

## EVALUATION OF SOME BLACKCURRANT CULTIVARS ACCORDING TO FRUIT QUALITY PARAMETERS

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### Abstract

*Ribes nigrum L. (blackcurrant) is widely cultivated across temperate Europe, Russia, New Zealand, parts of Asia, and to a lesser extent, North America. Blackcurrants are a valuable source of bioactive compounds like anthocyanins and polyphenols. The berries are used in the production of juices, nectars, jams and functional food. Cultivars with enhanced levels of anthocyanins are currently requested by juice processors and consumers due to their health benefits. The experiment has been carried out at the Research Institute for Fruit Growing Pitești - Mărăcineni, Romania, using six cultivars with different origins: 'Tiben', 'Ruben', 'Tisel', 'Gagatai', 'Titania', and 'Kalinka'. The following physical parameters were measured: yield, berry weight (BW), diameter, firmness, soluble solids content (SSC) and total sugar content (TSC). Also, a series of biochemical parameters were determined, such as: total polyphenols content (TPC), total flavonoids content (TFC), total tannins content (TTC), total anthocyanins content (TAC), and lycopene and  $\beta$ -carotene contents. All the determined parameters were correlated with the antioxidant activity values.*

**Key words:** blackcurrant, breeding, antioxidant activity, total polyphenol content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC), lycopene content,  $\beta$ -carotene content.

### INTRODUCTION

Blackcurrant (*Ribes nigrum L.*) is a significant fruit crop grown extensively in northern Europe (Griffiths et al., 1999; Brennan et al., 2008) and Russia (Mitchell et al., 2011) with a history of domestication dating back to at least 400 years ago. Poland has been the leading producer of blackcurrants in Europe (Pluta et al., 2012) for many years.

Blackcurrants are small, dark purple fruits that originate from medium-sized woody shrubs (Törrönen et al., 2012; Corrigan et al., 2014). The berries are highly valued for their high vitamin C and anthocyanin content (Szajdek et al., 2008), and are used in large-scale production of fruit juices, nectars, jams and functional food products (Griffiths et al., 1999; Brennan et al., 2008).

Various breeding programs in Romania and other countries have mainly concentrated on enhancing agronomic traits like: yield, pest resistance, and disease resistance (Corrigan et al., 2014). These programmes generate large

numbers of offspring, which are generally screened by the breeders to select candidates that meet the specific industry requirements for flavour typicality (Currie et al., 2013). The phenolic compounds dominantly locate in the berry skin whereas most sugars and organic acids are in the pulp (Sandell et al., 2009). Consequently, the berry pulp has a more intense flavour than the skin because the potentially astringent and bitter compounds are bound by polysaccharides.

The study aims at comparing the physical characteristics and chemical composition of blackcurrants, specifically focusing on: yield, berry weight, shape index, firmness, soluble solids content, total sugar content, total polyphenol content, total flavonoid content, total tannin content, total anthocyanin content, as well as on lycopene and  $\beta$ -carotene contents. The goal of the current study is providing comprehensive information as regards the selection of appropriate blackcurrant genotypes for breeding and fruit production toward a

potential development at the Research Institute for Fruit Growing in Pitești.

## MATERIALS AND METHODS

### Plant material

The experiment was carried out at the Research Institute for Fruit Growing (RIFG) Pitești-Mărăcineni in South of Romania 44°54'12" Northern latitude, and 24°52'18" Eastern longitude, 284 m altitude, over a period of 2 years (2022-2023).

The study included the following genotypes:

- three Polish cultivars: 'Tiben' obtained from crossing 'Titania' with 'Ben Nevis'; 'Tisel' obtained from crossing 'Titania' with itself; 'Ruben' obtained from crossing 'Bieloruskaja Slodkaja' with 'Ben Lomond';
- a Swedish cultivar 'Titania' was developed by Tamas (1984) from crossing 'Altayskaya Desertnaya' with 'Consort' × 'Kajaanin Musta',
- a Lithuanian cultivar: 'Gagatai' obtained from crossing 'Minaj Shmyriov' with 'Ojebyn';
- a Belarusian cultivar: 'Kalinka' with unknown genitors.

### Fruit quality parameters

During the peak fruit harvesting period we recorded various indicators from a sample of 50 fruits, with each measurement and analysis repeated 3 times.

**Fruit production** (g/plant) was calculated by weighing the fruits on each plant at every harvest and summing the yield.

