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CONTENT OF BIOLOGICALLY ACTIVE COMPOUNDS IN THE LEAVES OF 'WILLAMETTE' AND 'MEEKER' RASPBERRY CULTIVARS

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Abstract

The present study observed the content of biologically active compounds in raspberry leaves in different phenophases (full blossoming, fruit harvesting and after fruit harvesting) and two stages of agrotechnics (0.50 m, 0.30 m). The study was conducted during the period 2019-2020 in a collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture in Troyan with two introduced raspberry cultivars 'Willamette' and 'Meeker'. The field experiment is based at intra-row spacings between plants (0.50 m and 0.30 m) and inter-row spacings of 3.00 m. The content of biologically active compounds in the leaves was found during the phenophases: full blossoming, fruit harvesting and after fruit harvesting. The results show that the highest content of the studied indicators chlorophyll "a", chlorophyll "b" and β-carotene in the leaf samples is the cultivar Willamette, with a variant of 0.50 m, respectively (2.56 mg/g FW, 1.62 mg/g FW, 1.44 mg/g FW), from 2019 in the phenophase of full blossoming of plants. In the Meeker cultivar, the highest content of chlorophyll "a" and β-carotene was found from the variant of 0.50 m in 2019 in the phenophase of full blossoming (1.80 mg/g FW, 1.10 mg/g FW) and chlorophyll "b" from the same variant after fruit harvest for 2020-1.04 mg/g FW.

Key words: *raspberries, cultivars, agricultural techniques, biologically active compounds in the leaves.*

INTRODUCTION

Red raspberry (*Rubus idaeus* L.) is one of the most common berry crops in the world (Williams, 1960; Kazakov, 2001). The research and consumer interest in it is determined by its attractive appearance, gorgeous aroma and excellent nutritional and healing properties, which have a beneficial effect on human health. Recent studies have shown that the leaves of berry plants, such as strawberries, raspberries, blueberries and blackcurrants, are a potential source of biologically active compounds with strong healing, anti-cancer and anti-inflammatory properties (Oszmiański, et al., 2011; Costea, et al., 2016; Grochowski, 2016; Veljković, 2019). Red raspberry leaves contain biologically active polyphenols that can be used to supplement the daily intake of valuable natural antioxidants (Durgo et al., 2012). The main bioactive compounds in the leaves are pigments (chlorophylls and carotenoids). In living plants, chlorophyll plays an important role as the primary photosynthetic pigment for capturing light energy from the sun. Composed together with carotenoids (additional photosynthetic pigments) in pigment-protein complexes, it exhibits a color specific to each leaf and is even used as an indicator for maturity, quality and freshness of food crops. The beneficial effects of natural pigments on human health have led to increased interest in the study of these substances.
The aim of the present study is to observe the content of biologically active compounds in raspberry leaves in various agrotechnics of planting.

MATERIALS AND METHODS

The experiment was conducted during the period (2018-2020) in a collection plantation

of the Research Institute of Mountain Stockbreeding and Agriculture, Troyan. The objective of the study are the widely distributed raspberry cultivars, such as 'Willamette' and 'Meeker', characterized by high fruitfulness. The area is maintained in black fallow in the intra-row space and has naturally grassed inter-row spacing. Fertilizing was performed in the intra-row area to achieve optimal values of the individual nutrients for the raspberry crop. The plants were grown under irrigated conditions with drip irrigation. The experimental variants are:

I var. - planting at 0.50 m in the intra-row area;

II var. - planting at 0.30 m in the intra-row area;

In both variants the inter-row spacing is 3.00 m. The content of chlorophyll "a", chlorophyll "b" and ß-carotene was determined spectrophotometrically after extraction of the pigments with an organic solvent. Leaf samples were taken during the phenophases of full blossoming, fruit harvesting and after fruit harvesting.

Cut the fresh leaves of the plant -100 g. The sample is ground in a mortar with a little quartz sand and 1-2 drops of acetone. With the help of 10 ml of acetone in 2-3 portions, the mop is washed from the mortar and transferred to a test tube, where it stays for 5 minutes. The resulting green extract was filtered using a funnel and filter paper. Dilute the resulting extract 5 times by adding with in a test tube (Tsvetkova et al., 2017).

The methodology for studying plant resources in fruit plants was used to report the indicators (Nedev et al., 1979). The data processing was performed by the methods of two-factor analysis of variance, correlation and regression analysis (Lidanski, 1988), using the software product.

