



Photosynthetic Pigment Ratios in Spring Wheat (*Triticum aestivum* L.) Cultivars under Controlled Conditions

Mustafa Jasim Aljozary^{1*}, Oras Abdulsayed Mahdi², Ali Obaid³,
and Mikhail Yurievich Kasatkin⁴

¹Department of Applied Biotechnology, College of Biotechnology,
Al-Qasim Green University, Babylon 51013, Iraq

²Department of Biology, College of Sciences for Women, University of Babylon, Iraq

³Medical Instrumentation Techniques Engineering Department, College of Engineering
and Technologies, Al-Mustaqbal University, Hillah 51001, Babil, Iraq

⁴Department of Microbiology and Plant Physiology, Biological Faculty, National Research
Saratov State University, Saratov 410012, Russian Federation

Abstract: Photosynthetic pigment ratios are important physiological indicators of the structural and functional arrangement of the photosynthetic apparatus, indicating the relationship between light capture and photoprotective processes. The present study evaluated the chlorophyll a/b and total chlorophyll/carotenoid ratios in thirteen bread spring wheat (*Triticum aestivum* L.) cultivars developed through the Saratov breeding program under controlled conditions. The biological replicate dataset used here was previously reported in a concentration-based study; the present study specifically evaluates derived pigment ratios and their multivariate patterns. Photosynthetic pigment ratios were calculated from pigment concentration data obtained from four biological replicates per cultivar. Data were subjected to Welch's ANOVA, Pearson correlation, PCA, and HCA. Values of the chlorophyll a/b ratio varied between 2.47 and 3.33, whereas the total chlorophyll/carotenoids ratio ranged from 3.41 to 4.14, with cultivar differences not reaching significance ($p > 0.05$). The PCA based on the original pigment concentrations showed that PC1 explained 87.2% of the variation, with strong positive contributions from chlorophyll a, chlorophyll b, and carotenoids. HCA grouped the cultivars into three clusters according to similarities in their photosynthetic pigment profiles. Overall, the results showed that the photosynthetic pigment ratios remained within a narrow range under the controlled conditions tested, indicating limited variation in pigment-ratio characteristics among the studied cultivars.

Keywords: *Triticum aestivum* L., Chlorophyll a/b Ratio, Total Chlorophyll/Carotenoids Ratio, Principal Component Analysis, Spring Wheat Cultivars, Pigment Ratios.

1. INTRODUCTION

Among cereal crops, wheat is regarded as one of the major species and constitutes a primary food source for a large share of the world's population [1]. The photosynthetic pigment composition within chloroplasts and the proportion of each of these pigments affect the efficiency of the photosynthetic process [2, 3]. Light capture in plants is primarily mediated by chlorophylls a and

b, whereas carotenoids have two main functions, as they act as accessory pigments by capturing blue and green light wavelengths and transferring the excited energy to chlorophyll molecules, and they also protect the photosynthetic system against excessive light by dissipating surplus energy and reducing oxidative damage; the coordinated balance among these pigments is considered necessary for maintaining the efficiency of the photosynthetic process and photoprotection [4, 5].

Received: July 2026; Revised: September 2026; Accepted: September 2026

* Corresponding Author: Mustafa Jasim Aljozary <mustafajasim@biotech.uoqasim.edu.iq>

The ratio of chlorophyll a/b may serve as a measure of structural organization of the photosynthetic apparatus and the extent of plant adaptation to light intensity [6, 7]. Plant adaptation to high-light environments is associated with high values of this ratio [5-7]. Likewise, the ratio of total chlorophyll to carotenoids represents the balance between the capacity for light absorption and photoprotection [2, 8]. Changes in these ratios may provide additional information on relative pigment relationships that is not readily apparent from absolute pigment concentrations alone [9, 10]. Therefore, photosynthetic pigment ratios may serve as indirect physiological indicators associated with the functional organization of the photosynthetic apparatus and may contribute to characterizing physiological differences among different plant genotypes [6, 8, 11].