The average **fruit weight** was determined by using the HL-400 digital balance to weigh each fruit. Additionally, the **fruit diameter** was measured using a digital calliper, and the **berry firmness** was assessed non-destructively using a Bareiss HPE II Fff penetrometer for each sample.

Various quality parameters were measured, such as total soluble solids, total sugars content, total polyphenol content, total flavonoid content, total tannin content, total anthocyanin content, as well as lycopene and  $\beta$ -carotene contents.

**Total soluble solid content (TSS)** was measured using a Kruss DR201-95 refractometer, and the findings were expressed as °Brix at 20°C.

**Total sugar content (TSC)** was determined by the methodology suggested by Dubois et al. (1956).

**Total polyphenol content (TPC)** was measured using the method recommended by Matić et al. (2017). This involved the reaction of polyphenols with phosphotungstic acid in an alkaline medium, resulting in the formation of a blue-coloured compound. The newly formed compound has maximum absorption at 760 nm. The results were reported as mg gallic acid equivalent (GAE) per 100 g fresh weight (FW).

**Total flavonoid content (TFC)** was measured using the method proposed by Giosanu et al. (2016). Flavonoids reacted with aluminum chloride to form a yellow-orange compound which exhibited maximum absorption at 510 nm. The findings were reported as mg catechin equivalent (CE)/100 g fresh weight (FW).

To determine the **total anthocyanin content (TAC)**, the pH differential method suggested by Di Stefano and Cravero (1989) was used. According to this method, the total monomeric anthocyanin content is measured by taking into account the reversible structural transformation of the anthocyanin chromophore based on the pH. The absorbance at 520 nm was measured 30 minutes after preparing the blueberry extract samples in pH 0.6 (2% hydrochloric acid) and pH 3.5 (a phosphate buffer with 0.1 M citric acid and 0.2 M disodium phosphate). The results were expressed as cyanidin-3-O-glucoside equivalent (C3GE) per 100 g fresh weight (FW).

**Total tannin contents (TTC)** were measured following the method described by Cosmulescu et al. (2023). The results were reported as mg gallic acid equivalent (GAE) per 100 g fresh weight (FW).

The levels of **lycopene and  $\beta$ -carotene** were determined using the method proposed by Tudor-Radu et al. (2016). Carotenoids were extracted using a mixture of hexane, ethanol and acetone in a 2:1:1 ratio. The results were expressed in milligrams of lycopene or  $\beta$ -carotene per 100 grams of fresh weight (FW), utilizing the molar extinction coefficients of both compounds at 470 and 503 nm (Tudor-Radu et al., 2016).

**The antioxidant activity** was assessed using the DPPH assay method (Vijan et al., 2023). The results were expressed as a percentage of the inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH I%).

### Chemicals and Reagents

All the chemicals and reagents were purchased from Merck, Darmstadt, Germany. They include 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium hydroxide, sodium carbonate, sodium nitrite, disodium phosphate, aluminum chloride, acetone, n-hexane, ethanol, gallic acid, catechin, cyanidin-3-O-glucoside, and Folin-Ciocalteu reagent.

### Statistical Analysis

The analyses were conducted with three repetitions, and the data were presented as mean  $\pm$  standard deviation (SD). Excel 2021 (XLSTAT) was utilized for statistical analysis of the data. One-way analysis of variance (ANOVA), two-way ANOVA and Duncan's multiple range tests were carried out.

## RESULTS AND DISCUSSIONS

The Tables 1-3 show the values for yield, berry weight, diameter, firmness, total soluble solids (TSS), total sugar (TSC), total polyphenols

(TPC), total flavonoids (TFC), total tannins (TTC), total anthocyanins (TAC), lycopene and  $\beta$ -carotene contents, and the antioxidant activity (DPPH%) with indication of the values of mean and standard deviation for six blackcurrant cultivars.

The fruit mass ranged from 0.8 kg for 'Tiben' to 1.78 kg for 'Kalinka' (Table 1), with no significant variation observed among the different cultivars.

The weight of the blackcurrant is a crucial quality factor significantly influenced by the genotype, and also impacted by the growing conditions (Woznicki et al., 2016). The weight of the berries ranged from 0.73 g/fruit for 'Tisel' to 1.55 g/fruit for 'Titania'. A similar finding was reported by Kikas and Libek, 2023 for the Estonian blackcurrant cultivar 'Elmar'.

