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Betalain Content and Morphological Characteristics of Table Beet Accessions: Their Interplay with Abiotic Factors

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Abstract: Table beet (*Beta vulgaris* L.) is a source of the natural red-colored food dye (E162), highly demanded for the broad spectrum of its biological activity. The relevance of this study is dictated by the lack of knowledge about the dynamics of changes in the crop's betalain content during the growing season, which impedes identifying the optimal timing of harvesting in order to obtain the dye. This paper presents the results of research into betacyanins (BC) and betaxanthins (BX), separately in the peel and flesh of roots, in 15 differently colored table beet accessions from the collection of the N.I. Vavilov Institute (VIR). There was no statistically significant accumulation of betalains in beets during the growing season. The pigment's significant fluctuations associated with abiotic environmental factors were shown. The ratio of BC/BX in red-colored accessions was measured: 2.65 in the peel and 2.9 in the flesh. Strong positive relationships were found between BC and BX in the peel (r = 0.97) and flesh (r = 0.79) of red-colored biotypes, which stably persisted throughout the growing season. The beetroot peel was more sensitive to temperature changes, in contrast to the flesh. The negative effect of a temperature increase on betalains in red-colored beetroots intensified on the second or third day. The pigment composition of the flesh was less susceptible to the negative impact of increased temperatures, but reacted negatively to rainfall, becoming more expressed on the second or third day. A conclusion was made about the morphotype with high betalain content. Recommended cultivars are mid-ripening, with rounded and medium-sized roots, a large number of narrow leaf blades, and short and thin petioles.

Keywords: *Beta vulgaris* L.; betalains; betacyanins; betaxanthins; natural food coloring; correlations; morphological features; abiotic factors

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1. Introduction

Table beet (*Beta vulgaris* L. ssp. *vulgaris* var. *conditiva* Alef.) is an important source of the natural red-colored food dye betanin (E162). This crop is characterized by high root yield (50–60 t/ha), environmental plasticity, and pigment yield [1–3], preventing other sources of betanin, such as prickly pear fruits (*Opuntia vulgaris* Mill.) or red-colored forms of amaranth (*Amaranthus* L.), to compete with beet [4–6]. The dye betanin (betanidin 5-O-β-glucoside) isolated from beet occupies a dominant place (70–95%) in the group of betalains [7].

Betalains are nitrogen-containing plant pigments, characteristic of the *Caryophyllales* order representatives. They are water-soluble, tyrosine-derived pigments, forming two groups: red-violet betacyanins (BC) and yellow-orange betaxanthins (BX). BCs are mainly represented by betanin and isobetanin, while BXs are dominated by vulgaxanthins (I and II) [2].

The function of betalains in beetroots is not yet clear. The BC biosynthesis is known to be induced under the influence of UV radiation, high salinity, low temperature [8–10], mechanical damage or inoculation with pathogenic fungi [11,12]. The same stressors lead to the formation of reactive oxygen species (ROS), indicating that BCs are antioxidants that alleviate oxidative stress when plant cells have been damaged. It means that the accumulation of betalains (mainly BCs) is an adaptive survival strategy. We assume that it is the protective function of table beet betalains that allows the root to safely endure unfavorable environmental conditions in the soil before the onset of the next stage of ontogenesis—the growth of the seed bush—and at the same time, not to die from pathogenic soil microflora.

Betalains, in recent decades, has attracted the close attention of scientists not only because of the market orientation towards using natural food colors but also due to a wide range of their biological activities, including anti-inflammatory, hepatoprotective, antimicrobial and anticarcinogenic properties [13–18]. The pigment composition ranks the beet among the ten vegetables with the highest antioxidant activity [19–22]. The use of the beetroot dye combines its coloring effect with therapeutic properties, which is extremely important for human health improvement.

Choosing the time of table beet harvesting for food purposes mainly depends on the root size. Such practice is not suitable when this crop is grown to produce the dye. The external color of the beetroot does not change during its ontogenesis, and it is difficult to visually identify its readiness for harvesting. A group of Polish researchers studied the dynamics of changes in betalains during the growing season [23,24]. When the beet material was taken for analysis after 2 weeks, it was shown that the best harvesting time for high betalain content levels falls on the eighth and eleventh week of cultivation. A publication by a team of Spanish authors also showed that among the three stages of the growing season, the maximum amount of betalains was observed in the second stage [25]. These data indicate the absence of linear accumulation dynamics in the accumulation of pigments. Our previous studies did not observe the cumulative effect of betanin. At the same time, significant fluctuations in the pigment content associated with weather characteristics were described [26]. It is known, however, that higher temperatures during the storage of beetroots and a study of beet solutions under heating led to BC and BX decomposition [27,28]. Herbach and her team reported that, as temperature increased, BCs were cleaved to the form of betalamic acid and neobetacyanins [29]. The main dehydrogenation pathways associated with the decarboxylation of betanin/isobetanin and neobetanin were also described previously [30,31]. The present study was aimed at determining the limiting effect of environmental temperature on betalains during the growing season.

The biosynthesis of betalain pigments in table beet plants is a dynamic process that changes during ontogenesis and depends on the specific genotype, abiotic and edaphic factors, and agricultural practices [32]. In the works that studied the effect of beet raw material processing on the pigment content, the technique included mandatory peeling of the root [33,34]; this was certainly necessary when focusing on making juices for children and dietary food. However, the dye production technique does not imply the removal of the peel: the blanching of the whole root is used [35]. Therefore, close attention in this work was paid to identifying the trends in the BC and BX dynamics, separately in the peel and flesh of the beetroot, which, as far as we know, has not been done before.

The objective of this study was to trace the dynamic changes in the content of betacyanins and betaxanthins during the growing season, separately in the peel and flesh of table beetroots with different colors, and to identify the limiting effect of environmental factors.

2. Materials and Methods

2.1. Materials and Agricultural Details

The target material for this study was a set of 15 table beet accessions with different root colors (*Beta vulgaris* L. var. *conditiva* Alef.) from the collection held by the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR). Field experiments were performed following the unified guidelines [36] in 2021 at Pushkin and Pavlovsk Laboratories of VIR (latitude: 59°42′41.7″ N; longitude: 30°25′47.1″ E). Sowing was done on 31 May in a randomized row scheme, with three replications. Planting was carried out in six-meter rows following the 70 cm × 8 cm pattern. Seeds were sown manually to a depth of 2.5–3 cm. All cultivars were studied against a natural background, without fertilization or plant protection from pests and diseases. Morphometric parameters were measured once a week, from 13 July to 14 September.

