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Research Article

Variation and Correlation of Photosynthetic Pigments in Spring Wheat Cultivars under Controlled Growth Conditions

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Abstract: Photosynthetic pigments are an important factor in determining the physiological status and the efficiency of the photosynthetic process in plants. This study aimed to determine the contents of chlorophyll a, chlorophyll b, total chlorophyll (a + b), and carotenoids within the leaves of thirteen cultivars of spring wheat. Wheat seeds were grown under controlled conditions, and photosynthetic pigments were extracted using acetone. Their concentrations were measured using a spectrophotometer at different wavelengths. The data were evaluated using descriptive statistics and one-way analysis of variance (one-way ANOVA). The results indicated the presence of variation in the values of concentrations of photosynthetic pigments in the studied wheat cultivars. The highest chlorophyll a and carotenoid contents were recorded in cultivar Voevoda, while the highest chlorophyll b concentration was observed in cultivar L505. The two cultivars Lebedushka and Alexandrit showed lower levels of chlorophylls and carotenoids. A statistically significant difference was detected only for chlorophyll a, whereas chlorophyll b and carotenoids did not differ significantly among cultivars. Also, the study showed that the Pearson correlation coefficient between chlorophyll pigments was strongly positive, and the relationship of chlorophylls with carotenoids was moderately positive. The results showed variation in the content of photosynthetic pigments among the studied wheat cultivars under controlled conditions. The presence of a higher concentration of photosynthetic pigments in the Voevoda and L505 cultivars may indicate the presence of greater accumulation of these pigments and the possibility of photoprotection.

Keywords: Photosynthetic Pigments, Chlorophyll a and b, Carotenoids, Wheat Cultivars, *Triticum aestivum*, Physiological Variability, Plant Physiology.

1. INTRODUCTION

Chlorophylls (a and b) and carotenoids are considered as the photosynthetic pigments responsible for light absorption and play an important and central role (especially chlorophyll a and b) in absorbing light and carrying out the process of photosynthesis, while carotenoids function in protecting plants from oxidative damage resulting from excessive light intensity and are usually found in the chloroplasts of green leaves [1-3]. Therefore, the content of these pigments is considered an indicator of photosynthetic activity and the physiological status of the plant [4, 5].

Plants differ in photosynthetic pigment composition among cultivars of the same species due to differences in genetic composition, which

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lead to variations in leaf anatomy, chloroplast development, and metabolic activity [6-9]. Cultivar specificity is evident from early developmental stages, as significant differences in embryonic leaf growth and morphophysiological traits have been reported among spring wheat cultivars [10]. This variation may indicate the presence of differences in pigment composition and physiological responses to environmental conditions among the cultivars [11-13]. These variations represent an important source for selecting cultivars with higher pigment content and physiological performance [14, 15].

Wheat is considered a major agricultural crop worldwide and makes an important contribution to global food security [16-18]. Therefore, improving photosynthetic efficiency is considered an important strategy for increasing food productivity [19-21]. Recent studies confirmed the importance of photosynthetic pigments as indicators of plant physiological performance, and showed that variation in the concentration of chlorophyll and carotenoids may be associated to the photosynthetic activity and consequently with physiological performance [3]. Despite these developments, comparative studies on the concentrations of photosynthetic pigments among wheat cultivars, especially under laboratory conditions, are still limited. Information regarding pigment variation among Saratov spring wheat cultivars grown under identical controlled conditions remains scarce.

Photosynthetic pigments, especially chlorophylls (a and b) and carotenoids, are physiological indicators of great importance for the plant. Therefore, this study aimed to determine the variation in the levels of chlorophyll a, chlorophyll b, and carotenoids in spring wheat cultivars (*Triticum aestivum*) under controlled conditions. It also aimed to assess the physiological diversity among the cultivars and to determine the cultivars with different pigment accumulation patterns. In addition, correlations among photosynthetic pigments were evaluated.