Although the concentrations of photosynthetic pigments in spring bread wheat developed through the Saratov breeding program have been studied previously, the two ratios, namely chlorophyll a/b and total chlorophyll/carotenoids, were not evaluated in our previous study of these cultivars [12]. In that study, we examined the concentrations of chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll, together with their correlations among the studied cultivars. Because these ratios are mathematically derived from the previously measured pigment concentrations, they do not represent independent biological measurements; rather, they provide a complementary description of the relative relationships among pigment components. This ratio-based representation allows the relative balance between chlorophyll forms and between total chlorophyll and carotenoids to be examined as specific outcome variables, which was not the focus of the previous concentration-based study. In contrast, the present study uses the same biological replicate dataset to derive and evaluate the ratios of chlorophyll a/b and total chlorophyll to carotenoids, which were not analyzed in the previous study. In contrast to the previous concentration-based analysis, the present study evaluates the derived pigment ratios using Welch's ANOVA, Pearson correlation, PCA and hierarchical clustering (HCA) to describe ratio-based variation and multivariate patterns among the cultivars. Thus, the present work represents a distinct ratio-based analysis of the previously generated pigment dataset rather than a repetition of the previously reported

concentration-based analysis. The cultivars selected for this study carry Lr resistance genes against *Puccinia triticina* Eriks [13]. These cultivars were developed through the Saratov breeding program [14, 15]. Evaluating photosynthetic pigment ratios under controlled environmental conditions may provide complementary information about the relative relationships among pigment components [10, 11]. In addition, multivariate statistical approaches are widely used to reveal relationships and patterns of association among cultivars based on their physiological characteristics [16-18].

Accordingly, the present study sought to examine the ratios of chlorophyll a to b and total chlorophyll to carotenoids in genetically distinct spring bread wheat cultivars carrying different chromosomal introgressions and leaf rust resistance (Lr) genes. It also aimed to characterize their physiological relationships and variation under homogeneous controlled environmental conditions using correlation and multivariate analyses, to provide a more comprehensive understanding of the physiological variation among the cultivars.

2. MATERIALS AND METHODS

2.1. Plant Material

The study included a set of genetically diverse spring bread wheat (*Triticum aestivum* L.) cultivars originating from the Saratov breeding program. The cultivars were developed by the Laboratory of Soft Wheat the South-East Research Institute of Agriculture (FGBNU "FANC South-East") and included genotypes carrying different alien chromosomal introgressions and leaf rust resistance (Lr) genes [14, 15]. The study included the cultivars: L503, L503 Lr19+Lr26, L505, L505 656/11, Belyanka, Dobrynya, Dobrynya Lr19+Lr37, Favorit, Voevoda, Lebedushka, Alexandrit, Prokhorovka, and YuV-2. These cultivars represented diverse spring wheat germplasm and were selected for physiological evaluation under identical controlled growth conditions.

2.2. Calculation of Photosynthetic Pigment Ratios

The photosynthetic pigment concentration data used for the present analyses were generated in our previous study [12]. The original biological

replicate data were used to calculate the ratio of chlorophyll a to b, together with the ratio of total chlorophyll to carotenoids, for each biological replicate. Four independent biological replicates were available for each cultivar, with each replicate representing an independently sampled plant. Each biological replicate was treated as the experimental unit for the analyses conducted at the replicate level. These ratios were used for all subsequent statistical analyses. The original experiment was conducted under controlled growth conditions at 18 °C, with a 16 h light and 8 h dark cycle and a light intensity of 5000 lux; relative humidity was maintained at approximately 40–60%. Samples were obtained when the plants reached the three-leaf stage (BBCH 13), using the first two leaves that were fully expanded, with four biological replicates per cultivar. For pigment extraction, 0.07–0.08 g of fresh leaf material was subjected to extraction using 2–4 mL of 100% acetone, followed by filtration and adjustment to a total volume of 25 mL. Pigment absorbance measurements were obtained spectrophotometrically at 662, 644, and 470 nm, as described in our previous study [12].

2.3. Statistical Analysis

Results are reported as mean $\pm$ standard deviation (SD). Before statistical analysis, normality and homogeneity of variance were evaluated using the Shapiro–Wilk and Levene’s tests, respectively [19, 20]. As homogeneity of variance was not satisfied, differences in pigment ratios among the studied cultivars were evaluated using Welch’s analysis of variance (Welch’s ANOVA) [21], followed by the Games–Howell post-hoc procedure for pairwise comparisons. Pearson’s correlation analysis was used to examine associations among the studied pigment traits. Correlation analysis was performed at two analytical levels: individual biological replicates ($n = 52$) to assess replicate-level associations across cultivars, and cultivar mean values ($n = 13$) to assess associations at the cultivar level. In contrast, PCA and HCA were performed using the mean values for each cultivar. For PCA, chlorophyll a, chlorophyll b, and carotenoids were used as the original pigment concentrations to avoid redundancy among mathematically derived variables. Hierarchical cluster analysis was conducted on standardized chlorophyll a, chlorophyll b, and carotenoid concentrations, with Euclidean distance and Ward.D2 linkage applied.

