancer risk,[58−63] and the results from animal and cell-culture studies indicate even more beneficial cellular effects. These include antioxidant activity, inhibition of the cell cycle, and signaling pathways.[64]

The mean values for lycopene and β-carotene content in the blueberry fruits of 'Simultan' and 'Duke' cultivars and their progeny hybrids (Table 4) were 0.07 mg lycopene/100 g FW and 0.11 mg β-carotene/100 g FW, respectively. The lycopene level in the fruits of 'Simultan' and 'Duke' cvs. and their progeny hybrids oscillated between 0.02 mg/100 g FW (16-1-28 hybrid) and 0.16 mg/100 g FW ('Duke'). Also, the β-carotene level varied from 0.02 mg/100 g FW (16-1-15 hybrid) to 0.38 mg/100 g FW ('Duke'). Similar to vitamin C, both analyzed carotenoids registered lower levels in hybrids than those in genitors, with some exceptions.

In conformity with multiple studies, the antioxidants from fruits and vegetables protect lipids, proteins, and nucleic acids against the oxidative damage produced by free radicals, which represents an important step in the fight against cancer, heart disease, and vascular and neurodegenerative diseases.[65,66] Antioxidants are present in large quantities in blueberries (genus *Vaccinium*).[67] A few epidemiological studies showed that some types of cancer are caused by specific dietary habits, i.e., people consuming fruits and vegetables regularly have a lower risk of cancer.[67,68] It has been proven that berries inhibit many stages of carcinogenesis and stimulate the apoptosis of cancer cells.[67]

Reducing power is generally linked with reducing substances, which have been shown to exert antioxidant action by breaking the free radical chain and donating a hydrogen atom.[52] Anthocyanins, phenolic acids, and flavonoids are the main bioactive compounds of blueberries,[69] with the antioxidant activity of the fruit being an indication of the functional value of the fruit. Scibisz and Mitek[70] demonstrated that the antioxidant capacity was strongly correlated with the content of total anthocyanins and total phenolics in fruits of 14 cultivars of highbush blueberry (*V. corymbosum* L.) grown in Poland in the years 2002−2004.

To measure the antioxidant capacities of blueberries, in this study, we used the DPPH radical scavenging activity test. It is known that low DPPH values indicate a high antioxidant capacity. Regarding the antioxidant activity in blueberry fruits



(c)

Figure 1. Genotype × year interaction effect on berry weight (a), blueberry TSS (b), and pH (c).

of 'Simultan' and 'Duke' cultivars and their progeny hybrids, the mean value was 0.2022 mmol Trolox/100 g FW and reached its lowest limit, 0.1846 mmol Trolox/100 g FW, for the 16-1-49 hybrid, while the highest one was 0.2210 mmol Trolox/100 g FW, for the 16-1-29 hybrid. Finally, regarding the previously discussed 16-1-29 hybrid, a slightly above-average vitamin C content (15.47 mg/100 g) and low levels of lycopene and β-carotene (0.03 mg/100 g) were also determined.

As shown in Table 5, a tendency was observed for small berries to accumulate more soluble solids than larger ones. It has also been shown that as berry weight decreases, the total level of phenolic compounds, flavonoids, and carotenoids increases. This dynamics of the components with antioxidant activity could reflect the dilution of the berry juice (caused by the water accumulation) in larger berries. This observation is sustained by the significant positive correlations of total soluble solids (TSS) to polyphenols, lycopene, and β-carotene (to which is added positive but insignificant correlations of TSS to flavonoids and anthocyanins) and the negative (although insignificant) correlation of TSS and pH, which would rather characterize dilution but not insufficient ripening of the berries.

Anthocyanins correlated negatively with vitamin C and β-carotene (even insignificantly with lycopene).

Vitamin C showed opposite sign correlations with lycopene (negative) and β-carotene (positive).