2. MATERIALS AND METHODS

2.1. Experimental Plant Material and Conditions

Thirteen spring wheat (*Triticum aestivum* L.) cultivars (L505, L505 656/11, L503, L503 Lr19+Lr26, Belyanka, Dobrynya, Dobrynya Lr19+Lr37, YuV-2, Favorit, Voevoda, Lebedushka, Prokhorovka, and Alexandrit) were used in this study to evaluate and compare photosynthetic pigment content. These cultivars were selected because they represent spring wheat cultivars developed in the Saratov breeding program and were available for comparative evaluation of photosynthetic pigment variation under controlled laboratory conditions. Seeds were germinated on filter paper and seedlings were grown in a growth chamber at 18 °C under a 16 hours light and 8 hours dark photoperiod with an illumination intensity of 5000 lux. Relative humidity was not actively controlled and ranged between approximately 40–60%. Leaf samples were collected at the three-leaf stage (BBCH 13), and the first and second fully expanded leaves were used for pigment analysis. Each treatment consisted of four biological replicates.

2.2. Extraction of Photosynthetic Pigments

The central part of the leaf blade (0.07 - 0.08 g fresh weight) was used to determine the concentration of photosynthetic pigments [22]. The samples were ground well in a porcelain mortar with (2-4 ml) of 100% pure acetone, in addition to quartz sand and calcium carbonate (chalk). After that, the extract was filtered through a glass filter (No. 3) into a Büchner flask connected to a vacuum pump. Then, the obtained solution was transferred to a 25 mL volumetric flask, and the volume was made up to the mark by adding pure acetone [22].

2.3. Spectrophotometric Determination of Pigments

The content of chlorophyll a, chlorophyll b and carotenoids were determined according to the method described by Lew [22]. The absorbance (E) was measured at the wavelengths 662, 644 and 470 nm. As for the pigment concentration (C), it was calculated according to the following equations [22]:

$$\text{Chlorophyll a (mg L}^{-1}) = 9.784 \times E_{662} - 0.990 \times E_{644}$$

$$\text{Chlorophyll b (mg L}^{-1}) = 21.426 \times E_{644} - 4.650 \times E_{662}$$

$$\text{Carotenoids (mg L}^{-1}) = (1000 \times E_{470} - 1.9 \times C\text{ (chlorophyll a)} - 63.14 \times C\text{ (chlorophyll b)}) / 214$$

2.4. Calculation of Pigment Content

The pigment content was calculated based on the extract volume and the sample fresh weight according to the following equation [22]:

$$A = \frac{(C \times V)}{P}$$

A represents the pigment content expressed on the sample fresh weight, *C* represents the concentration of the sample (mg g^{-1} fresh weight), *V* represents the extract volume (ml), and *P* represents the sample fresh weight (g).

2.5. Statistical Analysis

Data are expressed as mean ± standard deviation (SD). The assumptions of normality and homogeneity of variance were evaluated using the Shapiro–Wilk and Levene's tests in Jamovi before selecting the appropriate statistical test. One-way ANOVA was applied when the assumptions were met, whereas Welch's ANOVA or the Kruskal–Wallis test was used when the assumptions were not met. Statistical significance was set at $p \leq 0.05$. In addition to this, the Pearson correlation coefficient was calculated between the photosynthetic pigments to determine the nature of the relationship between them.

3. RESULTS AND DISCUSSION

The results indicated variation in the concentrations of photosynthetic pigments among the 13 spring wheat cultivars, as shown in Figures 1-4. The clear variability in the levels of photosynthetic pigments among the cultivars (with a statistically significant difference detected only for chlorophyll a) may reflect the presence of underlying physiological differences. This may be due to differences in genetic background, differences in chloroplast development, as well as metabolic activity. This has been reported in previous studies that indicated that variation in pigment content is associated with differences in photosynthetic activity under laboratory conditions [6]. The values of chlorophyll-a concentrations ranged between 2.96 mg g^{-1} FW in the cultivar Voevoda and 1.94 mg g^{-1} FW in the cultivar Alexandrit (Figure 1). A significant difference among cultivars was detected for chlorophyll a (Kruskal–Wallis, $p = 0.040$).

