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VARIATION OF CHLOROPHYLL AND CAROTENOID PIGMENTS IN SOME WINTER WHEAT FLAG LEAF UNDER DIFFERENT FERTILIZER REGIMES

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Abstract

Five Romanian winter wheat genotypes were analyzed during two experimental years (2019-2020) under four different regime fertilizers for chlorophyll a, chlorophyll b and carotenoid contents from flag leaf, respectively, number of grains/spike, weight of grains/spike, thousand kernel weight. The results showed that the application of additional fertilization increase the analyzed traits, also causing an increase of traits variability. Regarding the variation of photosynthetic pigments from flag leaf as an effect of different fertilizer levels, their concentration is specific to each variety. The highest value of chlorophyll a type is observed in Cezara variety (665.83 mg/kg DW) at N_{150} kg s.a./ha fertilization level applied in a boot stage. The variation of chlorophyll b concentration in flag leaf is similar to chlorophyll a concentration as a reaction of different nitrogen dose aplication, the highest value for this type of chlorophyll being registered in T. 109-12 at F_4 (519.64 mg/kg DW). Carotenoid concentration from flag leaf varies from 3.93 mg/kg DW at Dumitra variety (F_3) to 97.63 mg/kg DW at Andrada variety in F_2 fertilization level.

Key words: *carotenoids, chlorophyll, flag leaf, yield components.*

INTRODUCTION

Fertilization, alongside the quality of genetic material and agrotechnical practices are the main tools of agricultural engineering (Tranavičiene et al., 2007). All vital processes are associated with protein, of which nitrogen play a central role, as an essential constituent (Leghari et al., 2016). Also, nitrogen availability and internal distribution play a critical role in the regulation of various growth-related and morphogenetic aspects of plant development (McIntyre, 2001). The supply of nutrients in the cultivation environment is directly related to the photosynthetic function of crop leaves (Tang et al., 2003).

Chlorophyll is a leaf pigment located at a cell organelle named chloroplasts, and their content was closely related to crop health, photosynthetic capacity and crop yield (Lukas et al., 2014). Chlorophyll is an important pigment involved in the absorption, transmission, and transformation of light energy in photosynthesis (Peng et al., 2021; Cheng and Liu, 2010). The photosynthetic pigments of higher plants include chlorophyll and carotenoids (Wang, 2019). Several studies have indicated that nitrogen nutrition plays an important role in the regulation of photosynthetic pigment synthesis in crop leaves, and that the level of nitrogen supply has a significant positive correlation with the chlorophyll content of crops (Peng et al., 2021; Zhang & Shangguan, 2007). Chlorophyll fluorescence emitted by plants is also closely related to various reaction processes in photosynthesis and may contain rich information about photosynthetic changes (Peng et al., 2021). Although carotenoids (car) also contribute to photoprotection and light collection in photosynthesis (Standfuss et al., 2005; Demmig-Adams et al., 1996) they affect the protection of unsaturated fatty acids, phospholipids, and galactolipids (Edge et al., 1997).

From all metabolic elements which plants use from soil, nitrogen needs in the largest amounts (Tucker, 2004). Nitrogen exists in organic and inorganic form and the greatest nitrogen content is in seeds, leaves, shoots and roots. Deficiency of nitrogen leads to loss green color in the leaves, decrease leaf area and intensity of photosynthesis (Bojović & Marković, 2009). Chlorophyll content is the appropriate parameter for the evaluation of nitrogen uptake (Shadchina & Dmitrieva, 1995). Cereals have different demands for nitrogen at different stages of growth (Akhter et al., 2016; Tarnavičiene et al., 2008). Nitrogen supply has large effect on leaf growth because it increases the leaf area of plants and, on that way, it influences on photosynthesis (Sinclair et al., 2019). Photosynthetic proteins represent a large proportion to total leaf N (Evans, 1989; Field & Mooney, 1986). Evans (1983) shows that chlorophyll content is approximately proportional to leaf nitrogen content. The importance of flag leaf in plant organogenesis is well recognized, the role of these being crucial especially in limited conditions. Due to his positions and vitality, the main task of these is to assure the optimal development of yield components.