2.2. Quantification of Betalains

Beetroots were sampled for the analysis twice a week, from 13 July through 14 September. All measurements were made within 3 h after the removal of roots from the soil. The pigment content was analyzed separately in the peel (cut with a knife, 1–2 mm) and flesh of ten roots per each accession, preliminarily washed and dried. The root filtrate was studied using spectrometry on a Shimadzu UV-1800 double-beam spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The phosphate buffer solution with pH 6.5 was used. The concentration of betalains was measured by applying the previously described technique [37] according to the following formula:

$$\text{betacyanins/betaxanthins} = \frac{\text{A} \times \text{DF} \times \text{NW} \times 1000}{\varepsilon \times \text{i}},$$

where:

A is the optical density, nm (for betacyanins: $A = A_{536nm} - A_{650nm}$, and for betaxanthins: $A = A_{485nm} - A_{650nm)}$; DF is the dilution factor; MW is the molecular weight (550 g/mol for betacyanins, and 339 g/mol for betaxanthins); ε is the molar extinction coefficient in $L \times mol^{-1} \times cm^{-1}$ (60,000 for betacyanins, and 48,000 for betaxanthins); i is the path length, cm. All measurements were performed in triplicate. The measurement at the wavelength of 650 nm was used to correct for impurities.

2.3. Statistical Analyses

All statistical analyses ($p < 0.05$) were performed using the Statistica v.8.0 for Windows software package, Excel software and R system. Descriptive statistics (mean, standard deviation, standard error of the mean, and coefficient of variation) were calculated for all parameters. Data means were compared using the one-way analysis of variance (ANOVA). The PCA and the correlogram were implemented in R. The values of the Pearson correlation coefficient at $r < 0.3$ were considered as weak, $0.3 > r > 0.5$ as moderate, $0.5 > r > 0.7$ as noticeable, $0.7 > r > 0.9$ as strong, and $r > 0.9$ as very strong.

3. Results and Discussion

The weather characteristics of the growing season in 2021 differed significantly from the mean long-term data (Figure 1). In May, with its low temperatures, the long-term rainfall data were exceeded by 14%, which resulted in the late sowing of beets (2 weeks later). June and July were extremely dry (−82% and −91% of the long-term mean, respectively). At the same time, the air temperature was 36–25% higher than the long-term mean level. Despite the extremely unfavorable conditions during these periods, the growth of leaf biomass proceeded quite well, pointing to the high drought resistance of the crop in these phases of ontogenesis. Due to the lack of soil moisture, the active phase of root growth started after 27 July, which was 2 weeks later than the long-term records.

A representative set of table beet samples was selected for testing through the screening of the VIR collection [38] from 15 accessions, classified according to the color of the root and its intensity into 4 groups: maroon (1), red (2), yellow (3), and white (4).

Despite the recorded unfavorable weather conditions in the first half of the summer, the mean yield on 14 September was 23.9 kg/10m^2, i.e., at the level of the long-term means for this region [39]. The weight of one beetroot varied significantly ($p < 0.05$) and averaged 137.3 g (Table 1). The highest yield was observed in the accessions with an elongated (cylindrical) root shape as well as in cvs. 'Bordo odnosemyannaya' (k-3151, Russia) and 'Boldor' (k-3880, The Netherlands).

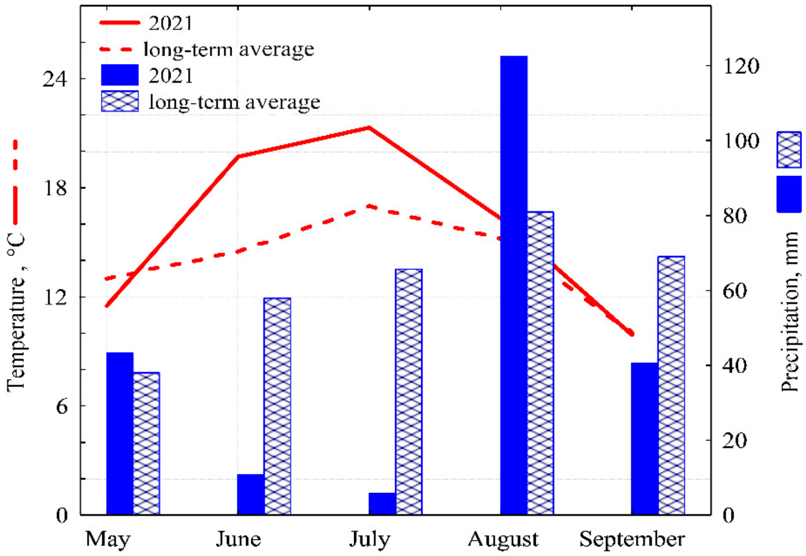


Figure 1. Mean monthly temperatures and rainfall in the experimental field during the 2021 growing season and long-term means for 1744–2020 (Pushkin and Pavlovsk Laboratories of VIR, Town of Pushkin, St. Petersburg, Russia). Source: Department of Automated Information Systems of Plant Genetic Resources, Hydrometeorological Station of VIR.

Table 1. Productivity characteristics of table beet accessions. Duration of the growing season: 105 days. Results are presented as mean values with a standard deviation (Mean ± SD), the coefficient of variation (%CV), mean values with standard error (Mean ± SE), and the least significant difference (LSD_{05}).

