https://doi.org/10.15407/frg2021.01.55
UDC 634.737:54.062

DYNAMICS OF PHOTOSYNTHETIC PIGMENTS CONTENT IN LEAVES OF *VACCINIUM CORYMBOSUM* L. DURING VEGETATION

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The quantitative indices of plastid photosynthetic pigments in the leaves of deciduous shrubbery *Vaccinium corymbosum* L., which grow in the same climatic conditions in the south of Belarus, were studied in order to quantify and compare seasonal changes in the efficiency of photosynthesis. For each variety of the studied plants *V. corymbosum* L., certain differences were noted on the content of photosynthetic pigments in regard to the ontogenetic stage, in particular to the natural light period, which manifested in the relative content of chlorophylls *a* and *b*, the ratio between them, the relative content of carotenoids to chlorophylls. The data obtained on the absolute and relative plastid pigments content, as well as on the ratio between them, indicate the presence of varietal specificity in the plant reaction on reducing the length of daylight hours.

Key words: *Vaccinium corymbosum* L., chlorophyll, carotenoids, ontogenesis.

Plants can often play a role as indicators of changes in ecosystem components that can be observed under the influence of natural and anthropogenic factors. Morphological changes in plants (leaf color, parameters of leaf blade, shoot growth, etc.) reflect biochemical processes. Various plant species have a certain pigments qantity and ratio, which change both in the process of phenological development and depending on the growing conditions (mainly climatic) [1]. Chlorophylls and carotenoids are important pigments in the assimilation tissues of higher plants and are responsible for the color change from dark green to yellow. Moreover, they play an important role in photosynthesis by capturing light energy, which is converted into chemical bond energy [2, 3]. In the process of photosynthesis, chlorophylls convert the radiant energy of sunlight into the chemical energy of organic carbon compounds in the cell [4]. Carotenoids are a class of natural fat-soluble pigments found primarily in plants, algae and photosynthetic bacteria, where they also play an important role in the process of photosynthesis. In some non-photosynthetic bacteria, yeast, and fungi, they can perform a protective function against damage by light and oxygen [5]. Animals cannot synthesize carotenoids, but they include carotenoids in

Citation: Deeva A.M., Lazaruk G.V., Pavlovskii N.B., Spiridovich A.V., Reshetnikov V.N. Dynamics of photosynthetic pigments content in leaves of Vaccinium corymbosum L. during vegetation. Fiziol. rast. genet., 2021, 53, No. 1, pp. 55-62. https://doi.org/10.15407/frg2021.01.55

diet. For animals, carotenoids serve as antioxidants and can be a source of vitamin A [6, 7]. Moreover, carotenoids perform important functions in plant reproduction due to their role in attracting pollinators and in seed dispersal [8]. The reserve of plastid pigments in plant leaves is one of the important conditions for the proper functioning of the photosynthetic apparatus. It is assumed that the quantitative content of pigments in photosynthetic organs can be successfully used as a characteristic index that determines the potential photosynthetic productivity of a species, annual atmospheric carbon sink, and as an index of the species response to changes in environmental conditions [9, 10, 11].

Plants of the genus *Vaccinium* L. are widespread both in the wild and as introduced taxa on different continents. Among the representatives of this genus, the North American species the highbush blueberry (*V. corymbosum* L.), which was introduced to Belarus in the early 1980s, is of great interest [12, 13]. By 2017, more than 1,000 hectares of industrial plantings of highbush blueberries were created in Belarus [14]. Research carried out in the world and in Belarus on the biochemical composition of the fruits and leaves of this culture has shown that it is a valuable food raw material and a potential source of a number of biologically active substances [15, 16].

The aim of our work was to investigate the dynamics of photosynthetic pigments content in leaves of *Vaccinium corymbosum* L. during vegetation in order to quantify and compare seasonal changes in the efficiency of photosynthesis.