All statistical analyses were performed using Jamovi (version 2.7.38).

3. RESULTS AND DISCUSSION

The analysis of photosynthetic pigment ratios displayed only limited variation across spring wheat cultivars under controlled laboratory conditions. The assumption tests indicated that the chlorophyll a/b ratio did not significantly depart from normality (Shapiro–Wilk $W = 0.967$, $p = 0.155$), whereas the total chlorophyll-to-carotenoid ratio exhibited a significant departure from normality ($W = 0.952$, $p = 0.035$). Levene’s test indicated unequal variances for both the chlorophyll a/b ratio ($F = 2.65$, $p = 0.011$) and the total chlorophyll-to-carotenoid ratio ($F = 2.24$, $p = 0.029$). Therefore, Welch’s ANOVA was applied to address unequal variances, with the Games–Howell test used for pairwise comparisons.

3.1. Chlorophyll a/b Ratio

The greatest mean value for the chlorophyll a/b ratio was found in the cultivar Voevoda (3.33 ± 0.14), whereas the lowest ratio was recorded in the cultivars Alexandrit (2.47 ± 0.70) and L505 (2.47 ± 0.72) (Figure 1). Despite this slight variation, Welch’s ANOVA indicated no statistically significant difference ($p = 0.417$; Table 1).

The observed variation in the chlorophyll a/b ratio may reflect cultivar-related variation [22, 23]. The chlorophyll a/b ratio is broadly recognized as a useful physiological measure of the structural arrangement of the photosynthetic apparatus, as it reflects the relative allocation of chlorophyll between the photosynthetic reaction centers and the light-harvesting antenna system rather than simply the total pigment content [6, 24]. The lack of significant differences among the cultivars indicated that the chlorophyll a/b ratio remained relatively stable under the standardized laboratory conditions used in the present study.

Table 1. Welch’s ANOVA results for pigment ratios among spring wheat cultivars.

Parameter	F Welch	P value
Chl. a/b	1.11	0.417
Total Chl./Caro.	1.87	0.126

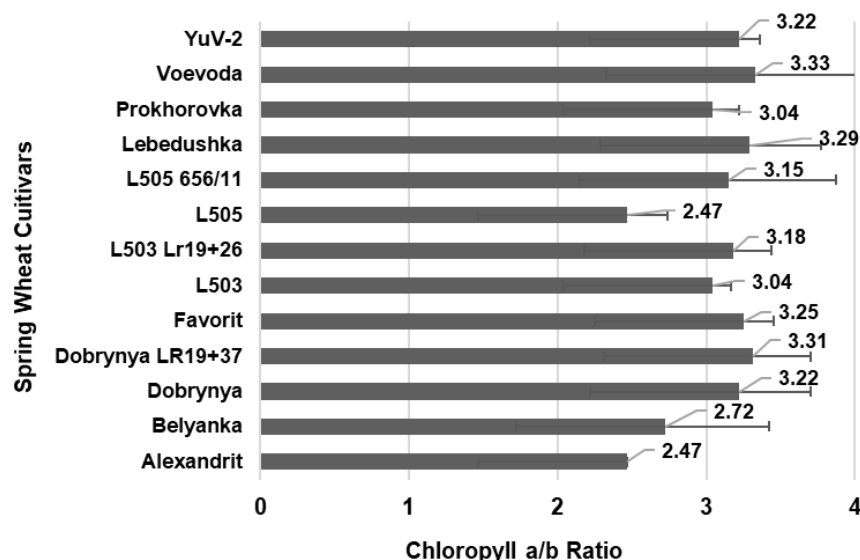


Fig. 1. Chlorophyll a/b ratio in spring wheat cultivars (mean ± SD).