MATERIALS AND METHODS

Five Romanian winter wheat genotypes (Andrada, Cezara, Dumitra, Taisa and line T. 109-12) were tested in a field condition at Agricultural Research and Development Station at Turda (longitude 23°47′, latitude 46°35′, altitude 427 m) during two experimental years (2019 and 2020) for their reaction to different nitrogen dose. The biologic material was tested on chernozem soil with a pH of 6.81 and 3.73% humus content. The nitrogen content in soil are above 0.205 mg kg^{-1}, mobile phosphorus 35 mg kg^{-1}, and mobile potassium 320 mg kg^{-1}. The sowing plant density was 550 seeds m^{-2} on harvesting plot surface of 7.5 m^2. The experiment was three factorials combining the experimental year (2 graduation), fertilization (4 graduation) and genotypes (5 graduation). Regarding the fertilization levels they were:

- F_1 - basic fertilization apllied after plant emergency- $N_{50}P_{50}$ kg s.a./ha (control);
- F_2-F_1 + additional fertilization (N_{50} kg s.a./ha) applied in boot stage (GS 40);
- F_3-F_1 + additional fertilization (N_{100} kg s.a./ha) applied in boot stage (GS 40);
- F_4-F_1 + additional fertilization (N_{150} kg s.a./ha) applied in a boot stage (GS 40).

Thirty plants from each repetition were analised for main yield components- number of grain per spike (NG), weight of grains per spike (WG) and thousand kernel weight (TKW) as a reaction of different fertilizer dose.

The chlorophyll (Chl a and Chl b) and carotenoid (Car) contents were determinated from a mix of fresh flag leaves using acetone (98%) for extraction, followed by vaccum filtration and measuring the absorbance of the extract at 470 nm, 646 nm and 663 nm with a spectophotometer (T80+ UV/VIS spectrophotometer - PG Instruments Ltd). The statistical analysis was performed using Microsoft Excel and Mathlab program.

The aim of this study was to quantify the relationship among application of different rate of nitrogen fertilizer, flag leaf chlorophyll content and main yield components.

RESULTS AND DISCUSSIONS

Number of grains per spike has a medium variability (< 20) for the most studied genotypes in different nitrogen application, except Dumitra genotype which has a high variability coefficient for this trait at all fertilizer levels and T. 109-12 at additional fertilization with 100 kg ha^{-1} N s.a. applied in plants boot stage (Table 1). Also, application of additional fertilization has determinate a medium variability for weight of grain per spike, the mean values of this character registering a constant increase with the increase of the fertilization dose. Generally, low variability of thousand kernel weight (<10) were registered for most studied genotypes under different fertilization level, this reaction is due to the high genetic determinism of this character. Dumitra variety has also a different reaction for thousand kernel weight character (>10) in case of F_1 and F_2, which means that in achieving this character the contribution of the environmental factor has a higher contribution. Regarding the weight of grains per spike, additional fertilization applied in boot stage determine an increase of this traits regardless of the dose applied.

Variation of chlorophyll pigments in the flag leaf is specific for each variety, generally the application of increasing fertilizer doses has a favorable effect on them (Figure 1).

Table 1. The mean values and variability coefficient of yield components of studied genotypes under different fertilization levels

	F_1		F_2		F_3		F_4	
Andrada								
	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV
NG	39.46	17.87	39.03	16.83	39.84	16.28	41.64	15.94
WG	1.598	19.07	1.612	17.84	1.624	14.93	1.745	17.74
TKW	40.58	8.67	41.38	7.55	40.93	8.60	41.91	7.68
Cezara								
NG	39.07	17.88	38.83	18.66	36.83	19.97	43.03	17.05
WG	1.567	20.63	1.582	19.22	1.469	18.08	1.779	18.46
TKW	40.05	8.60	40.89	8.74	40.24	8.68	41.39	7.63
Taisa								
NG	39.24	19.40	40.9	19.78	41.63	19.19	43.6	18.58
WG	1.593	20.63	1.677	20.98	1.705	19.85	1.803	19.58
TKW	40.64	8.10	41.13	9.86	41.07	9.10	41.44	8.81
Dumitra								
NG	37.81	24.27	38.89	26.66	38.39	25.69	38.64	21.28
WG	1.547	25.55	1.613	27.85	1.588	26.55	1.607	23.15
TKW	41.13	11.58	41.72	11.60	41.44	7.89	41.63	9.05
T.109-12								
NG	42.9	18.24	43.85	16.95	39.97	20.43	43.37	16.52
WG	1.885	19.37	1.924	17.76	1.743	18.97	1.902	17.20
TKW	43.94	6.27	43.88	5.48	43.91	7.79	43.89	5.88