Among the components with antioxidant activity, vitamin C was significantly positively correlated to antioxidant activity,

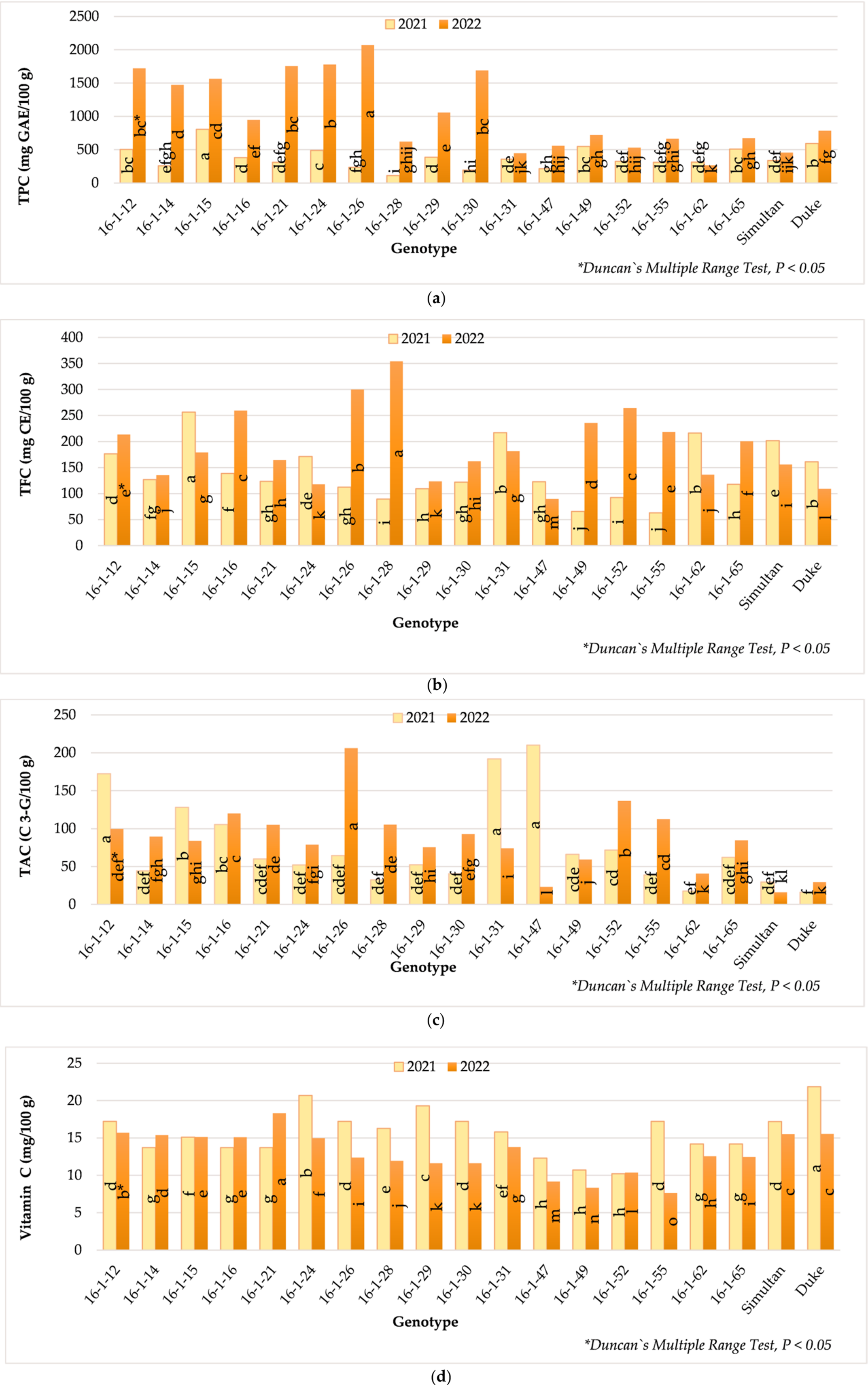
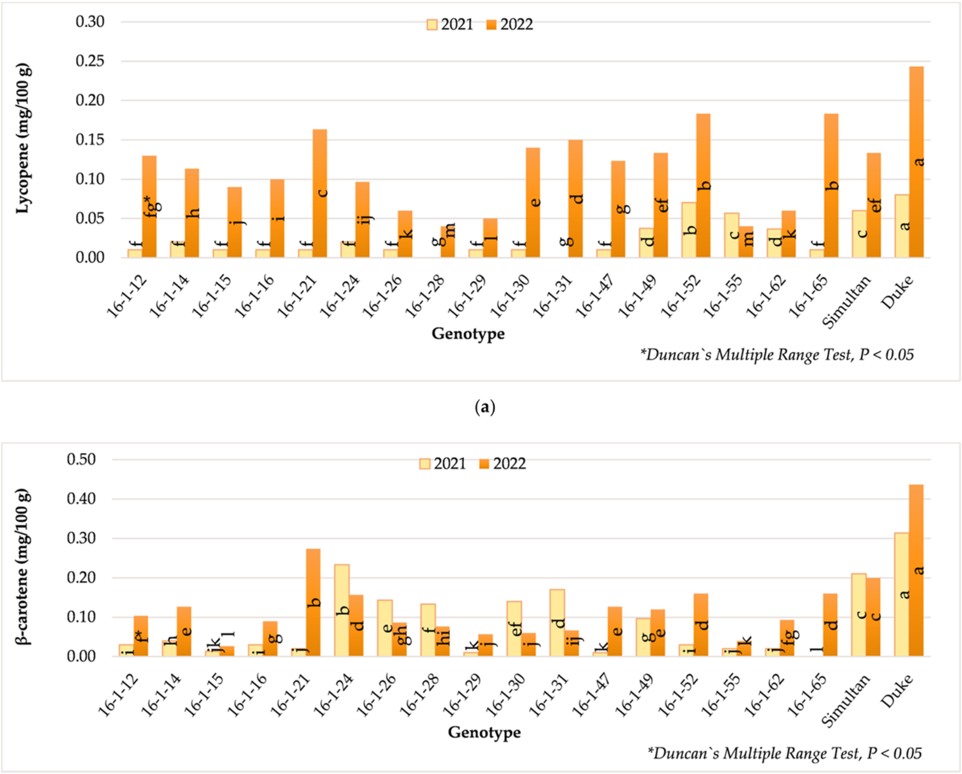