The fruit size is a key factor that determines the quality of the yield (Ochmian et al., 2014). 'Gagatai' had the largest fruit diameter, a diameter of 13.02 mm, while 'Tisel' had a diameter of 9.93 mm, which was the smallest fruit diameter. The firmness, susceptibility to damage, and the force needed to detach the fruit from the stem are key factors that influence the suitability of different cultivars for mechanical harvesting (Ochmian et al., 2014).

Table 1. Fruit biometric characteristics of blackcurrant genotypes (average 2022-2023)

Genotype	Yield (kg/plant)	Berry weight (g/fruit)	Diameter (mm)	Firmness (N)	TSS ( $^{\circ}$ Brix)
Ruben	1.22 $\pm$ 0.78 <sup>a</sup>	0.84 $\pm$ 0.09 <sup>c</sup>	11.66 $\pm$ 0.60 <sup>b</sup>	26.97 $\pm$ 3.23 <sup>b</sup>	19.13 $\pm$ 2.00 <sup>ab</sup>
Tisel	1.68 $\pm$ 0.89 <sup>a</sup>	0.73 $\pm$ 0.10 <sup>c</sup>	9.93 $\pm$ 0.91 <sup>d</sup>	29.13 $\pm$ 7.27 <sup>bc</sup>	22.93 $\pm$ 2.14 <sup>a</sup>
Kalinka	1.78 $\pm$ 0.31 <sup>a</sup>	0.86 $\pm$ 0.22 <sup>c</sup>	10.38 $\pm$ 0.69 <sup>cd</sup>	30.90 $\pm$ 7.04 <sup>b</sup>	21.23 $\pm$ 4.48 <sup>a</sup>
Gagatai	1.24 $\pm$ 0.90 <sup>a</sup>	1.29 $\pm$ 0.12 <sup>ab</sup>	13.02 $\pm$ 0.48 <sup>a</sup>	46.53 $\pm$ 4.75 <sup>a</sup>	15.23 $\pm$ 1.46 <sup>b</sup>
Titania	1.62 $\pm$ 0.13 <sup>a</sup>	1.55 $\pm$ 0.35 <sup>a</sup>	11.08 $\pm$ 0.28 <sup>bc</sup>	37.97 $\pm$ 5.75 <sup>ab</sup>	18.53 $\pm$ 1.78 <sup>ab</sup>
Tiben	0.80 $\pm$ 0.09 <sup>a</sup>	1.07 $\pm$ 0.19 <sup>bc</sup>	11.53 $\pm$ 0.10 <sup>b</sup>	36.40 $\pm$ 5.30 <sup>ab</sup>	16.03 $\pm$ 2.10 <sup>b</sup>

Note: <sup>a</sup>Different letters between cultivars denote significant differences (Duncan test.  $p < 0.05$ ).

Different letters between susceptible and resistant cultivars denote significant differences (LSD test.  $P < 0.05$ ).

The berry firmness oscillated between 26.97 N for 'Ruben' and 46.53 N for 'Gagatai'.

Over the two years, the highest TSS concentration was found in the fruits of 'Tisel' (22.93 $^{\circ}$ Brix), and the lowest in the fruits of 'Gagatai' (15.23 $^{\circ}$ Brix).

Other parameters such as: total sugar (TSC), total polyphenols (TPC), total flavonoids (TFC), total tannins (TTC), total anthocyanins (TAC), along with lycopene and  $\beta$ -carotene contents

and the antioxidant activity (DPPH%) are vital in assessing the taste of fruits and their suitability for fresh consumption. Blackcurrants are rich in phytochemicals, thus possessing strong antioxidant, anti-inflammatory, and anti-microbial properties (Tabart et al., 2006; Nour et al., 2014).

The impact of environmental conditions on the concentration of active components in different plants is well-documented. Factors like light,

temperature, water availability, mineral nutrition, grafting, increased atmospheric CO<sub>2</sub>, elevated ozone levels, and agricultural practices directly influence biochemical processes, consequently impacting the production of these secondary metabolites. Additionally, genetic makeup can significantly influence the antioxidant, micro-

nutrient, and phytochemical composition of plants (Tabart et al., 2006; Treutter, 2010; Nour et al., 2014). Total sugars content (TSC) of blackcurrant fruit varied from 33.37 g glucose/100 g for 'Tiben' to 80.53 g glucose/100 g for 'Tisel' (Table 2).