The highest values of chlorophyll pigments are present in Cezara variety flag leaf at F_4 fertilizer dose in case of chlorophyll a (665.83 mg/kg DW) and at T. 109-12 genotype at the same fertilized dose for chlorophyll b (519.64 mg/kg DW). The variation of the chlorophyll content in the flag leaf as effect of the application of different fertilization treatments represents their photosynthetic potential. Thus, for the Andrada variety application of additional fertilization improves all pigments content (chl a, ahl b and carotenoids) with a higher content at F_2 fertilization for carotenoids (97.63 mg/kg DW), chlorophyll a content at F_3 fertilization (559.98 mg/kg DW), respectively higher chlorophyll b at F_4 fertilization dose. A different reaction of leaf pigments content can be observed in case of Cezara variety where the application of additional fertilization has a negative effect of those, the only positive effect being observed in case of F_4 treatment both for chlorophyll a and chlorophyll b. In case of Taisa and T. 109-12 genotypes application of F_4 dose of fertilization determined the highest concentrations of chlorophyll a and chlorophyll b. A slight decrease of these two types of chlorophylls can be observed in case of F_3 fertilization dose for these two genotypes, while in the case of the Dumitra variety the increase of the applied fertilizer dose entails also the increase of the chlorophyll of type a and of type b, respectively. A general effect of additional fertilization is observed in case of carotenoid whose concentration decreases with increasing fertilizer doses.

The biplot analysis of studied genotypes in relationship with different dose of fertilization (Figure 2) shows that additional fertilization has a positive impact on the main yield components and flag leaf chlorophyll concentration (chl a and chl b). Application of F_4 treatment improve agronomic performances in case of Andrada, Cezara, Taisa and Dumitra genotypes which means these varieties have the ability to capitalize on managed inputs. A specific reaction can be observed in the case of T.109-12 perspective line, whose morphological and biochemical performances are superior even in a low fertilization condition. The Cezara and Andrada varieties have a high carotenoid content, which means that these genotypes have an increased adaptability to different environment conditions.