VIR Catalogue No.	Accession Name	Origin	Photosynthetic Surface Area, cm^2	Root Color Group	Yield, kg/10 m^2	Root Weight, g **	
						Mean ± SE	% CV
2011	Betterowe Potagere ***	Algeria	2070.0	1	33.6	263.2 ± 100.9	96.3
3677	Detroit rubinovy	Russia	700.10	1	24.5	136.0 ± 36.2	65.2
3151	Bordo odnosemyannaya	Russia	868.47	1	31.2	121.5 ± 15.1	30.4
3698	Russkaya odnosemyannaya	Russia	873.14	1	28.9	158.5 ± 36.4	56.2
3206	Joijaj	Lithuania	561.46	1	16.5	91.8 ± 11.2	27.2
3207	Red Cloud	Netherlands	693.58	1	24.3	135.0 ± 19.6	35.5
3204	Rubidius ***	Hungary	884.00	1	23.5	130.7 ± 34.7	65.1
3209	Mona	Russia	1685.81	1	22.3	123.8 ± 25.3	45.6
1967	Kubanskaya borshchevaya	Russia	1041.25	2	15.0	83.2 ± 11.7	14.0
3201	Long Canner ***	Botswana	1292.00	2	30.9	171.6 ± 69.6	90.7
3105	Dvusemyannaya 4-53	Ukraine	1143.89	2	10.5	47.3 ± 6.8	25.2
3880	Boldor	Netherlands	840.13	3	31.4	174.3 ± 25.8	36.3
-	L1 yellow *	Russia	707.69	3	25.5	141.5 ± 31.7	54.9
3881	Avalanch	Netherlands	785.94	4	24.6	141.0 ± 27.3	22.2
-	L1 white *	Russia	555.21	4	25.2	140.0 ± 46.5	61.4
Mean ± SD			980.2 ± 422.2		23.9 ± 7.0	137.3 ± 12.4	
LSD_{05}			238.7		4.6	29.9	

* VIR breeding material; ** values were obtained on 10 beetroots of each accession; *** cylindrical root shape.

The content of betalains in the accessions was measured separately in the peel and flesh of their roots (Table 2). It was shown that in group 1, the amount of betalains in the peel averaged 1246.2 mg/L, and in the flesh, 819.1 mg/L, i.e., pigments in the peel were 1.5 times higher than those in the flesh. The accessions from group 2 demonstrated a similar ratio (1.5), with the total betalain content averaging 984.6 mg/L in the peel and 638.5 mg/L in the flesh. These data appeared consistent with previous results [26,40]. The highest content of betalains was observed in the accessions 'Bordo odnosemyannaya' (k-3151, Russia), 'Red Cloud' (k-3207, The Netherlands) and 'Detroit rubinovy' (k-3677, Russia).

Table 2. Betalain content in the tested table beet accessions (mg/L FM). Duration of the growing season: 105 days. Results are presented as mean values with a standard error (Mean ± SE). Values were obtained from 10 roots of each accession.

Root Color Groups			Peel Pigments			Flesh Pigments		
			BC *	BX **	Σ	BC	BX	Σ
1		Mean ± SE Min ÷ Max %CV	889.0 ± 58.7 647.2 ÷ 1077.1 18.7	357.1 ± 23.5 270.8 ÷ 435.9 23.5	1246.2 ± 81.4 941.1 ÷ 1498.6 18.4	593.1 ± 62.7 386.7 ÷ 824.7 29.1	225.9 ± 27.3 143.1 ÷ 354.1 32.2	819.1 ± 89.1 529.7 ÷ 1178.8 30.8
2		Mean ± SE Min ÷ Max %CV	665.9 ± 163.8 [a] 384.1 ÷ 951.5 42.6	318.7 ± 33.4 178.9 ÷ 385.1 18.1	984.6 ± 195.2 [a] 563.0 ÷ 1336.6 34.3	450.5 ± 143.6 [a] 241.8 ÷ 725.6 55.2	188.0 ± 48.9 [a] 128.1 ÷ 184.9 45.1	638.5 ± 189.2 [a] 392.7 ÷ 1010.5 51.3
3		Mean ± SE Min ÷ Max %CV	83,8 ± 25.1 58.7 ÷ 108.8 42.3	256.0 ± 65.1 191.0 ÷ 321.1 35.9	339.8 ± 90.1 249.7 ÷ 430.0 37.5	20,1 ± 10.7 9.4 ÷ 30.8 75.4	33.3 ± 8.0 25.3 ÷ 41.3 34.0	53.4 ± 18.3 34.7 ÷ 72.1 49.65
4		Mean	not detected					

* BC—betacyanins; ** BX—betaxanthins; values between accessions with the superscript letter "a" were significantly different ($p < 0.05$).

Betalains (mainly BXs) in the yellow-colored accessions were also synthesized mainly in the peel, and to a much lesser extent in the flesh: the BX/BC ratio was 6.4. In those with white roots, the pigment composition was not identified on the spectrophotometer.

The ratio of betacyanins to betaxanthins (BC/BX) in all red-colored accessions averaged 2.65 in the peel and 2.9 in the flesh (Figure 2A,B). This indicator varied slightly during the growing season: the coefficient of variation (%CV), depending on the cultivar, was 14.2–19.1%. Any conjugation between the ratio and the intensity of the red color was not recorded. For example, the accessions with the lightest (k-1967) and maroon colors (k-3105) had similar BC/BX parameters in the peel. Similar results were shown in earlier studies [24,41]. The ratio ranged from 1.9 to 2.8, depending on the year of cultivation, and averaged at 2.3. Similar results were obtained when studying three table beet cultivars grown in Slovenia: the BC/BX ratio was about 2.1 [42]. However, in a study performed by other authors, the BC/BX ratio was close to 1.8 [43,44]. Supposedly, this indicator is a fairly stable value that characterizes a specific genotype and, possibly, is associated with the site of growing. There is a possibility that it is a threshold BC level, after which further biosynthesis is restrained. The limit to the accumulation of betalains in ontogenesis was also shown by Montes-Lora et al. [25]. A noteworthy fact is that no versions of ratios close to 1:1 were found.

The yellow-colored accessions demonstrated the predomination of BXs, while BCs were present in smaller amounts. The BX/BC ratio was 2.86 in the peel and 2.13 in the root flesh (Figure 2C).

There are very few works concerning betalains differentiated according to the root areas. An interesting study was implemented by a group of Polish authors [7], who identified the betalain profile in the peel and six rings of the beetroot flesh. The BC/BX ratio in this study changed from the peel to the center of the root in descending order: 2.8–2.5–2.49–2.1–2.0–1.9–1.6. At the same time, BXs decreased from the peel to the center by 39% and BCs by 66%. There is a conclusion that the decrease in the amount of betalains in the roots of red-colored biotypes occurred from the peel to the root's center due to a more active decrease in BCs.