The highest value of chlorophyll b concentration for cultivar L505, where it reached (1.21 mg g^{-1} FW), and the lowest value was from cultivar Lebedushka (0.6 mg g^{-1} FW) (Figure 2).

Carotenoids also showed variation in their results, where the highest value was for the cultivars Voevoda (0.97 mg g^{-1} FW) and L505 (0.95 mg g^{-1} FW), and the lowest concentrations were for the cultivar Lebedushka (0.69 mg g^{-1} FW) and Alexandrit (0.70 mg g^{-1} FW) (Figure 3). However, the observed variation was not statistically significant (Kruskal–Wallis, $p = 0.166$).

As for total chlorophyll, the highest concentration was for the cultivar L505 (3.91 mg g^{-1} FW) and the cultivar Voevoda (3.85 mg g^{-1} FW), while the lowest concentrations were for the cultivars Lebedushka (2.56 mg g^{-1} FW) and Dobrynya Lr19+Lr37 (2.68 mg g^{-1} FW) (Figure 4).

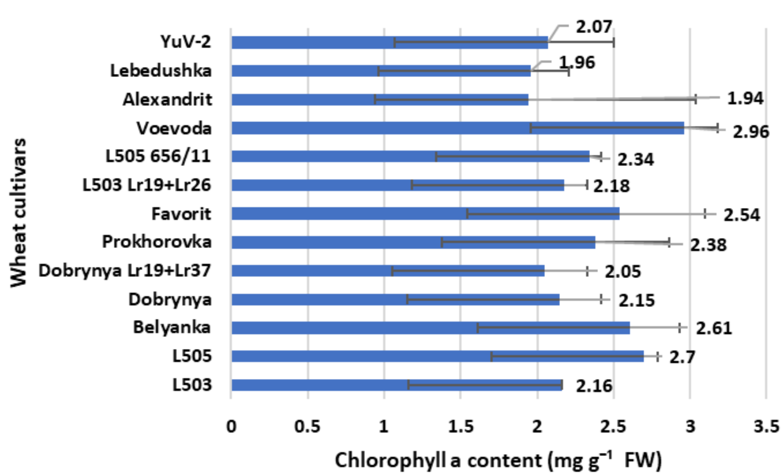

Fig. 1. Mean (± SD) values of chlorophyll a content in leaves of thirteen wheat cultivars.

Total chlorophyll reflects the chlorophyll status of the leaf, as it represents the combined contribution of chlorophyll a and b [3]. Despite the numerical differences, the variability within cultivars in the values, as shown in Figures 1- 4, may reflect physiological stability. The values indicated that the least variable cultivars were Favorit and L503 Lr19+Lr26, and this may indicate stability of pigment accumulation in them, while the highest variability in values was shown by the cultivars Voevoda and Dobrynya Lr19+Lr37, which may reflect greater variability in pigment accumulation. The higher chlorophyll a and carotenoid contents observed in the cultivar Voevoda may reflect cultivar-specific genetic characteristics that promote the accumulation of these pigments under controlled laboratory conditions. Genetic differences among cultivars have previously been associated with variation in chlorophyll content and photosynthesis-related traits, even when the plants are grown under identical environmental conditions [6]. Overall, a statistically significant difference was detected only for chlorophyll a, whereas chlorophyll b and carotenoids did not show statistically significant differences among cultivars. Although chlorophyll a showed a statistically significant difference among cultivars, chlorophyll b and carotenoids did not differ significantly ($p > 0.05$). This may be due to the controlled laboratory conditions that reduce differences in physiological performance, as well as the similarity in genetic and structural characteristics among some cultivars [9, 23].