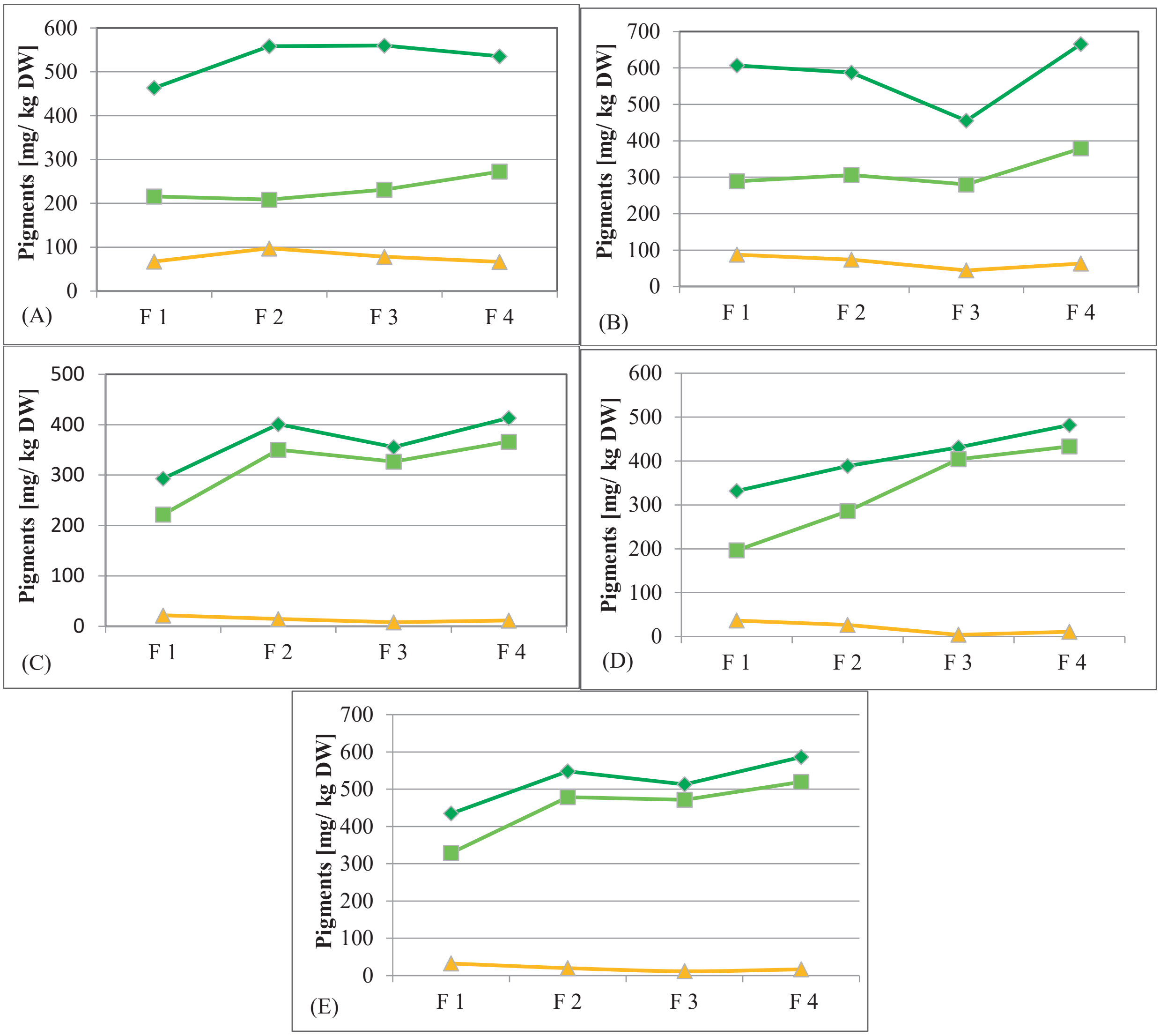

Figure 1. Variation of the flag leaf pigments as a reaction of different level of fertilizer: (A)- Andrada; (B)- Cezara; (C)- Taisa; (D)- Dumitra; (E)- T. 109-12; ♦ chorophyll a; ■ chlorophyll b; ▲ carotenoids

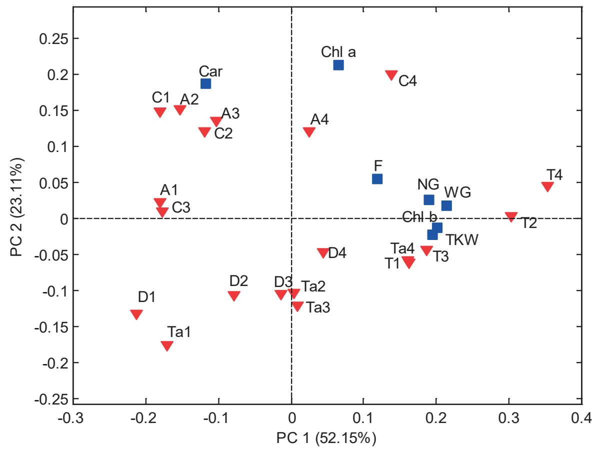

Figure 2. The biplot analysis for morphological and biochemical compounds of studied genotypes under different fertilization regime (▼A1- Andrada at F_1; ▼A2- Andrada at F_2; ▼A3- Andrada at F_3; ▼A4- Andrada at F_4; ▼C1- Cezara at F_1; ▼C2- Cezara at F_2; ▼C3- Cezara at F_3; ▼C4- Cezara at F_4; ▼Ta1- Taisa at F_1; ▼Ta2- Taisa at F_2; ▼Ta3- Taisa at F_3; ▼Ta4- Taisa at F_4; ▼D1- Dumitra at F_1; ▼D2- Dumitra at F_2; ▼D3- Dumitra at F_3; ▼D4- Dumitra at F_4 ; ;▼T1- T. 109-12 at F_1; ▼T2- T. 109-12 at F_2; ▼T3- T. 109-12 at F_3; ▼T4- T. 109-12 at F_4; ■NG- number of grains/spike; ■ WG- weight of grains/spike; ■TKW- Thousand Kernel Weight; ■F- fertilization; ■Chl a- Chlorophyll a; ■Chl b- Chlorophyll b; ■Car-Carotenoids)

The application of additional fertilization in booting stage determines the increase of the analyzed characters for all of the studied genotypes, exception for the carotenoid content in which case the highest concentration content is registred in low or medium fertilizer dose. This reaction can be attributed to the protective plants mechanism which is more effective in the case of reduced metabolic activity.

CONCLUSIONS

The application of additional nitrogen fertilizer improves flag leaf photosynthetic activity through increasing the chlorophyll a concentration and chlorophyll b concentration with a positive effect on the main yield components. The level of photosynthetic pigments in flag leaf has a genetic determinism but these traits can be improved by fertilizer application. Generally, the variability of yield components, especially number of grains/spike and weight of grains/spike decrease with the application of additional fertilization which increasing the plant ability to adapt to stress conditions by keeping or improving the productivity elements.