Figure 2. Genotype × year interaction effect on blueberry TPC (a), TFS (b), TAC (c), and vitamin C content (d).

although positive but insignificant correlations were established between phenolics (TPC, TFC, and TAC) and antioxidant activity. Carotenoids were negatively correlated to antioxidant activity (significant, lycopene and insignificant, β-carotene).



(a)

(b)

Figure 3. Genotype × year interaction effect on blueberry lycopene (a) and β-carotene content (b).

Table 6. One-Way ANOVA Test Results Regarding the Genotype Influence on Blueberry Quality Indicators, Depending on the Experimental Year

dependent variable	year		sum of squares	df	mean square	*F*	sig.
berry weight (g)	2021	between groups	12.125	18	0.674	3.645	0.000
	2022	between groups	12.205	18	0.678	38.096	0.000
total soluble solids (°Brix)	2021	between groups	474.062	18	26.337	1.219	0.295
	2022	between groups	297.021	18	16.501	5.329	0.000
pH	2021	between groups	517.933	18	28.774	188.734	0.000
	2022	between groups	24 719.145	18	1373.286	9595.745	0.000
polyphenols (mg GAE/100 g)	2021	between groups	1 469 407.286	18	81 633.738	27.527	0.000
	2022	between groups	17 635 256.189	18	979 736.455	74.360	0.000
flavonoids (mg CE/100 g)	2021	between groups	154 382.170	18	8576.787	127.464	0.000
	2022	between groups	263 806.003	18	14 655.889	627.102	0.000
TAC (mg C3-G/100 g)	2021	between groups	182 347.588	18	10 130.422	15.844	0.000
	2022	between groups	104 937.045	18	5829.836	93.866	0.000
vitamin C (mg/100 g)	2021	between groups	511.046	18	28.391	137.120	0.000
	2022	between groups	435.343	18	24.186	4893.811	0.000
lycopene (mg/100 g)	2021	between groups	0.033	18	0.002	73.217	0.000
	2022	between groups	0.161	18	0.009	339.163	0.000
β-carotene (mg/100 g)	2021	between groups	0.459	18	0.026	1307.228	0.000
	2022	between groups	0.491	18	0.027	470.859	0.000

As represented in Figures 1−3, significant differences were recorded between the 2 years of the study in terms of the determined parameters, while also highlighting a significant genotype × year interaction.