RESULTS AND DISCUSSIONS

Carotenoids and chlorophylls (lipid-soluble pigments) are natural pigments responsible for yellow, orange, red and green colous, widely distributed in various plant organs (leaves, blossoms, stems). Chlorophylls are pigments capable of capturing light energy from sunlight and producing carbohydrates. They are responsible for the process of photosynthesis and green hues in plants. Among the several types of chlorophyll pigments, chlorophyll a and b are the most common pigments that are mainly involved in photosynthesis. Carotenoids are chemical compounds that reflect yellow, orange and red colors. They can be used as an indicator of maturity, quality and freshness of food crops (Limantara et al., 2015).

In 2019, in the phenophase of full blossoming, higher content of chlorophyll "a", chlorophyll "b" and β-carotene were reported in the leaves of both variants of 'Willamette' (Table 1).

In both genotypes studied, the pigments had higher values at longer planting distances. Variation coefficient was high with 20.57% (chlorophyll "a") and 28.09% (chlorophyll "b"). Statistical evidence between genotypes was reported only in terms of chlorophyll "a" content, and the differences between the variants are unproven.

The content of chlorophyll "a" and chlorophyll "b" during the period of fruit harvesting was less than the blossoming period. It was in the range from 1.00 to 1.69 mg/g FW (chlorophyll "a") and from 0.48 to 0.78 mg/g FW (chlorophyll "b"). In 'Willamette', the highest (in the second variant) and the lowest values of the two pigments (in the first variant) of planting were reported. The differences in chlorophyll values were minimal in both agricultural techniques used for 'Meeker'. During the fruit harvesting period, the coefficient of variation of chlorophyll "a" and "b" pigments was high at values above 20%. There is no mathematical proof between the variants and genotypes of both cultivars regarding the presence of chlorophyll.

The content of ß-carotene during fruit harvesting was in the range 0.63 - 0.83 mg/g FW, values lower than the blossoming period. The first versions of "Willamette"

(0.81 mg/g FW) and "Meeker" (0.83 mg/g FW) have a higher content.

During the next phenophase after fruit harvesting, the tendency to decrease the amount of the studied indicators continues, as again in the leaves of "Willamette" higher values of chlorophyll "a", "b" and carotene were reported compared to "Meeker". Variation coefficient was in the range 22.64-34.96%. Statistically, differences between genotypes were proven (P<0.05), and between variants were insignificant.

Table 1. Biologically active compounds in the leaves of raspberry genotypes in 2019

Cultivars/indicators	Chlorophyll "a" (mg/g FW), $X \pm S_x$	Chlorophyll "b" (mg/g FW), $X \pm S_x$	ß-carotene (mg/g FW), $X \pm S_x$
Blossoming period			
Willamette-0.50 m	2.56 ± 0.02	1.62 ± 0.14	1.44 ± 0.04
Willamette-0.30 m	2.21 ± 0.12	1.33 ± 0.19	1.24 ± 0.05
Meeker-0.50 m	1.80 ± 0.25	1.03 ± 0.27	1.10 ± 0.13
Meeker-0.30 m	1.62 ± 0.35	0.85 ± 0.30	0.96 ± 0.18
VC %	20.57	28.09	17.34
Minimum	1.62	0.85	0.96
Maximum	2.56	1.62	1.44
Proving the differences (P) among variants	n.s	n.s	n.s
Proving the differences (P) among genotypes	P<0.05	n.s	n.s
Fruit harvesting period			
Willamette-0.50 m	1.00 ± 0.10	0.48 ± 0.10	0.81 ± 0.02
Willamette-0.30 m	1.69 ± 0.07	0.78 ± 0.09	0.63 ± 0.07
Meeker-0.50 m	1.39 ± 0.02	0.68 ± 0.01	0.83 ± 0.02
Meeker-0.30 m	1.09 ± 0.14	0.60 ± 0.04	0.68 ± 0.04
Minimum	1.00	0.48	0.63
Maximum	1.69	0.78	0.83
VC %	24.46	20.16	13.12
Proving the differences (P) among variants	n.s	n.s	P<0.05
Proving the differences (P) among genotypes	n.s	n.s	n.s
After fruit harvesting			
Willamette-0.50 m	1.10 ± 0.24	0.56 ± 0.07	0.65 ± 0.12
Willamette-0.30 m	1.27 ± 0.07	0.58 ± 0.00	0.68 ± 0.03
Meeker-0.50 m	0.61 ± 0.02	0.35 ± 0.01	0.44 ± 0.03
Meeker-0.30 m	0.68 ± 0.15	0.40 ± 0.06	0.46 ± 0.06
Minimum	0.61	0.35	0.44
Maximum	1.27	0.58	0.68
VC %	34.96	24.42	22.64
Proving the differences (P) among variants	n.s	n.s	n.s
Proving the differences (P) among genotypes	P<0.05	P<0.05	P<0.05