The best adaptation of a crop to a new environment depends on a large stock of genetic polymorphisms [45]. Table beet is polymorphic in the main morphological features, such as the shape of the leaf rosette, leaf color, root shape, length and color of the petiole, the diameter of the root neck, and color of the root flesh, which allows it to adapt to changing environments.

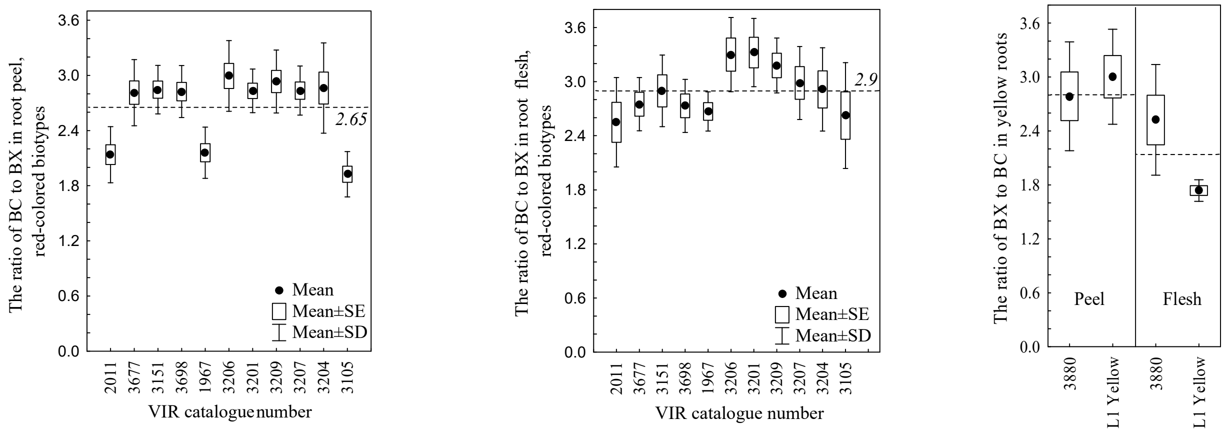


Figure 2. The BC/BX ratio in the peel (**A**) and flesh (**B**) of red-colored table beet accessions during the growing season. The BX/BC ratio in yellow-colored accessions (**C**).

The principal component analysis (PCA) was used to trace the patterns of variability between morphological features and the content of betalains in the peel and flesh of beetroots and to identify the main components (Figure 3). The first component, PC1 (33.9% of the total variance), encompassed the weight of the whole plant with tops, the weight of the root, its parameters, leaf length and width, leaf area, and the entire photosynthetic surface. The aggregate set of these indicators can be interpreted as the yield factors for table beet, including its basic components. The highest factor load (>0.90) was observed for the following morphological characters: the plant weight with tops, photosynthetic surface area and the number of adult leaves. The second component, PC2 (19.4% of the total variance), was associated with the pigment composition of table beet: the BC and BX content in the peel and flesh. The highest factor load was recorded for betalains in the root flesh. A clear splitting among the tested accessions with different root colors was observed in relation to the PC2 component of the analysis.

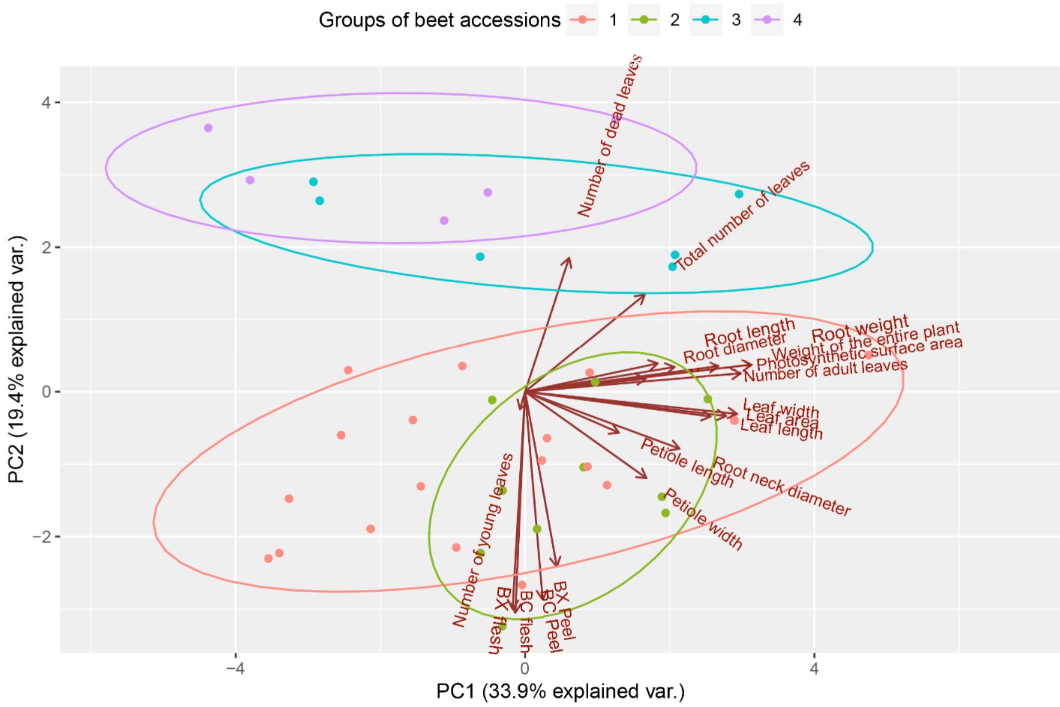


Figure 3. PCA plot showing beetroot color groupings.