In general, berry weight and vitamin C content were higher in the first year of the study, while an increasing trend in the second year was observed for total soluble solids, polyphenols, and lycopene. Except for β-carotene, a greater influence of the genotypes was observed in the second year of the study on the fruit quality indicators (Table 6).

Therefore, as represented in Figures 1−3 and Tables S1−S3 in the Supporting Information, the highest berry weights were recorded in 2021 for hybrids 16-1-47, 16-1-55, 16-1-52 and in 2022 for 16-1-14, 16-1-15, and 16-1-49. Hybrids 16-1-30 and 16-1-31 had in both years of study high TSS values. Also, in 2022, among hybrids 16-1-26, 16-1-24, 16-1-21, 16-1-12, and 16-1-30, with a high level of polyphenols, only 16-1-26 stood out for its high flavonoid content. Last but not least, in 2021, hybrids 16-1-24 and 16-1-29 presented only slightly lower vitamin C levels than Duke but significantly higher than the other descendants.

In the first year of the study (2021), the shrubs (in the third year from planting) produced few but large berries. In 2022, the number of fruit buds and implicitly of berries was higher, but their weight decreased. This can be explained by the fact that the plants did not have the proper photosynthetic capacity to invest in the berries produced in the second year of the study, especially in the conditions of more pronounced thermal stress in 2022 than that in 2021. In 2022, the absolute maxima in May, June, and July (31.5, 36.8, and 38.3 °C) were higher than those in 2021 (28.4, 34.0, and 36.8 °C). Since the plantation was irrigated and the atmospheric humidity was not lower in 2022, it cannot be a question of berry dehydration (and therefore a reduction of the berry weight and a concentration of the soluble solids caused by the dehydration). Cell division is sensitive to temperature, and therefore, it can influence the growth of the fruits. In this case, it is necessary to mention that the absolute minima in 2022 (−9.3 °C in March and −3.8 °C in April) were lower than those in 2021 (−6.2 °C in March and −3.3 °C in April). Higher temperatures in 2022 could also explain the higher TPC of the blueberries according to López et al.[71] and the reduction in vitamin C content.

In 2022, starting from May and until harvest, the number of hours with temperatures over 30 °C was higher than that in 2021. However, the minimum temperatures of this period were frequently below 15 °C (being generally lower than 2021), which determined higher amplitudes of temperature variation in the second year of the study. Practically, the plants benefited not only from warm and sunny days but also from cooler nights. Under these conditions, TPC recorded higher levels in 2022 than those in 2021. In addition, according to the authors cited by Nicholas et al.,[72] the increase in sunshine hours correlated with an increase in TPC, especially TAC (which belongs to the class of flavonoids). Dinis et al.[73] cited authors who referred to the fact that there was a well-documented relationship between total sugar content (TSC) and total anthocyanin content (TAC). Thus, higher levels of TSC in 2022 than those in 2021 proved that in 2022, the climatic conditions were more favorable for the physiological process development than in 2021. Additionally, there are some observations regarding the high temperatures to which the blueberry species are adapted. According to Zheng et al.,[74] the increase in environmental temperature from 25 to 30 °C (more frequent in the present study in 2022) contributed to the regular distribution of stomata, but not the subsequent increase in temperature from 30 to 40 °C. The authors also found that the optimal temperature for transpiration in the northern highbush blueberry is 38 °C. This means that the plants suffer less from the heat due to the superior cooling capacity compared to the southern highbush blueberry, at which the optimum temperature for transpiration was established at 34 °C. In general, the data in the literature refer to situations where the growth temperature correlates with the reduction of the TPC level not only in blueberries but also in other species, for example, grapevine.[73,75−78] These discrepancies may be due to temperature oscillations above the optimum of the respective species, taking into account the fact that an increase in temperatures below the optimum stimulates plant physiological processes, and an increase in temperatures above the optimum can cause their reduction.