Descriptively, the cultivars can be grouped according to their pigment content into high pigment cultivars (Voevoda, L505, and Belyanka), medium pigment cultivars (L503, Dobrynya, YuV-2, L505 656/11), and low pigment cultivars (Alexandrit and Lebedushka). The high content of photosynthetic

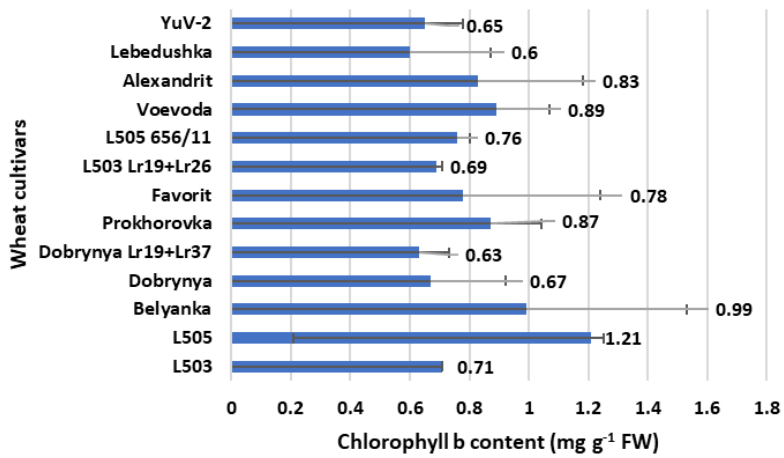

Fig. 2. Mean (± SD) values of chlorophyll b content in leaves of thirteen wheat cultivars.

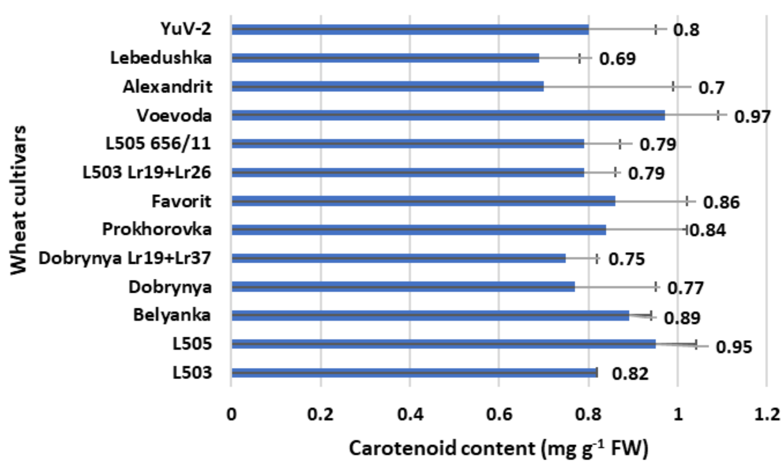

Fig. 3. Mean (± SD) values of carotenoid content in leaves of 13 wheat cultivars.

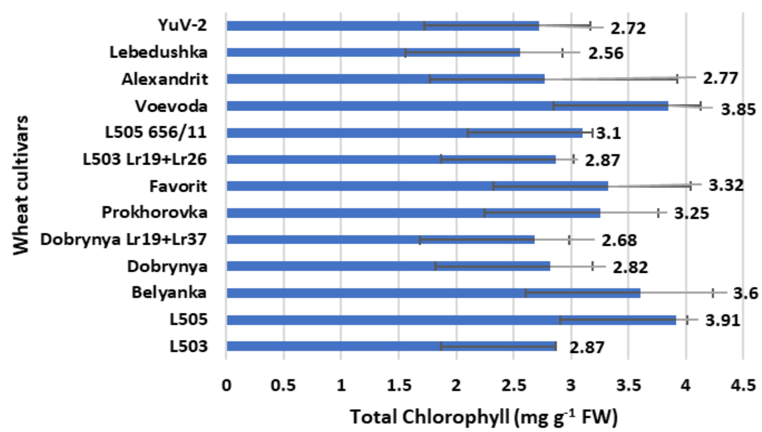

Fig. 4. Mean (± SD) values of total chlorophyll (chlorophyll a + b) content in leaves of 13 wheat cultivars.

pigments, especially total chlorophyll, may indicate a greater pigment accumulation, which has been reported to be associated with biomass and productivity in previous studies [3, 7]. Chlorophyll pigments play a main role represented by light absorption and energy transfer during the process of photosynthesis, while carotenoids play a central role in dissipating excess energy as well as protecting the photosynthetic apparatus from oxidative damage. Therefore, the increase in pigment content may reflect an improvement in the light absorption system and protection [6]. As for carotenoids, their high concentration indicates efficiency of photoprotection, meaning that the higher their concentration, the greater the protection and thus the increased ability of the plant to tolerate harsh conditions [3, 2].