Metabolic processes in a plant are largely associated with anatomical and morphological features, such as the structure of the leaf apparatus and the storage organ. The plant habitus depends on the combination of such leaf biomass characteristics, such as the shape and size of the leaf, number of leaves, and the thickness and length of the petiole. They build up the morphotype of the aboveground part of table beet plants and attest to their photosynthetic activity. The number of young and dead leaves on a plant is one of the markers used to form an idea of the table beet earliness level [39,46]. A correlation analysis among the groups of red-, yellow-, and white-colored table beet biotypes was performed to disclose the nuances of phenotypic differences in betalain-synthesizing accessions. Morphological parameters were measured once a week, from 12 July to 14 September, on 10 roots of each cultivar ($n = 100$). The measurements showed that during the growing season, the vector of correlations did not change: negative relationships varied only within the negative range, and positive within the positive one, confirming the general tendency.

There were strong positive relationships between BC and BX in both the peel (r = 0.97, $p < 0.001$) and the flesh (r = 0.79, $p < 0.05$) among all red-colored accessions (Figure 4). The yellow-colored biotypes also showed strong positive correlations between betalains in the peel (r = 0.97, $p < 0.001$) and notable ones in the flesh (r = 0.53, $p < 0.05$) (Figure 5). In the year of testing, a noticeable positive correlation of betalains appeared with the weight of the root, its parameters and the number of leaves on the plant, which had not previously been observed by us or other authors [24,26,38,47]. It had earlier been shown that beetroots with a diameter of 4.6–6.4 cm contained more betalains than larger ones, with a diameter of 9 cm. Our results were an exception to the rule that did not change the known pattern and were associated with an abnormally hot and dry summer in 2022. Despite the absence of a direct negative correlation between betalains and yield parameters, its indirect signs were found. The difference in the red-colored biotypes was a strong negative dependence of BC and BX on the width of the leaf blade (r = −0.85–0.88, $p < 0.01$) and a significant dependence on the length and width of the petiole. The leaf width, in its turn, negatively correlated with the root weight (r = −0.61), root length (r = −0.77, $p < 0.05$) and diameter (r = −0.57), with the total number of leaves per plant (r = −0.73, $p < 0.05$), and the number of dead leaves (r = −0.86, $p < 0.01$). It is important to note, however, that these relationships were typical only for the red-colored biotypes and were not observed in the accessions from groups 3 and 4 (Figures 5 and 6). Similar indirect evidence was observed regarding the length and width of the leaf petiole. Thus, biotypes with narrow leaves and thin and short petioles may point to an increased content of pigments in red-colored beetroots. At the same time, the number of leaves should be 12–14, providing a photosynthetic surface area of 960–990 cm^2 (Table 1) and a root neck of at least 2.5 cm (Figure 7).

Judging from the results of the correlation analysis, when choosing a cultivar for dye extraction, preference should be given to mid-ripening cultivars, because biotypes with flat and cylindrical roots are not prone to significant pigment accumulation, which is explained by their earliness level [48]. Flat beet forms belonging to the Egyptian flat cultivar type are, as a rule, early, while cylindrical ones are late-ripening. It should be mentioned that this general tendency has exceptions [49].

Numerous external and internal factors affect the accumulation and stability of betalains: natural determinants, such as the temperature, rainfall, soil composition, number of sunny days, spectral light composition and the presence/absence of oxygen, enzymes, metal cations and nitrogen, and the degree of glycosylation and acylation [4,15,50–53]. In most cases, betalains are extracted at the end of the growing season from mature beetroots and other sources of betalains: pitahaya (*Hylocereus polyrhizus* Weber) and prickly pear (*Opuntia vulgaris* Mill.) fruits, were studied. It was interesting to find out, however, the effect of mean daily air temperatures and rainfall on table beet betalains during the growing season in order to determine the optimal harvesting time to obtain dyes.

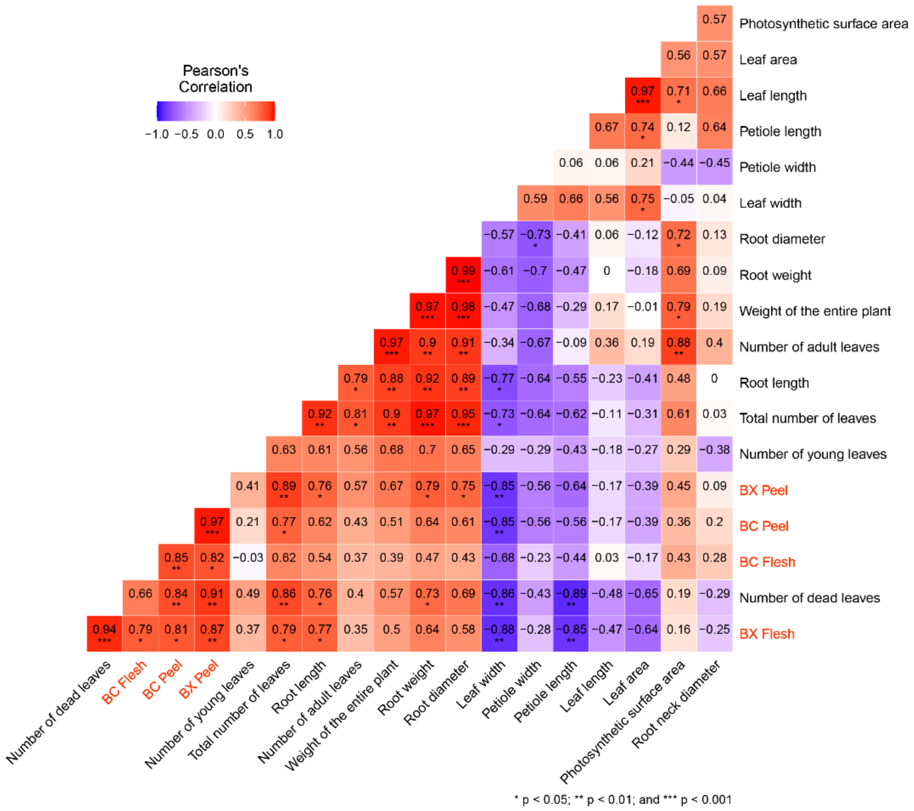


Figure 4. Correlogram showing the correlations between different morphological characters and table beet betalains in the red-colored groups 1 and 2 (n = 100 replications). The numbers inside each square show the Pearson R correlation values.