3.2. Total Chlorophyll to Carotenoids Ratio

The total chlorophyll/carotenoids ratio showed variation within narrow limits. The highest mean value was recorded in the cultivars Belyanka (4.14 ± 0.61) and L505 (4.08 ± 0.72). In contrast, the lowest mean value was observed for the cultivar YuV-2 (3.41 ± 0.16) (Figure 2). Welch's ANOVA indicated that no statistically significant differences were detected ($p = 0.126$; Table 1). This limited variation among the cultivars indicates that the total chlorophyll/carotenoids ratio remained relatively stable under standardized laboratory conditions.

The observed variation was small and did not reach statistical significance.

The relative stability of the total chlorophyll/carotenoids ratio may reflect a relatively stable relationship between chlorophyll and carotenoid concentrations under the studied conditions [4]. Although cultivars such as YuV-2 showed slightly lower total chlorophyll/carotenoids ratios, suggesting a higher proportion of carotenoids, the differences observed were not statistically significant. The overall stability of this ratio indicates that pigment composition remained

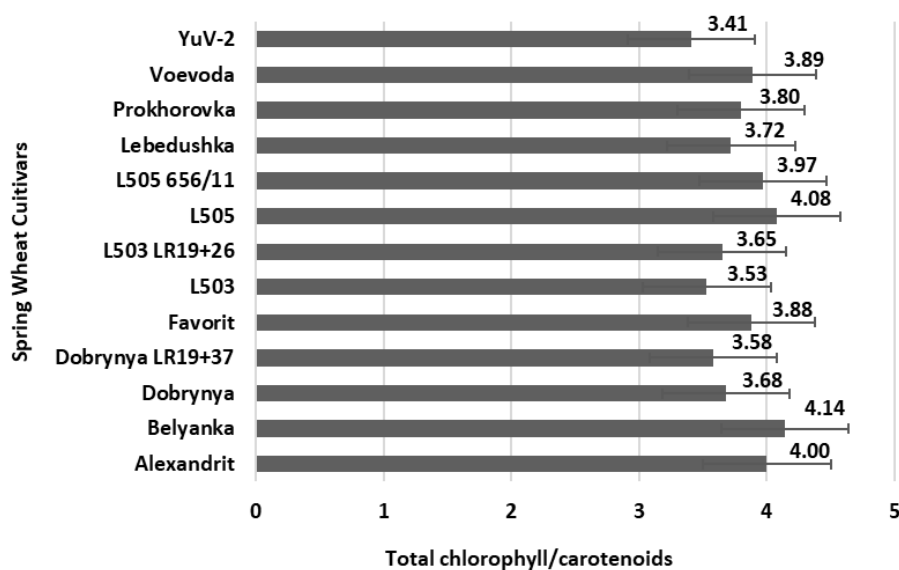


Fig. 2. Total chlorophyll/carotenoids ratio in spring wheat cultivars (mean ± SD).

relatively consistent among the studied cultivars under favorable growth conditions.

3.3. Correlation Analysis

Pearson correlation analysis (Table 2) based on individual biological observations ($n = 52$) revealed several strong correlations among the measured pigment concentrations. Correlations among the original pigment concentrations were considered in the biological interpretation, including those involving chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids. Correlations involving the derived pigment ratios and their component variables were not interpreted as independent biological associations because these relationships are partly determined by the mathematical construction of the ratios.

Correlation analysis (Table 3) based on cultivar mean values ($n = 13$) showed several patterns that were consistent with those obtained from the individual observations. These results provided an additional assessment of the relationships among the pigment traits at the cultivar level. For biological interpretation at the cultivar level, the correlation analysis based on cultivar mean values was considered the primary analysis, whereas the replicate-level analysis was used to describe associations across individual observations.

Overall, the correlation analysis indicated consistent relationships among the original pigment concentrations across individual observations and cultivar means. Correlations involving derived pigment ratios were interpreted cautiously because of their inherent mathematical relationships with the variables used to calculate them.

Table 2. Pearson's r coefficients and corresponding p -values based on individual biological observations ($n = 52$).