Correlation analysis showed the presence of a coordinated relationship among photosynthetic pigments (Table 1). Chlorophyll a showed a strong positive correlation with chlorophyll b (r = 0.78). There was also a strong correlation between total chlorophyll and chlorophyll a (r = 0.96) and chlorophyll b (r = 0.94). While the correlations between carotenoids and chlorophyll a (r = 0.67), chlorophyll b (r = 0.72), and total chlorophyll (r = 0.75) were moderate. This indicates the presence of functional coordination between light absorption pigments and photoprotective pigments [24]. These results suggest coordinated relationships among photosynthetic pigments under controlled laboratory conditions [24].

The strong correlations between chlorophyll a, chlorophyll b, and total chlorophyll reflect the functional and structural coordination in the work of the photosynthetic apparatus, as these pigments are associated within light-harvesting complexes. As for the moderate correlation between carotenoids and chlorophylls, it may be due to the dual role of carotenoids in light harvesting and photoprotection, which indicates the presence of a partially independent regulatory mechanism [24].

4. CONCLUSIONS

The present study revealed differences in photosynthetic pigment concentrations among 13 spring bread wheat cultivars under controlled laboratory conditions. These differences may be due to physiological differences in the genetic composition of the cultivars. The variation in chlorophyll a, chlorophyll b, carotenoids,

Table 1. Pearson correlation coefficients among photosynthetic pigments.

Parameter	Chl. a	Chl. b	Total Chl.	Carotenoids
Chl. a	1	0.78	0.96	0.67
Chl. b	0.78	1	0.94	0.72
Total Chl.	0.96	0.94	1	0.75
Carotenoids	0.67	0.72	0.75	1

and total chlorophyll indicates variation in pigment accumulation among cultivars, their light-harvesting characteristics, as well as photoprotective capacity.Cultivars Voevoda, L505, and Belyanka showed high pigment content, which may indicate a higher pigment levels. In contrast, cultivars such as Alexandrit and Lebedushka showed lower pigment concentrations, which may indicate relatively lower pigment content. In addition, the differences among cultivars reflect variation in physiological stability under different environmental conditions. The strong positive correlations between chlorophyll a, chlorophyll b, and total chlorophyll, as well as the moderate positive correlations between chlorophylls and carotenoids, suggest the presence of physiological coordination between photosynthetic systems and photoprotective systems. The coordination existing between these systems reflects the presence of a physiological system that may be associated with the physiological characteristics of these cultivars. This study is of importance in selecting cultivars with a better genetic composition and higher ability in the process of photosynthesis as well as photoprotection. We recommend conducting more studies on these cultivars under field conditions and stress conditions to inform their performance, which helps in supporting targeted breeding as well as assisting in selecting high-quality cultivars.

5. ETHICAL STATEMENT

This study did not involve any human or animal participants; therefore, ethical approval was not required.

6. FUNDING

The research was carried out without any external financial support.

7. AUTHOR CONTRIBUTIONS

Oras Abdulsayed Mahdi developed the study concept, conducted the experimental work, performed the data analysis, and drafted the manuscript. M.J. Aljozary contributed to the experimental design and manuscript editing. Ali Obaid performed the statistical analysis, assisted with the interpretation of the findings, and assisted in manuscript preparation. M.Y. Kasatkin supervised the research work and critically reviewed the manuscript.

The authors confirm the use of generative AI tools only to improve the language and grammar of the manuscript and to facilitate its readability. These tools played no role in the preparation of the scientific content, analysis of the data, interpretation of the results, or the drawing of the conclusions. The authors retain full responsibility for the correctness and integrity of the manuscript.

8. CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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