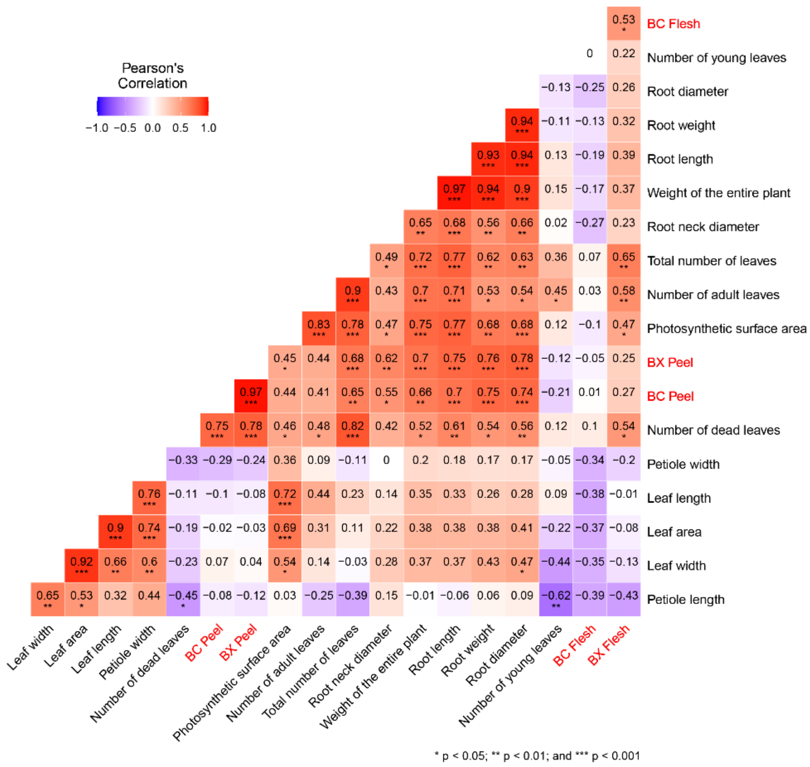


Figure 5. Correlogram showing the correlations between different morphological characters and table beet betalains in the yellow-colored group 3 (n = 100 replications). The numbers inside each square show the Pearson R correlation values.

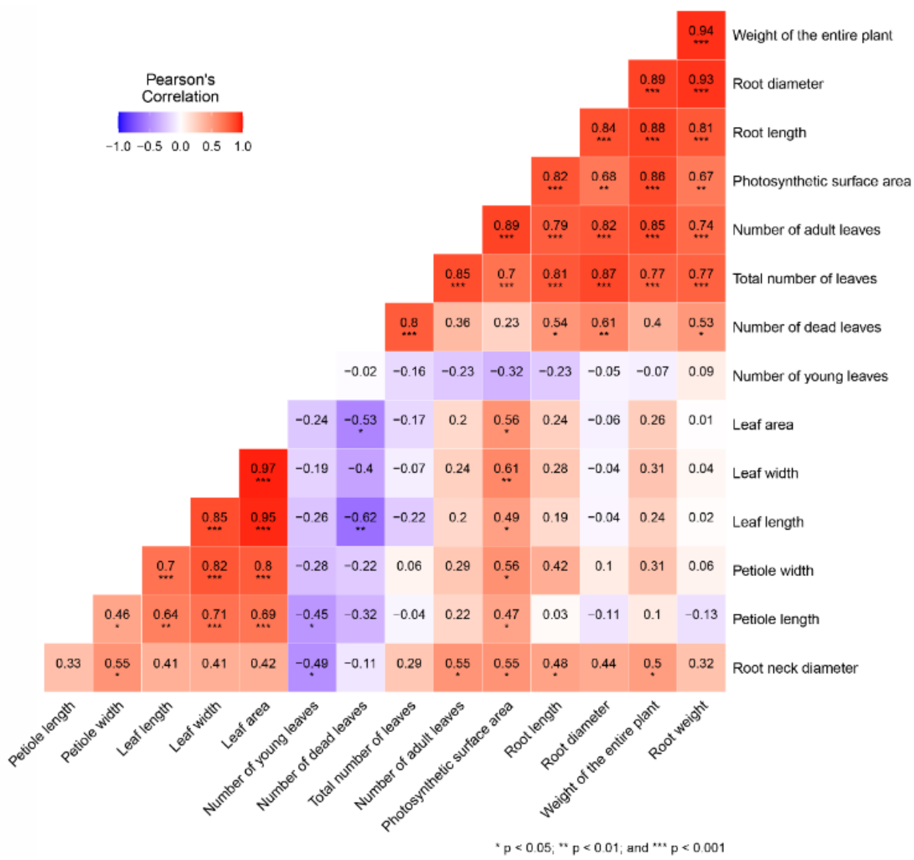


Figure 6. Correlogram showing the correlations between different morphological characters in the white-colored beetroot group 4 (n = 100 replications). The numbers inside each square show the Pearson R correlation values.