Parameter	Chl. a	Chl. b	Total Chl.	Carotenoids	Chl. a/b	Total Chl./Caro
Chl. a p-value	1	0.725 (<0.001)	0.963 (<0.001)	0.887 (<0.001)	-0.197 (0.161)	0.469 (<0.001)
Chl. b p-value		1	0.884 (<0.001)	0.631 (<0.001)	-0.773 (<0.001)	0.693 (<0.001)
Total Chl. p-value			1	0.849 (<0.001)	-0.437 (0.001)	0.589 (<0.001)
Carotenoids p-value				1	-0.177 (0.209)	0.083 (0.560)
Chl. a/b p-value					1	-0.559 (<0.001)
Total Chl./Caro						1

Table 3. Pearson's r coefficients and corresponding p -values based on cultivar mean values ($n = 13$).

Parameter	Chl. a	Chl. b	Total Chl.	Carotenoids	Chl. a/b	Total Chl./Caro
Chl. a p-value	1	0.725 (0.005)	0.966 (<0.001)	0.951 (<0.001)	-0.134 (0.662)	0.637 (0.019)
Chl. b p-value		1	0.879 (<0.001)	0.740 (0.004)	-0.768 (0.002)	0.816 (<0.001)
Total Chl. p-value			1	0.937 (<0.001)	-0.383 (0.196)	0.749 (0.003)
Carotenoids p-value				1	-0.184 (0.547)	0.471 (0.104)
Chl. a/b p-value					1	-0.624 (0.023)
Total Chl./Caro						1

3.4. Principal Component Analysis (PCA)

The PCA was based on three original photosynthetic pigment traits, namely chlorophyll a, chlorophyll b, and carotenoid concentrations, measured across thirteen spring wheat cultivars to explore the major patterns of variation among the cultivars [25, 26]. The analysis identified one major component with an eigenvalue exceeding 1, accounting for 87.2% of the overall variation. PC1 explained 87.2% of the variation (eigenvalue = 2.62).

The scree plot (Figure 3) illustrated the eigenvalues of the principal components and supported the retention of PC1, which was the only component with an eigenvalue greater than 1 [26, 27]. PC1 was strongly influenced by carotenoid, chlorophyll a, and chlorophyll b concentrations, with positive loadings of 0.965, 0.960, and 0.873, respectively. Because the pigment ratios and total chlorophyll were derived from the measured pigment concentrations, they were not included in this PCA to avoid redundancy among the variables. These results indicate that the multivariate pattern among cultivars was mainly associated with variation in the original pigment concentrations.

3.5. Hierarchical Cluster Analysis (HCA)

The HCA was conducted using standardized photosynthetic traits, with Euclidean distance and the Ward.D2 clustering method. This analysis divided the thirteen cultivars into three clusters according to their photosynthetic pigment characteristics (Figure 4).

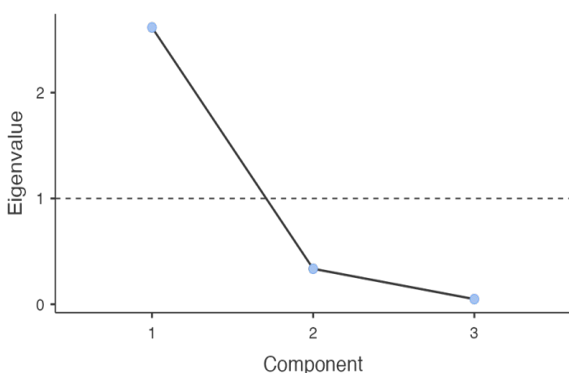


Fig. 3. Scree plot of eigenvalues from principal component analysis based on chlorophyll a, chlorophyll b, and carotenoid concentrations.

The first cluster included three cultivars, namely Voevoda, L505, and Belyanka, which were grouped together based on their photosynthetic pigment profiles. The second cluster included L505 656/11, Prokhorovka, and Favorit, whereas the third cluster included the remaining seven cultivars, namely L503, YuV-2, L503 Lr19+L26, Dobrynya, Alexandrit, Dobrynya Lr19+L37, and Lebedushka. This grouping reflected similarities and differences in the multivariate patterns of pigment concentrations among the studied cultivars. This pattern was broadly consistent with the PCA results, which also showed variation among cultivars based on their photosynthetic pigment profiles. The heatmap representation illustrated the relationships among the cultivars and the pigment variables. The clustering pattern also reflected similarities in the original photosynthetic pigment concentrations among the studied cultivars. This analysis was consistent with the principal component analysis, indicating that the studied cultivars share similar photosynthetic pigment profiles, while showing variation among cultivars under controlled conditions. Although three clusters were identified, the clustering pattern indicated that the cultivars were grouped according to similarities in their pigment concentrations under the studied conditions.