In 2020, in the phenophase of full blossoming, the content of chlorophyll “a” in the leaves was in the range 1.56-1.88 mg/g FW (Table 2). Higher values were reported for longer planting distances, 1.88 mg/g FW for Willamette (0.50 m) and 1.72 mg/g FW for ‘Meeker’ (0.50 m), respectively. Statistical differences between variants and genotypes are unproven. The second variant of ‘Meeker’ had the highest variant of chlorophyll "b" (0.81 mg/g FW). Moreover, both variants of planting of ‘Willamette’ contain the same amount (0.75 mg/g FW). The presence of carotene in the leaves of the first variants was 0.96 mg/g FW (‘Willamette’) and 0.94 mg/g FW (‘Meeker’), respectively. Variation coefficient is low for the three studied indicators. Statistical differences between variants and genotypes are unproven.

Table 2. Biologically active compounds in leaves of raspberry genotypes in 2020

Cultivars/indicators	Chlorophil "a" (mg/g FW), $X \pm S_x$	Chlorophil "b" (mg/g FW), $X \pm S_x$	ß-carotene (mg/g FW), $X \pm S_x$
Blossoming period			
Willamette-0.50 m	1.88 ± 0.05	0.75 ± 0.02	0.96 ± 0.00
Willamette-0.30 m	1.66 ± 0.18	0.75 ± 0.06	0.88 ± 0.10
Meeker-0.50 m	1.72 ± 0.00	0.72 ± 0.03	0.94 ± 0.02
Meeker-0.30 m	1.56 ± 0.05	0.81 ± 0.06	0.88 ± 0.02
VC %	7.86	5.24	4.42
Minimum	1.56	0.72	0.88
Maximum	1.88	0.81	0.96
Proving the differences (P) among variants	n.s	n.s	n.s
Proving the differences (P) among genotypes	n.s	n.s	n.s
Fruit harvesting period			
Willamette-0.50 m	1.59 ± 0.04	0.71 ± 0.05	0.88 ± 0.01
Willamette-0.30 m	1.55 ± 0.11	0.70 ± 0.01	0.86 ± 0.04
Meeker-0.50 m	1.28 ± 0.14	0.56 ± 0.06	0.77 ± 0.08
Meeker-0.30 m	1.12 ± 0.10	0.52 ± 0.05	0.64 ± 0.07
Minimum	1.12	0.52	0.64
Maximum	1.59	0.71	0.88
VC %	16.13	15.25	13.76
Proving the differences (P) among variants	n.s	n.s	n.s
Proving the differences (P) among genotypes	P<0.05	P<0.05	n.s
After fruit harvesting			
Willamette-0.50 m	1.13 ± 0.11	0.94 ± 0.07	0.68 ± 0.05
Willamette-0.30 m	1.01 ± 0.25	0.73 ± 0.17	0.65 ± 0.18
Meeker-0.50 m	1.37 ± 0.23	1.04 ± 0.02	0.81 ± 0.12
Meeker-0.30 m	1.05 ± 0.27	0.92 ± 0.14	0.64 ± 0.11
Minimum	1.01	0.73	0.64
Maximum	1.37	1.04	0.81
VC %	34.96	24.42	22.64
Proving the differences (P) among variants	n.s	n.s	n.s
Proving the differences (P) among genotypes	n.s	P<0.01	n.s

During the phenophase fruit harvesting (2020) the highest content was found in 'Willamette' in both variants, as the longer planting distances had a predominance, although minimal. Variation coefficient is mean. Mathematically, differences were demonstrated only between genotypes ($P < 0.05$).

After fruit harvesting, the highest levels of chlorophyll "a", chlorophyll "b" and ß-carotene were reported in the first variant of 'Meeker' (1.37 mg/g FW; 1.04 mg/g FW; 0.81 mg/g FW). Although the content of biologically active compounds in the leaves of 'Willamette' is lower than in 'Meeker', higher genotypes were found in both genotypes at longer planting distances. Variation coefficient is high with values, respectively for chlorophyll "a" - 34.96%; chlorophyll "b" - 24.42%; ß-carotene - 22.64%. Statistically, the differences between variants and genotypes with respect to the biologically active compounds studied were insignificant. The results of scientific research show that the content of chlorophylls and carotenoids also depends on the technology of growing raspberries (conventional and organic). In this regard, a study by Ponder et al. (2019) showed that raspberry leaves from conventional farming contained significantly more ß-carotene (1.22 mg 100 g^{-1} FW and 0.46 mg 100 g^{-1} FW), chlorophyll "a" (8.09 mg 100 g^{-1} FW and 3.96 mg 100 g^{-1} FW), chlorophyll "b (2.43 mg 100 g^{-1} FW and 1.79 mg 100 g^{-1} FW), than did the leaves from organic farming.