Table 2. Fruit biochemical characteristics of blackcurrant genotypes (average 2022-2023)

Genotype	TSC (g glucose/100 g)	TPC (mg GAE/100 g)	TFC (mg CE/100 g)	TTC (mg GAE/100 g)	TAC (mg C3GE/100 g)
Ruben	41.55±33.88 <sup>a*</sup>	718.03±7.80 <sup>a</sup>	161.8±0.82 <sup>b</sup>	541.08±9.62 <sup>c</sup>	40.98±3.81 <sup>b</sup>
Tisel	80.53±0.25 <sup>a</sup>	727.11±0.10 <sup>a</sup>	164.06±0.65 <sup>b</sup>	530.05±0.04 <sup>d</sup>	36.87±0.11 <sup>bc</sup>
Kalinka	49.34±19.92 <sup>b</sup>	687.18±22.47 <sup>b</sup>	216.08±50.56 <sup>a</sup>	579.68±8.30 <sup>b</sup>	62.45±14.44 <sup>a</sup>
Gagatai	46.78±0.09 <sup>b</sup>	712.92±0.11 <sup>a</sup>	143.48±0.67 <sup>b</sup>	596.36±0.18 <sup>a</sup>	14.73±0.01 <sup>dc</sup>
Titania	55.70±0.07 <sup>b</sup>	493.09±0.12 <sup>d</sup>	80.28±0.40 <sup>c</sup>	389.20±0.10 <sup>f</sup>	9.18±0.18 <sup>c</sup>
Tiben	33.37±0.04 <sup>b</sup>	653.71±0.22 <sup>c</sup>	125.15±0.67 <sup>bc</sup>	440.73±0.04 <sup>e</sup>	23.81±0.15 <sup>cd</sup>

Note: \*Different letters between cultivars denote significant differences (Duncan test.  $p < 0.05$ ).

Different letters between susceptible and resistant cultivars denote significant differences (LSD test.  $P < 0.05$ ).

During the two years of experimentation, the total polyphenols content (TPC) in the fruits of the blackcurrant cultivars ranged from an average of 493.09 mg GAE/100 g for 'Titania' to 727.11 mg GAE/100 g for the 'Tisel'. Similar results for TPC were reported by Kikas et al., 2020 for 37 blackcurrant cultivars of different geographical origin (Belarus, Estonia, Finland, Latvia, Lithuania, Norway, Poland, Russia, Scotland, Sweden, and Ukraine) grown in Estonia.

The flavonoid content (TFC) in the analyzed blackcurrants varied from 80.28 mg CE/100 g for 'Titania' to 216.08 mg CE/100 g for 'Kalinka'. The TFC results obtained in our study fall between the previously reported data by Mikkonen et al. (2001), Laczko-Zöld et al. (2018), and Milić et al. (2022) for blackcurrants and redcurrants.

The total tannin content (TTC) ranged from 389.20 mg GAE/100 g for 'Titania' to 596.36 mg GAE/100 g for 'Gagatai'.

Our results revealed that blackcurrants have high levels of tannins and low levels of flavonoids.

The total anthocyanin content (TAC) in the fruits of the blackcurrant cultivars showed significant differences over the two years of research. The average total anthocyanin content (TAC) was 9.18 mg C3GE/100 g for 'Titania' and 62.45 mg C3GE/100 g for 'Kalinka'. Sellappan et al. (2002) noted significant differences and inconsistencies among cultivars over different periods of time. These variations may be attributed to genetic disparities, harvest maturity, agricultural techniques, as well as to variations in extraction and laboratory procedures (Clark et al., 2002).

As regards the content of carotenoids (Table 3), the lowest level of  $\beta$ -carotene was of 0.20 mg/100 g in 'Tisel', while the highest level was of 0.39 mg/100 g in 'Kalinka'. The lycopene content ranged from 0.003 mg/100 g for 'Titania' to 0.03 mg/100 g for 'Kalinka' and 'Tiben'.

Table 3. Fruit biochemical characteristics of blackcurrant genotypes (average 2022-2023)

Genotype	$\beta$ -carotene (mg/100 g)	Lycopene (mg/100 g)	DDPH %
Ruben	0.24±0.04 <sup>b*</sup>	0.01±0.01 <sup>c</sup>	93.44±1.12 <sup>ab</sup>
Tisel	0.20±0.01 <sup>b</sup>	0.02±0.01 <sup>c</sup>	94.65±0.04 <sup>a</sup>
Kalinka	0.39±0.05 <sup>a</sup>	0.03±0.01 <sup>a</sup>	91.78±2.14 <sup>b</sup>
Gagatai	0.34±0.01 <sup>a</sup>	0.02±0.01 <sup>bc</sup>	95.52±0.05 <sup>a</sup>
Titania	0.22±0.01 <sup>b</sup>	0.003±0.01 <sup>d</sup>	95.41±0.05 <sup>a</sup>
Tiben	0.25±0.01 <sup>b</sup>	0.03±0.01 <sup>ab</sup>	94.64±0.05 <sup>a</sup>

Note: \*Different letters between cultivars denote significant differences (Duncan test.  $p < 0.05$ ).