3.6. Overall Interpretation

Although slight variation in photosynthetic pigment ratios was observed among the cultivars, these ratios

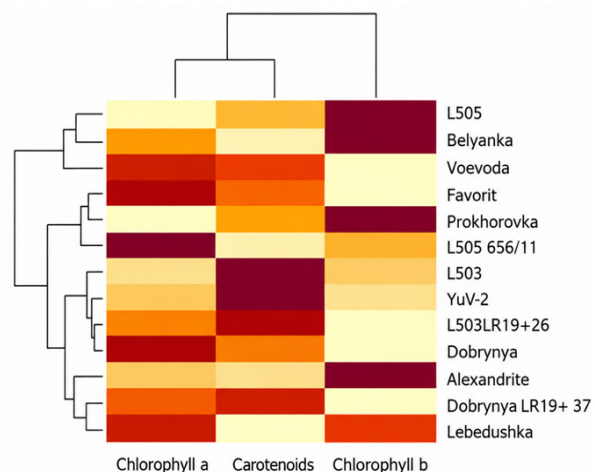


Fig. 4. Heatmap and hierarchical clustering of spring wheat cultivars based on standardized chlorophyll a, chlorophyll b, and carotenoid concentrations. Euclidean distance and Ward.D2 clustering were used.

remained within a narrow range under controlled conditions. Statistically significant differences were not observed in the pigment ratios, indicating that the relative pigment balance remained relatively stable under controlled laboratory conditions. The present findings agree with earlier studies demonstrating the stability of photosynthetic pigment ratios under favorable growth conditions [6, 28]. Earlier research has demonstrated that wheat genotypes can vary in their physiological and photosynthetic responses to drought and heat stress [17, 18]. The main contribution of the present re-analysis is the characterization of the relative relationships between chlorophyll a and chlorophyll b, and between total chlorophyll and carotenoids, under the specific controlled conditions tested. These ratios provide a complementary description of pigment composition based on the previously reported pigment concentration data. However, neither ratio showed statistically significant differences among cultivars (chlorophyll a/b, $p = 0.417$; total chlorophyll/carotenoids, $p = 0.126$). Therefore, the present findings characterize pigment-ratio patterns under the tested conditions but do not establish genotype-dependent physiological effects.

4. CONCLUSIONS

This investigation demonstrated that the measured pigment ratios in spring wheat cultivars from the Saratov breeding program and maintained under controlled laboratory conditions varied only within a narrow range. Although slight variation in the chlorophyll a-to-b ratio was observed among cultivars, the differences were not statistically significant ($p = 0.417$).

The total chlorophyll-to-carotenoid ratio also exhibited limited variation, with no statistically significant differences among cultivars ($p = 0.126$). The relative relationships among photosynthetic pigments remained relatively consistent under the studied conditions. Under non-stress conditions, this pigment balance may reflect a relatively stable organization of photosynthetic pigments. In general, the results indicate that pigment ratios showed relatively limited variation under the studied conditions. The present findings provide a baseline for photosynthetic pigment-ratio characteristics under the controlled conditions tested and may serve as a reference for future studies investigating cultivar responses under environmental stress.

5. ETHICAL STATEMENT

No human participants or experimental animals were involved in this study.

6. ACKNOWLEDGMENTS

The author gratefully acknowledges the Soft Wheat Laboratory of the South-East Research Institute of Agriculture (FGBNU “FANC South-East”) for providing the plant material and technical support. The author also acknowledges Saratov State University named after N.G. Chernyshevsky (SSU), Saratov, for academic support. The author used ChatGPT (OpenAI) for improving the English language of the manuscript.

7. CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose

8. FUNDING

No external funding was received for this research.

9. AUTHOR CONTRIBUTIONS STATEMENT

Conceptualization: M.J.A.; Methodology: M.J.A.; Investigation: M.J.A.; Formal analysis: A.O.; Data curation: M.J.A.; Visualization: M.J.A.; Writing original draft: M.J.A., O.A.M.; Writing – review and editing: M.J.A., O.A.M.; Resources: M.Y.K.; Validation: M.Y.K.