A correlation analysis was conducted to follow the relationships between the biologically active compounds on average for the three phenophases over the years.

In the first experimental year in the (0.50 m) variant of the variety ‘Willamette’, there is a very strong positive correlation between chlorophyll "a" and "b", as well as between chlorophyll and carotene (r = 0.94; r = 0.92; r = 0.91) (Table 3). In variant (0.30 m) a very strong correlation between chlorophyll (r = 0.96) and a strong correlation between chlorophyll "a" and "b" with carotene (r = 0.83; r = 0.88) was again reported.

Table 3. Correlation dependences between biologically active compounds of ‘Willamette’ for the period (2019-2020)

Indicators	Chlorophyll “a” (mg/g FW)	Chlorophyll “b” (mg/g FW)	ß-carotene (mg/g FW)
2019	**0.50 m**		
Chlorophyll “a”	1		
Chlorophyll “b”	0.94	1	
ß-carotene (mg/g)	0.92	0.91	1
0.30 m			
Chlorophyll “a”	1		
Chlorophyll “b”	0.96	1	
ß-carotene (mg/g)	0.83	0.88	1
2020	**0.50 m**		
Chlorophyll “a”	1		
Chlorophyll “b”	-0.83	1	
ß-carotene (mg/g)	0.99	-0.88	1
0.30 m			
Chlorophyll “a”	1		
Chlorophyll “b”	0.49	1	
ß-carotene (mg/g)	0.96	0.69	1

In the second year of the experiment with the two planting variants of ‘Willamette’, a very strong positive correlation was observed only between chlorophyll “a” and ß-carotene (r = 0.99; r = 0.96). In the 0.50 m variant between chlorophyll "a" and "b" and between chlorophyll "b" and carotene, a strong but negative dependence was reported (r = -0.83; r = -0.88).

In the first experimental year, in both planting variants of ‘Meeker’, there was a very strong positive correlation dependence between all studied indicators with values ranging from r = 0.93 to r = 0.99 (Table 4). In the second year, in both variants of “Meeker”, a high dependence was found only between chlorophyll “a” and ß-carotene (r = 0.98; r = 0.99).

Table 4. Correlation dependences between biologically active compounds of ‘Meeker’ for the period (2019-2020)

Indicators	Chlorophyll “a” (mg/g FW)	Chlorophyll “b” (mg/g FW)	ß-carotene (mg/g FW)
2019	**0.50 m**		
Chlorophyll “a”	1		
Chlorophyll “b”	0.96	1	
ß-carotene (mg/g)	0.99	0.96	1
0.30 m			
Chlorophyll “a”	1		
Chlorophyll “b”	0.93	1	
ß-carotene (mg/g)	0.96	0.96	1
2020	**0.50 m**		
Chlorophyll “a”	1		
Chlorophyll “b”	0.07	1	
ß-carotene (mg/g)	0.98	0.12	1
0.30 m			
Chlorophyll “a”	1		
Chlorophyll “b”	0.36	1	
ß-carotene (mg/g)	0.99	0.42	1

CONCLUSIONS

In both experimental years, the highest content of biologically active compounds in the leaves of raspberry cultivars ‘Willamette’ and ‘Meeker’ were registered at the phenophase of full blossoming.

In 2019, in both studied genotypes the pigments had higher values at longer planting distances at the phenophase of full blossoming.

During the phenophase of full blossoming and after fruit harvesting in 2019, a higher content of pigments in ‘Willamette’ fruit was reported, and during the phenophase harvesting of ‘Meeker’.

The correlation analyzes of the biologically active compounds chlorophyll "a", chlorophyll "b" and ß-carotene in the leaf samples determine the following dependences at over r <0.7:

In the first experimental year in both variants in the genotypes, a strong correlation between the three studied indicators was reported.

In 2020, only the first variant of ‘Willamette’ was found. In the case of the 0.30 m planting variant, a strong pattern was observed only between chlorophyll “a” and ß- carotene.

In 2019, in the phenophase of full blossoming, in both studied genotypes, the pigments had higher values at longer planting distances.

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