Different letters between susceptible and resistant cultivars denote significant differences (LSD test.  $P < 0.05$ ).

The antioxidative activity of all the tested berry extracts showed appropriate values, ranging from 91.78% for 'Kalinka' to 95.52% for 'Gagatai'. No significant differences were observed among the six cultivars. Similar values were also reported by Anisimovienė in 2013. In our study, TPC was positively correlated with TTC ( $r=0.834^{**}$ ), TFC ( $r=0.696^{**}$ ), lycopene ( $r=0.578^*$ ), and TAC ( $r=0.524^*$ ) (Table 4). TFC

presented a positive correlation with TAC ( $r=0.931^{**}$ ), TTC ( $r=0.753^{**}$ ), and both carotenoids: lycopene ( $r=0.780^{**}$ ), and  $\beta$ -carotene ( $r=0.704^{**}$ ). In terms of antioxidant activity (DPPH %), there was a significant negative correlation with TFC ( $r=-0.867^{**}$ ), TAC ( $r=-0.931^{**}$ ), both lycopene ( $r=-0.655^{**}$ ) and  $\beta$ -carotene ( $r=-0.629^{**}$ ).

Table 4. Correlation matrix of TSC, TPC, TFC, TTC, TAC,  $\beta$ -carotene, lycopene and DPPH % in blackcurrant fruits

		TSC	TPC	TFC	TTC	TAC	$\beta$ -carotene	lycopene	DPPH %
TSC (g/100 g)	Pearson Correlation	1	-0.047	-0.148	-0.011	-0.136	-0.380	-0.331	0.416
TPC (mg GAE/100g)	Pearson Correlation		1	0.696**	0.834**	0.524*	0.347	0.578*	-0.351
TFC (mg CE/100 g)	Pearson Correlation			1	0.753**	0.931**	0.704**	0.780**	-0.867**
TTC (mg GAE/100 g)	Pearson Correlation				1	0.559*	0.685**	0.520*	-0.422
TAC (mg C3GE/100 g)	Pearson Correlation					1	0.568*	0.684**	-0.931**
$\beta$ -carotene	Pearson Correlation						1	0.709**	-0.629**
lycopene	Pearson Correlation							1	-0.655**

\*\*Correlation is significant at the 0.01 level (2-tailed).

\*Correlation is significant at the 0.05 level (2-tailed).

## CONCLUSIONS

The cultivar significantly influenced the composition of blackcurrant fruits regarding the total phenolic content, in general, and tannins, flavonoids and anthocyanins, in particular. In addition, a significant fluctuation in lycopene content was observed. The antioxidant activity of all the tested blackcurrant extracts showed high values (91.78-95.52%), but no significant differences among the six cultivars.

The current study indicated that 100 g of fresh blackcurrant from 'Ruben', 'Tisel', 'Kalinka', 'Gagatai', 'Titania' and 'Tiben' contained 33.37-80.53 g of total sugars, 493.09-727.11 mg GAE of polyphenolic compounds, 389.20-596.36 mg GAE of tannins, 80.28-216.08 mg CE of flavonoids, 9.18-62.45 mg C3GE of monomeric anthocyanins, 0.003-0.03 mg of lycopene, and 0.20-0.39 mg of  $\beta$ -carotene. These results indicate that blackcurrants have high level of tannins, medium level of

flavonoids, low levels of anthocyanins and especially of carotenoids. Presented data confirmed the usefulness of *Ribes nigrum* L. species as a rich source of bioactive phenolic compounds with the potential to be used in food and pharmaceutical industries.

Regarding all the studied cultivars, 'Tisel' stood out with the highest values for weight/plant, TSS, TSC and TPC. Also, 'Kalinka' stood out with the highest values for TAC and carotenoids. The interest in breeding programs will increase due to their potential as sources of genetic variability.

However, further research is needed to determine the other antioxidant compounds from the blackcurrant fruits.

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