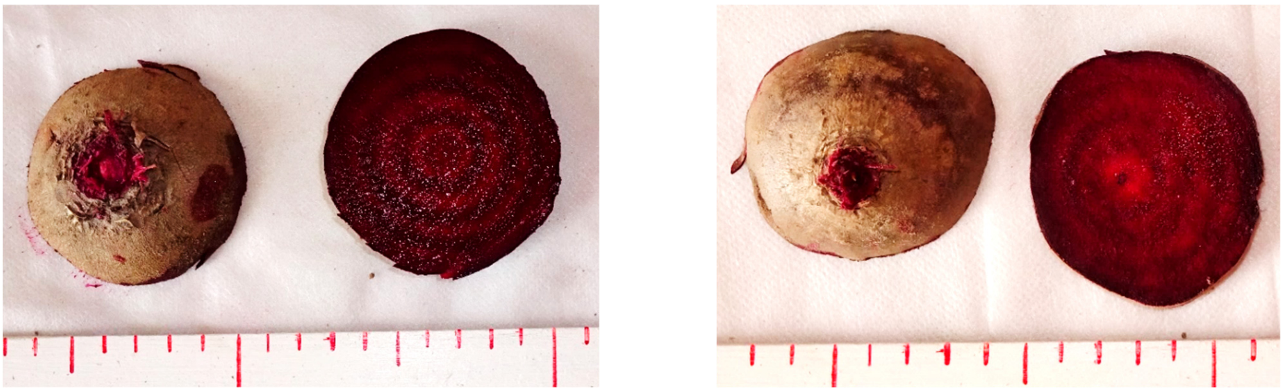
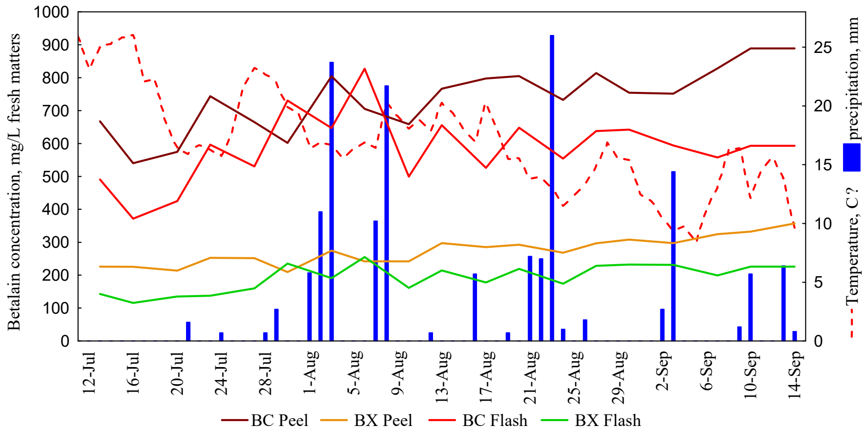
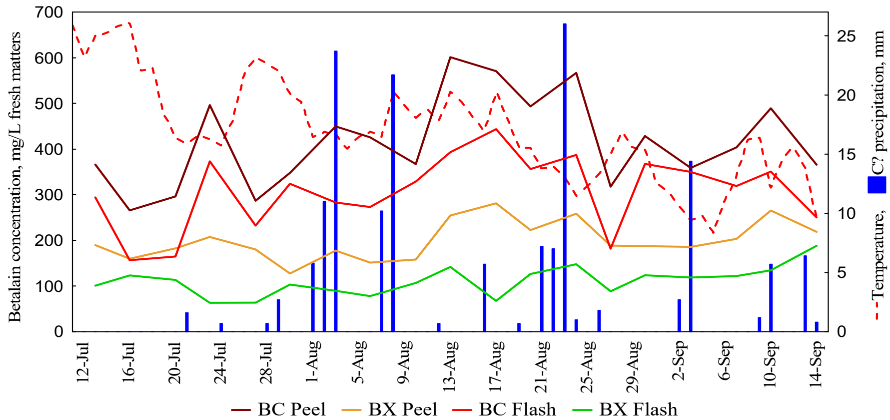


Figure 7. The size of the table beetroot neck and the intensity of the color ((**left**): 3 cm; (**right**): 1.5 cm).

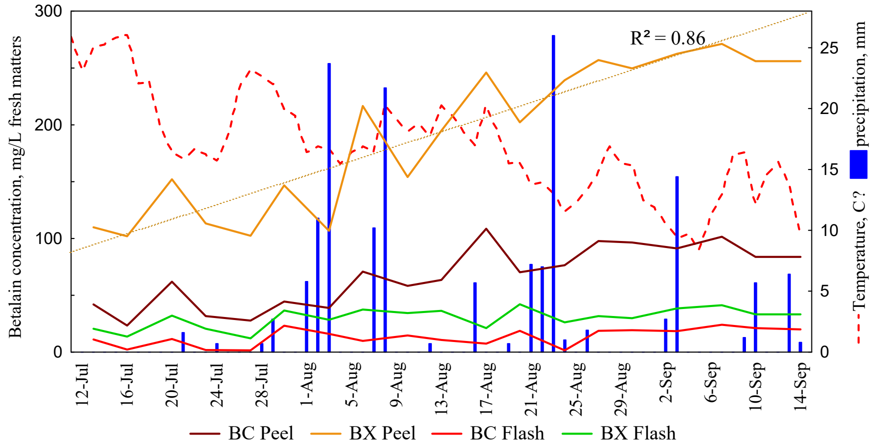
The dynamics curve showed significant ($p < 0.05$) variations in BC and BX content during the growing season in all color groups (Figure 8A–C). There were weak BC accumulation dynamics in the root peel within group 1 (Figure 8A), which we had not observed in our previous studies [26,38]. Probably, this was caused by the weather conditions in the summer of 2021, when the abnormally long hot and dry period in the first half of the summer held back the biosynthesis of pigments and the growth of beetroots. No significant cumulative effect of BC accumulation in the root flesh was found in all accessions from groups 1 and 2 ($p < 0.05$). The curve of BC accumulation in the peel and flesh had a similar pattern and a negative correlation with air temperature (r = −0.62–0.72). Moreover, BCs in the peel showed greater sensitivity to temperature changes: it can be explained by its direct contact with the environment, which was noted by Sawicki et al. [7]. The negative effect of elevated temperatures on betalains was previously highlighted by a number of researchers [23,24,52,54,55].



(**A**)



(**B**)



(**C**)

Figure 8. Dynamic changes in weather characteristics and betalain content in table beet accessions. (**A**) Group 1, (**B**) group 2, (**C**) group 3 (R^2 means the coefficient of determination).

In contrast to the groups with dominating red coloration, the accessions from group 3 showed a significant positive change in BXs in the peel (R^2 = 0.86, $p < 0.05$), confirming the observations by Stintzing and Carle [56] regarding the presence of accumulation in yellow-colored biotypes (Figure 8C).

The response of metabolic processes to the impact of environmental factors does not occur instantly in plants; it can manifest itself after several hours or days. In order to clarify the response of accessions with different dominant colors to air temperature and rainfall, a correlation analysis was applied in dynamics with a shift in environmental parameters for up to 4 days. Figures 9 and 10 demonstrate that the interrelation between betalains both in the peel and flesh and an increase in temperature is in the negative area. The peel is more sensitive to abiotic factors: in all accessions, the negative response of BCs and BXs in the peel was stronger, increasing on the next day. The BC response in the peel of red-colored accessions persisted until the third day, gradually weakening (Figure 9). As for BCs in the flesh, the effect increased gradually for two days but showed a much weaker correlation with the temperature (r = −0.39–0.5). It can be concluded that the pigment composition in the beetroot flesh is less susceptible to the negative effects of higher temperatures. In general, there were no significant differences in the BC and BX responses among all tested accessions from groups 1, 2 or 3: their reactions to temperature were identical.