10. REFERENCES

1. P.R. Shewry and S.J. Hey. The contribution of wheat to human diet and health. *Food and Energy Security* 4(3): 178-202 (2015). <https://doi.org/10.1002/fes3.64>
2. D. Fu, C. Zhao, X. Huang, and P. Wang. Chloroplast proteostasis: Coupling chlorophyll and carotenoid metabolism with chlorophyll-binding protein homeostasis in plants. *Journal of Experimental Botany*: erag307 (2026). <https://doi.org/10.1093/jxb/erag307>
3. M.P. Johnson. Structure, regulation and assembly of the photosynthetic electron transport chain. *Nature Reviews Molecular Cell Biology* 26(9): 667-690 (2025). <https://doi.org/10.1038/s41580-025-00847-y>
4. D. Vetoshkina, N. Balashov, B. Ivanov, A. Ashikhmin, and M. Borisova-Mubarakshina. Light harvesting regulation: A versatile network

- of key components operating under various stress conditions in higher plants. *Plant Physiology and Biochemistry* 194: 576-588 (2023). <https://doi.org/10.1016/j.plaphy.2022.12.002>
5. F. Gao, J. Guo, and Y. Shen. Advances from chlorophyll biosynthesis to photosynthetic adaptation, evolution and signaling. *Plant Stress* 12: 100470 (2024). <https://doi.org/10.1016/j.stress.2024.100470>
 6. G.D. Rashkov, M.A. Stefanov, A.N. Misra, and E.L. Apostolova. The role of light-harvesting complex II organization in the Efficiency of Light-Dependent Reactions in the Photosynthetic Apparatus of *Pisum sativum* L. *Plants* 14(12): 1846 (2025). <https://doi.org/10.3390/plants14121846>
 7. P.I. Calzadilla, J. Song, P. Gallois, and G.N. Johnson. Proximity to Photosystem II is necessary for activation of Plastid Terminal Oxidase (PTOX) for photoprotection. *Nature Communications* 15: 287 (2024). <https://doi.org/10.1038/s41467-023-44454-x>
 8. T. Sun, S. Rao, X. Zhou, and L. Li. Plant carotenoids: recent advances and future perspectives. *Molecular Horticulture* 2: 3 (2022). <https://doi.org/10.1186/s43897-022-00023-2>
 9. M. Son, A. Pinnola, S.C. Gordon, R. Bassi, and G.S. Schlau-Cohen. Observation of dissipative chlorophyll-to-carotenoid energy transfer. *Nature Communications* 11: 1295 (2020). <https://doi.org/10.1038/s41467-020-15074-6>
 10. K. Hikosaka. Leaf pigment composition: a window into how plants cope with environmental stress. *Tree Physiology* 46(4): tpag037 (2026). <https://doi.org/10.1093/treephys/tpag037>
 11. R. Croce and H. van Amerongen. Natural strategies for photosynthetic light harvesting. *Nature Chemical Biology* 10: 492-501 (2014). <https://doi.org/10.1038/nchembio.1555>
 12. O.A. Mahdi, M.J. Aljozary, A. Obaid, and M.Y. Kasatkin. Variation and correlation of photosynthetic pigments in spring wheat cultivars under controlled growth conditions. *Proceedings of the Pakistan Academy of Sciences: B. Life and Environmental Sciences* 63(2): 85-91 (2026). [https://doi.org/10.53560/PPASB\(63-2\)1131](https://doi.org/10.53560/PPASB(63-2)1131)
 13. E. Gulyaeva, E. Shaydayuk, and P. Gannibal. Leaf rust resistance genes in wheat cultivars registered in Russia and their influence on adaptation processes in pathogen populations. *Agriculture* 11: 319 (2021). <https://doi.org/10.3390/agriculture11040319>
 14. E.I. Gulyaeva, S.N. Sibikeev, A.E. Druzhin, and E.L. Shaydayuk. Enlargement of genetic diversity of spring bread wheat resistance to leaf rust (*Puccinia triticina* Eriks.) in Lower Volga region. *Agricultural Biology* 55(1): 27-44 (2020). <https://doi.org/10.15389/agrobiology.2020.1.27eng>
 15. S.N. Sibikeev and A.E. Druzhin. Prebreeding research of near-isogenic lines of spring bread wheat with a combination of translocations from *Agropyron elongatum* (Host.) P.B. and *Aegilops ventricosa* Tausch. *Vavilovskii Zhurnal Genetiki i Selektzii – Vavilov Journal of Genetics and Breeding* 19(3): 310-315 (2015). <https://doi.org/10.18699/VJ15.040>
 16. D. Baranwal, R. Kumar, C.S. Azad, and R. Munjal. Assessment of terminal heat tolerance among wheat (*Triticum aestivum*) genotypes based on multivariate analysis and selection indices. *The Indian Journal of Agricultural Sciences* 94(9): 923-928 (2024). <https://doi.org/10.56093/ijas.v94i9.143443>
 17. R.A. Awadalla, A. Sallam, A. Börner, M.M. Elshamy, and Y.M. Heikal. The role of salicylic acid in modulating phenotyping in spring wheat varieties for mitigating drought stress. *BMC Plant Biology* 24(1): 948 (2024). <https://doi.org/10.1186/s12870-024-05620-5>
 18. M.M. Hasan, M.A. Baset Mia, J.U. Ahmed, M.A. Karim, A.K.M.A. Islam, and M. Mohi-Ud-Din. Heat stress tolerance in wheat seedling: Clustering genotypes and identifying key traits using multivariate analysis. *Heliyon* 10(19): e38623 (2024). <https://doi.org/10.1016/j.heliyon.2024.e38623>
 19. S.S. Shapiro and M.B. Wilk. An analysis of variance test for normality (complete samples). *Biometrika* 52(3-4): 591-611 (1965). <https://doi.org/10.1093/biomet/52.3-4.591>
 20. H. Levene. Robust tests for equality of variances. In: I. Olkin, S.G. Ghurye, W. Hoeffding, W.G. Madow, and H.B. Mann (Eds.). *Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling*. Stanford University Press, Palo Alto, CA, USA, pp. 278-292 (1960).
 21. B.L. Welch. On the comparison of several mean values: an alternative approach. *Biometrika* 38(3-4): 330-336 (1951). <https://doi.org/10.1093/biomet/38.3-4.330>
 22. K. Guo, T. Chen, P. Zhang, Y. Liu, Z. Che, F. Shahinnia, and D. Yang. Meta-QTL analysis and in-silico transcriptome assessment for controlling chlorophyll traits in common wheat. *The Plant Genome* 16: e20294 (2023). <https://doi.org/10.1002/tpg2.20294>
 23. H. Xu, J. Dong, X. Meng, H. Jiang, G. Ding, Z. Wang,