Materials and methods

The experimental material was taken at the collection plantations of the branch Laboratory for the Introduction and Technology of Unconventional Berry Plants of the Central Botanical Garden of the National Academy of Sciences of Belarus, located in the Gantsevichi district of the Brest region (N 52° 44', E 26° 22'). The object of research was 9 varieties of highbush blueberries with different harvest ripening periods.

Variety	Ripening period
Bluecrop	Medium ripening variety
Bluegold	Early ripening variety
Brigitta Blue	Late ripening variety
Denise Blue	Medium ripening variety
Duke	Early ripening variety
Hardyblue	Medium ripening variety
Nelson	Late ripening variety
Northland	Early ripening variety
Elizabeth	Late ripening variety

Blueberry plantations were created by 2-year-old self-rooted saplings in 1999. The planting pattern is 2.0 × 1.5 m. The soil on the plot is mineral with a pH (H_2O) 4.5, underlain by loose, uneven-grained sand. The near-trunk strip of plantations was mulched with sawdust with a layer of 10 cm and a width of 1 m. The row spacing was kept in natural turf. Blueberry plantations are equipped with irrigation, which was used during dry periods.

Biochemical studies of plant samples were carried out in the Department of Biotechnology of the Central Botanical Garden (Minsk). The pigment content was determined in freshly harvested leaves. The plant material for research was taken from 10—15 plants, from the middle part of the shoots by a random sample. The sampling was carried out three times during the growing season at the passage of the main stages of plant phenological development: flowering (May), fruiting (August), and after changing the color of the leaves in autumn (October).

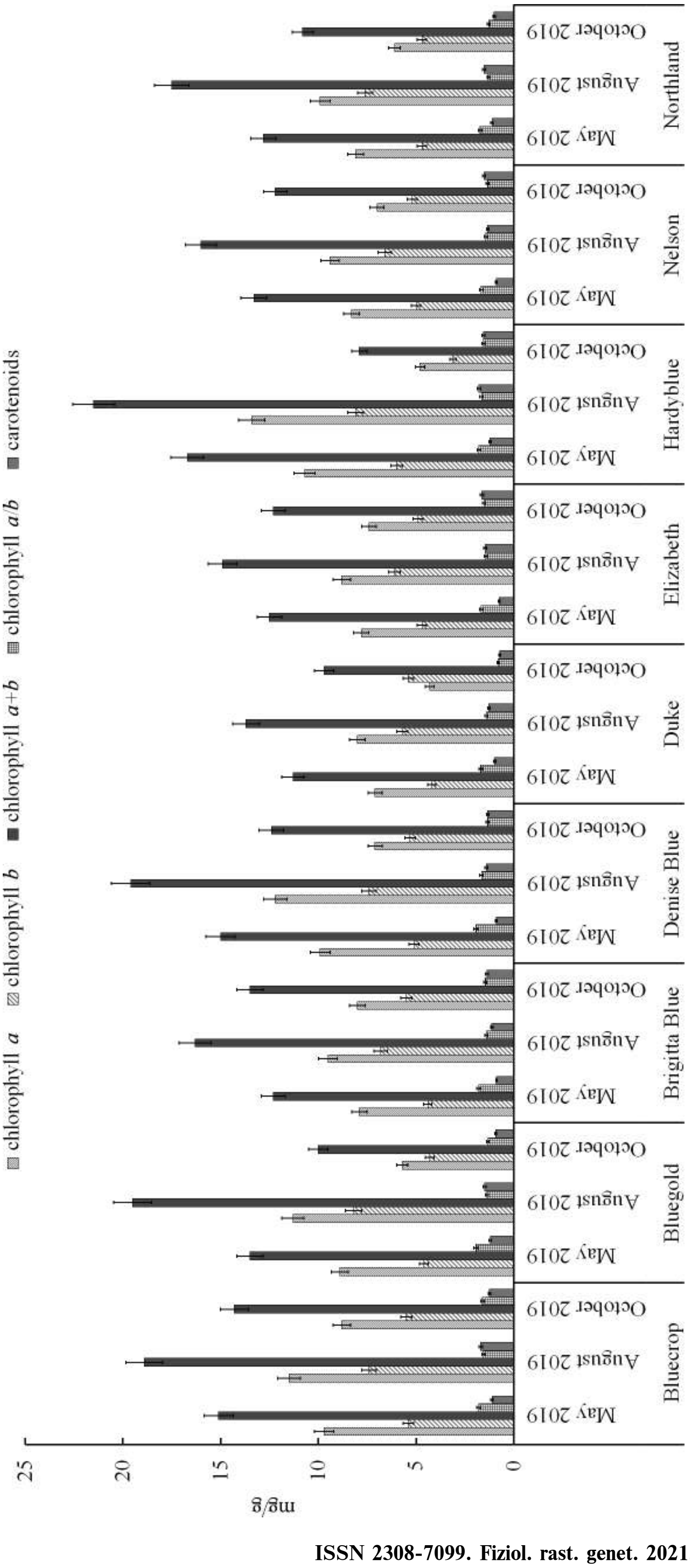
The quantitative content of photosynthetic pigments was determined spectrophotometrically [17]. For the analysis, a weighed portion of the leaves of about 5 grams was taken and grounded using a homogenizer with the addition of a small amount of acetone in the presence of calcium carbonate. The prepared plant mass was transferred to a Schott glass filter and filtered into a Bunsen flask. Extraction of pigments was carried out in small portions of acetone until the filtrate was discolored. The total volume of the filtrate was fixed and the optical density of the extract was measured, specifying the maximum absorption of chlorophylls *a* and *b,* if necessary. The concentration of chlorophylls and carotenoids in the obtained extracts was calculated using the formulas [17].