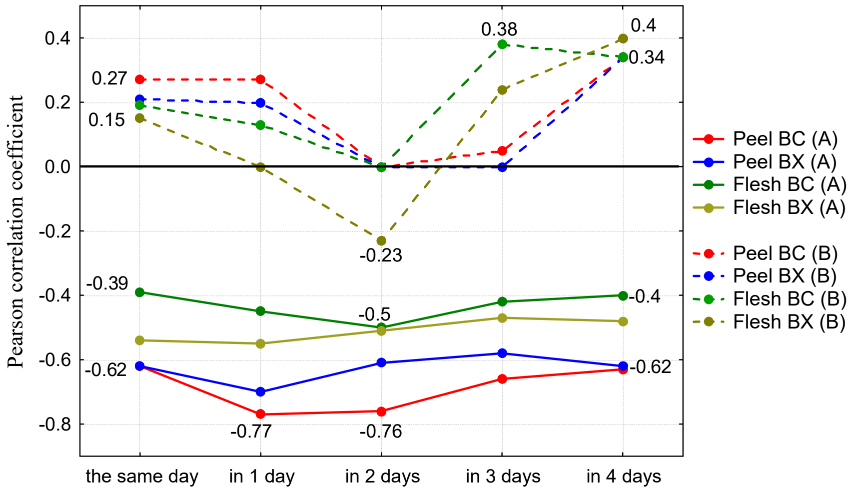


Figure 9. Dynamics of the interplay between betalains in red-colored table beet accessions (groups 1 and 2) and abiotic factors (A—the correlation with air temperature; B—the correlation with precipitation).

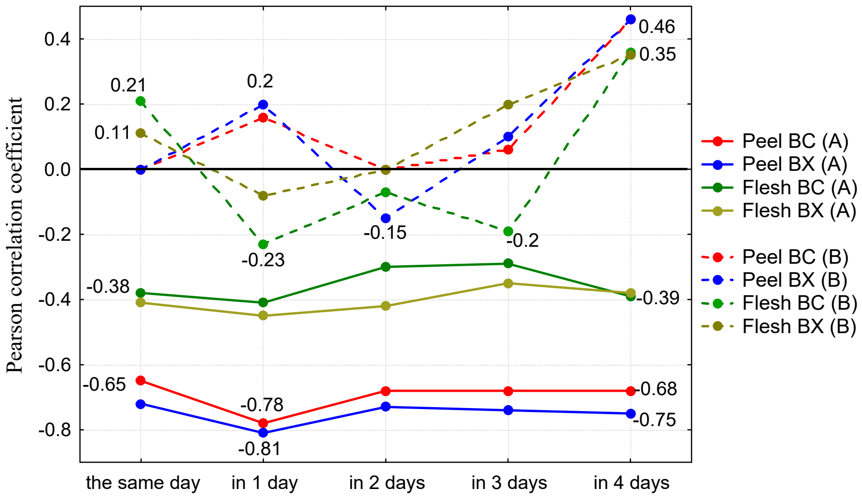


Figure 10. Dynamics of the interplay between betalains in yellow-colored table beet accessions (group 3) and abiotic factors (A—the correlation with air temperature; B—the correlation with rainfall).

The period after a rainfall is favorable for the active growth of table beet. Overall, a rather weak or moderate effect of precipitation on the pigment composition was shown for all accessions: the correlation ranged from −0.23 to +0.38 for four days. Groups 1 and 2 demonstrated an abrupt weakening of positive correlations on the second day. Previously, it was shown that indirect relationships of betalains with the weight, diameter and length of the root in red-colored biotypes were negative. Therefore, the root weight growth after a rainfall led to a negative betalain response. The response was somewhat different in group 3: the pigments in the root flesh, especially BCs, reacted negatively to rainfall on the next day (Figure 10). With this, the BC and BX response in the peel, where the yellow-colored accessions contained the main amount of pigments with a predominance of BXs, did not significantly manifest itself. Moderate positive correlations with rainfall were recorded for all groups after 4 days, as the growth of the root subsided (r = 0.34–0.46).

4. Conclusions

This study is the first analysis of the detailed dynamic changes in betacyanins and betaxanthins contained in the table beet peel and flesh during the growing season. In the active growth period of beetroots, no significant betalain accumulation was observed in red-colored table beet accessions, with the exception of a slight tendency towards BC accumulation in the peel. It was shown that the pigments in the peel of red-colored biotypes were 1.5 times higher than the pigments in the flesh. As far as the yellow-colored accessions were concerned, BXs were synthesized mainly in the peel and had a cumulative effect throughout the growing season.

The BC/BX ratio in all red-colored accessions averaged 2.65 in the peel and 2.9 in the flesh. Betaxanthins prevailed in the BX/BC ratio in the peel (2.86) and in the flesh (2.13) of yellow-colored accessions. The results of the correlation analysis showed that red-colored accessions exhibited strong positive relationships between BCs and BXs in the peel (r = 0.97, $p < 0.001$) and in the flesh (r = 0.79, $p < 0.05$), which remained stable throughout the growing season. A conclusion was made about a morphotype with a high content of betalains.

Furthermore, significant variations in the content of BCs and BXs associated with abiotic environmental factors were shown. The peel of the beetroot was more sensitive to temperature changes than the flesh. The negative effect of higher environmental temperatures on betalains increased on the second or third day in all accessions. The pigment composition in the flesh was less susceptible to the negative impact of higher temperatures, but responded negatively to rainfall, manifesting this tendency on the second or third day. There were no significant differences between the BC and BX responses among all tested accessions from groups 1, 2 and 3: their reactions to the temperature factor were identical.

The identified relationships are important for selecting a table beet cultivar to extract the E162 dye: preference should be given to mid-ripening cultivars, with round and medium-sized roots, a large number of narrow leaf blades, and short and thin petioles. The observed correlations with weather conditions should be taken into account when choosing a specific harvesting date.

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