- F. Wang, Y. Wang, M. Wu, L. Ma, G. Miao, R. Qin, C. Zhao, H. Sun, F. Cui, and Y. Wu. TaHAL3-7A increases grain yield by enhancing photosynthetic pigment content in wheat (*Triticum aestivum* L.). *The Plant Journal* 122(5): e70274 (2025). <https://doi.org/10.1111/tpj.70274>
24. A. Kume, T. Akitsu, and K.N. Nasahara. Why is chlorophyll b only used in light-harvesting systems? *Journal of Plant Research* 131(6): 961-972 (2018). <https://doi.org/10.1007/s10265-018-1052-7>
25. K.M. Yeater, S.E. Duke, and W.E. Riedell. Multivariate analysis: greater insights into complex systems. *Agronomy Journal* 107: 799-810 (2015). <https://doi.org/10.2134/agronj14.0017>
26. V. Peršić, A. Ament, J. Antunović Dunić, G. Drezner, and V. Cesar. PEG-induced physiological drought for screening winter wheat genotypes sensitivity – integrated biochemical and chlorophyll a fluorescence analysis. *Frontiers in Plant Science* 13: 987702 (2022). <https://doi.org/10.3389/fpls.2022.987702>
27. C. Matthew. Notes on the use of principal component analysis in agronomic research. *Grassland Research* 4: 1-6 (2025). <https://doi.org/10.1002/qlr2.70003>
28. X. Li, W. Zhang, D. Niu, and X. Liu. Effects of abiotic stress on chlorophyll metabolism. *Plant Science* 342: 112030 (2024). <https://doi.org/10.1016/j.plantsci.2024.112030>

ACCEPTED MANUSCRIPT