All measurements were carried out in four replicates. The reliability of the experimental data obtained was confirmed by the methods of biological statistics [18]. For the statistical processing of the results and the creation of figure, the Microsoft Excel software packages were used. The hypotheses about the equality of the two means were tested using the Student t-test. The condition necessary for using the Student t-test was the normality of the distribution and the equality of general variances, which was estimated by the value of the F-test [18]. The results obtained were considered reliable at a given level of significance $p < 0.05$.

Results and discussion

Analysis of meteorological data for 2018—2019 showed that in the Gantsevichi region there were favorable climatic conditions for the growth and development of highbush blueberry plants [19]. A gradual decrease in air temperature in the autumn period contributed to the ripening and hardening of late shoots (from the beginning of the second decade of November to the end of this month of 2018, a slow decrease in air temperature was noted, from +4 °C to −9 °C), which contributed to the maturation of the top shoots formation and replacement. In the winter period 2018—2019, no sharp temperature jumps were observed, periods of thaw were replaced by a gradual decrease in temperature. Thus, the thermal conditions of the winter period did not have a detrimental effect on blueberry plants. It should be noted that the development of highbush blueberry plants and the passage of stages of phenological development during the growing season took place at the time usual for this culture [20].

It was found that the studied varieties of highbush blueberry differ significantly in the content and dynamics of pigment accumulation in the leaves (Figure). The pigment content per unit dry weight varies from 3.1±0.1 mg/g for Hardyblue to 13.4±0.4 mg/g for Denise Blue. The pigment content in blueberry leaves was also determined by the stage of sea-



Absolute and relative plastid pigments content in leaves of different varieties of *Vaccinium corymbosum* L. depending on the phenophase sonal plant development. The maximum amount of pigments was accumulated by the leaves of the medium-ripening variety Hardyblue in the fruiting stage. It should be noted that for cultivars of early and medium ripening periods, an increase in the level of chlorophylls in the fruiting stage is characteristic by 11—20 % and 23—60 %, respectively, in comparison with these indices in the flowering stage and during the beginning of leaf fall. Decrease in the efficiency of photosynthesis in deciduous shrubs during the period of leaf fall is associated with a weakening of the ability to carry out photosynthesis and growth processes [21].