Some authors state that the vitamin C level of blueberries is a characteristic of the variety, but it is also influenced by environmental conditions.[56,57] Regarding the oscillations of vitamin C, Correia et al.[57] reported, similar to our study, a reduction in the level of this compound under conditions of increasing temperature and duration of sunshine. Last but not least, Shevchuk et al.[56] found the highest levels of vitamin C in 'Reca', 'Elisabeth', and 'Bluegold' blueberry cultivars in conditions of lower temperatures and poor rainfall regimes.

■ CONCLUSIONS AND PERSPECTIVES

The strategy adopted for the improvement of additive polygenic parameters is the crossing of "similar" × "similar" parents, which already have a good level of parameters. The success of the selection was revealed by the individualization of superior genotypes for the character of the selection objective. The highest average weight was determined for hybrids 16-1-47 (2.47 g), 16-1-14 (2.46 g), 16-1-15 (2.38 g), and 16-1-55 (2.33 g), exceeding their genitors (1.93 g of Simultan and 1.96 g of Duke). Overall, among genotypes with high berry weight, eight had above-average other quality indicators: 16-1-12 (TPC, TFC, TAC, vitamin C, and antioxidant activity), 16-1-14 (TPC, vitamin C, and antioxidant activity), 16-1-15 (TPC, TFC, TAC, and vitamin C), 16-1-16 (TFC, TAC, and antioxidant activity), 16-1-21 (TSS, lycopene, β-carotene, TPC, TAC, vitamin C, and antioxidant activity), 16-1-29 (TSS, TPC, vitamin C, and antioxidant activity), 16-1-49 (lycopene, β-carotene, and TPC), and 16-1-52 (lycopene, TFC, TAC, and antioxidant activity). In most cases (except for carotenoids and vitamin C), the mentioned hybrids exceeded their genitors. These will be evaluated in the next breeding stages for registration as new cultivars.

Smaller blueberry hybrids had higher values of total soluble solids, total polyphenol and flavonoid content, lycopene, and β-carotene. Although berry size can influence the purchase decision and therefore the success of the blueberry market, it is important to remember that smaller fruits are richer in compounds with biological activity. As an overview, it can be concluded that berry quality was significantly influenced by both genotype and study year.

Even though the appearance (quantified by size, color, taste, and aroma) is still a decisive element for consumers regarding the fruit quality estimation, the results of our study highlighted that small blueberries accumulated more soluble solids and presented higher content of polyphenols, lycopene, and β-carotene than big ones.

Our results will strengthen the breeding process in the Research Institute for Fruit Growing Pitesti-Arges, which aims to create new valuable varieties of *V. corymbosum* L. with a significant complex of biologically active compounds, which makes the fruits of this crop a trendy food product. Similar to their genitors, hybrids have the advantage of showing early fruiting. Their berry quality is a genetically determined character. However, the significant variations between the two years of the study indicate the need to continue this research over a longer period, in order to observe the behavior of the plants in the environmental conditions of the Pitesti-Arges area, especially during summers with statistically assured warming trends.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c00466.

Variations in average berry weight, total soluble solids (TSS), and pH of the blueberry fruits of 'Simultan' and 'Duke' cultivars and their progeny hybrids (Table S1); variations in the total content of polyphenols (TPC), flavonoids (TFC), anthocyanins (TAC), and vitamin C in blueberry fruits of 'Simultan' and 'Duke' cultivars and their progeny hybrids (Table S2); and variations in the total content of lycopene and β-carotene, and antioxidant activity (AA) of the blueberry fruits of 'Simultan' and 'Duke' cultivars and their progeny hybrids (Table S3) (PDF)

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Author Contributions

L.E.V.: conceptualization, methodology, validation, investigation, writing—review and editing, and supervision; O.H.: conceptualization, software, investigation, resources, and writing—original draft preparation; M.S.: validation, resources, and writing—review and editing; V.T.: software; and R.T.: conceptualization and supervision. All authors have read and agreed to the published version of the manuscript

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported by the project "PROINVENT", Contract no. 62487/03.06.2022 POCU/993/6/13—cod SMIS: 153299 financed by The Human Capital Operational Programme 2014−2020 (POCU), Romania.

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