A high amount of carotenoid pigments also accumulates during the fruiting stage in the leaves of early and medium-ripening varieties. From the analysis of the results obtained, it should be noted that for the late-ripening varieties (Brigitta Blue, Elizabeth and Nelson), the accumulation of the greatest amount of carotenoids was in October. High amount of carotenoids, more than 1.5 mg/g dry weight, noted for mid-season varieties: Bluegold, Northland, Bluecrop, Hardyblue, in August; and for late-maturing Elizabeth and Nelson cultivars, in October. The quantitative content of carotenoids less than 0.75 mg/g dry weight noted for Duke and Elizabeth, which plant material was taken in May.

It is known that the quantitative characteristics of plastid pigments accumulation in plants is a hereditary characteristic. It's believed that the chlorophylls *a* and *b* content in the foliage of shade-loving plant species is higher than that of light-loving plants, and shade-loving plants exhibit the highest photosynthesis rate under moderate light [22]. However, it has been established that the ability to accumulate pigments in the leaves of deciduous plants also depends on the characteristics of the aging processes of the leaf, and the time of stopping its growth in the autumn, in plants with different periods of crop maturation [23].

The results obtained indicate a different plastid pigments biosynthesis intensity in the leaves of highbush blueberry varieties, which differ in periods of fruit ripening. Leaves of early and middle ripening varieties contain chlorophyll *a* more, on average, by 25 %, and chlorophyll *b* — by 50 %. At the same time, the content of carotenoids in the analyzed samples differed insignificantly.

According to the conclusion of the Crimean scientists who performed a similar study of plastid pigments [24], in order to establish statistically significant differences between the compared plant taxa, it is preferable to analyze not only the absolute values of the chlorophylls and carotenoids content in leaves, but their ratios to the sum of pigments. The results of our calculations indicate the genetic stability of the pigment composition ratios in the analyzed blueberry varieties, taking into account seasonal changes (Table 1). Thus, the chlorophyll *a* percentage in the total pigments content ranges from 41 % (Duke) to 58 % (Denise Blue), chlorophyll *b* — from 30 % (Bluegold) to 51 % (Duke), carotenoids — from 5 % (Denise Blue) to 16 % (Hardyblue).

In the seasonal variability of pigments, there is a pattern associated with age-related changes in leaves and phenophases of plant development. Thus, in the leaves of *V. corymbosum* L. varieties Bluegold, Duke, Northland, Bluecrop, Hardyblue, Denise Blue, the minimum amount of

TABLE 1. Chlorophylls and carotenoids percentage in the total pigments content in blueberry leaves, %

Variety	Chlorophyll *a*		Chlorophyll *b*		Carotenoids	
	min	max	min	max	min	max
Bluecrop	55.3	57.4	31.9	36.2	6.6	8.3
Bluegold	50.3	57.7	29.8	39.3	7.2	8.6
Brigitta Blue	51.7	55.5	33.4	38.4	6.7	9.1
Denise Blue	51.7	58.4	30.1	38.6	5.3	9.7
Duke	41.0	54.7	32.3	51.4	6.9	8.4
Elizabeth	51.7	58.4	30.1	38.6	6.4	11.8
Hardyblue	50.8	57.9	32.5	35.0	6.5	16.4
Nelson	53.6	55.0	33.2	37.3	7.2	11.1
Northland	50.4	55.8	32.4	40.4	7.6	8.3

all pigments was observed in October, during the period of leaf dying. In varieties Elizabeth, Nelson and Brigitta Blue, the minimum was observed in May, during the stage of leaf formation and the formation of reproductive organs. During this period, the formation of the green pigments pool in the leaves of all varieties proceeds with a significant excess of chlorophyll *a*, which can be seen from the ratio of chlorophyll *a* and *b* (from 1.7 to 2.0).

The maximum amount of plastid pigments in blueberry leaves was found in samples collected in August during the stage of mass ripening of berries. In blueberry plants during this period, lutein and carotene accumulate most actively among carotenoids [11].

Based on the analysis of variance data (Table 2), the value of the Fisher criterion (F-criterion) of factor 1, which includes information on all varietal gradations of plastid pigments — F_{exp} = 33.3, and F_{tabl} lies in the interval (3.1; +∞). F_{exp} lies in the critical region, therefore, this index affects the content of pigments in highbush blueberry leaves. The sample coefficient of determination for factor 1 is 6.9 %, which also confirms the conclusion about the effect of this factor on the studied values approximately in the amount of the indicated value.

The same with respect to factor 2 — F_{exp} = 392.4, and F_{tabl} = 3.12, the sample coefficient of determination for factor 2 — the time of sampling (ontogenesis stage) is 81.7 %, therefore, the ontogeny stage significantly affects the plastid pigments content (efficiency of photosynthesis) in the leaves of the studied varieties *V. corymbosum* L. And in addition, we deter-

TABLE 2. Results of analysis of variance of the obtained data on pigments content in blueberry leaves

Source of variation	SS	df	MS	F_{exp}	P	F_{tabl}
Samples	62.46	2	31.23	33.26	5.86E-11	3.12
Columns	736.76	2	368.38	392.38	1.90E-39	3.12
Interaction	35.31	4	8.82	9.40	3.57E-06	2.49
Inside	67.596	72	0.93	–	–	–
Total	902.13	80	–	–	–	–

mined the interaction of factors F_{exp} = 9.4, and F_{tabl} = 2.5. Since F_{exp} is included in the interval (2.5; +∞), it means that there is an interaction between the factors.

Thus, evaluation of quantitative indices of plastid photosynthetic pigments in the leaves of 9 highbush blueberry cultivars growing in Belarus showed the presence of varietal characteristics in the content of these substances in regard to the stage of seasonal development, in particular to the natural light period, which manifested in the content of chlorophylls *a* and *b*, the ratio between them and carotenoids. The data obtained on the absolute and relative plastid pigments content, and on the ratio between them indicate the presence of varietal specificity during the growing season.

Ackowledgement. The authors are grateful to PhD in biology T.V. Kurlovich for help in collecting experimental data.

Financial support. The work was carried out within the framework of the project of the Belarusian Foundation for Basic Research Б19М-053 (ГР 20192180) «Accumulation and composition of biologically active compounds of phenolic nature in the leaves in varieties of *Vaccinium corymbosum* L.».

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Received 07.02.2021

ДИНАМІКА ВМІСТУ ФОТОСИНТЕТИЧНИХ ПІГМЕНТІВ У ЛИСТКАХ *VACCINIUM CORYMBOSUM* L. ПІД ЧАС ВЕГЕТАЦІЇ

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Кількісні показники пластидних фотосинтетичних пігментів у листках листяних чагарникових рослин *Vaccinium corymbosum* L., які ростуть в однакових кліматичних умовах на півдні Білорусі, були вивчені з метою кількісної оцінки та порівняння сезонних змін ефективності фотосинтезу. Для кожного сорту досліджуваних рослин *V. corymbosum* L. зафіксовано певні відмінності за вмістом фотосинтетичних пігментів щодо онтогенетичної стадії, зокрема періоду природного освітлення, що проявляється у відносному вмісті хлорофілів *a* і *b*, співвідношенні між ними, відносному вмісті каротиноїдів до хлорофілів. Отримані дані про абсолютний та відносний вміст пластидних пігментів, а також співвідношення між ними вказують на наявність видової специфічності в реакції рослин на зменшення тривалості світлового дня.

Ключові слова: Vaccinium corymbosum L., хлорофіл, каротиноїди, онтогенез.