On the other hands, additional fertilization determines a specific variation on carotenoid content caused by the plant self-control capacity to manage the increasing photosynthetic activity.

Also, additional fertilization gives more nutritional stability to wheat plants which means that the carotenoids content, as one the main plant photoprotector, can suffer a decrease level of leaf content.

REFERENCES

Akhter, M. M., Hosain, A., Timsina, J., Teixeira da Silva, A., Islam, M. S. (2016). Chlorophyll meter – a decision-making toll for nitrogen application in wheat under light soils. *International Journal of Plant Production*, *10*(3), 289–302. DOI: 10.22069/ijpp.2016.2898.

Bojovič, B., Markivič, A. (2009). Correlation between nitrogen and chlorophyll content in wheat (*Triticum aestivum* L.). *Kragujevac Journal of Science*, *31*, 69–74.

Cheng, Z. H., Liu, L. Y. (2010). Detection of vegetation light use efficiency based on chlorophyll fluorescence spectrum. *Trans. Chin. Soc. Agric. Eng., 26*, 74–80.

Demmig-Adams, B., Gilmore, A. M., Adams, W. W. (1996). Carotenoids 3: In vivo functions of carotenoids in higher plants. *Faseb Journal*, *10*, 403–412.

Edge, R., McGarvey, D. J., Truscott, T. G. (1997). The carotenoids as anti-oxidants - A review. *J. Photochem. Photobiol. B Biol*., *41*, 189–200.

Evans, J. R. (1983). Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). *Plant Physiology*, *72*: 297-302.

Evans, J. R. (1989). Photosynthesis and nitrogen relationship in leaves of C3 plants. *Oecologia*, *20*: 9-19.

Field, C., Mooney, H. A. (1986). The photosynthesis – nitrogen relationship in wild plants. In: *On the economy of plant form* (Givnish T. J., Ed.). Cambridge, University Press, 25-53.

Leghari, S. J., Wahocho, N. A., Laghari, G. M., Laghari, A. H., Bhabhan, G. M., Talpur, K. H., Bhutto, T. A., Wahocho, S. A., Lashari A. A. (2016). Role of Nitrogen for Planth Growth and Development: A Review. *Advances in Environmental Biology*, *10*(9): 209-218.

McIntyre, G. I. (2001). Control of plant development by limiting factors: a nutritional perspective. *Physiol Plantarum*, *113*: 165–175.

Peng, J., Feng, Y., Wang, X., Li, J., Xu, G., Phonenasay, S., Luo, Q., Han, Z., Lu, W. (2021). Effects of nitrogen application rate on the photosynthetic pigment, leaf fluorescence characteristics, and yield of indica hybrid rice and their interrelations. *Sci Rep*., *11*, 7485. https://doi.org/10.1038/s41598-021-86858-z.

Shadchina, T. M., Dmitrieva, V. V. (1995). Leaf chlorophyll content as a possible diagnostic mean for the evaluation of plant nitrogen uptake from the soil. *Journal of Plant Nutrition*, *18*(7), 1427-1437. Doi:http://dx.doi.org/10.1080/01904169509364992.

Sinclair, T. R., Rufty, T. W., Lewis, R. S. (2019). Increasing photosynthesis: unlikely solution for world food problem. *Trends Plant Sci., 24*(11):1032–1039.

Standfuss, J., Terwisscha van Scheltinga, A. C., Lamborghini, M., Kühlbrandt, W. (2005). Mechanisms of photoprotection and nonphotochemical quenching in pea light-harvesting complex at 2.5 Å resolution. *EMBO J.*, *24*: 919-928. doi.org/10.1038/sj.emboj.7600585.

Tang, Y. L., Wang, J. H., Huang, J. F., Wang, R. C., He, Q. X. (2003). Variation law of hyperspectral data and chlorophyll and carotenoid for rice in mature process. *Trans. Chin. Soc. Agric. Eng. 19*, 167–173.

Tranavičiene, T., Šikšnianienė, J., Urbonavičiūtė, A., Vagusevičiene, I., Samoulienė, Duchovskis, P., Sliesaravičius, A. (2007). Effects of nitrogen fertilizers on wheat photosynthetic pigment and carbohydrate contents. *Biologija*, Vol. *53*(4): 80–84.

Tranavičiene, T., Urbonavičiūte, A., Samouliene, G., Duchovskis, P., Vagusevičiene, I., Sliesaravičius, A. (2008). The effect of differential nitrogen fertilization on photosynthetic pigment and carbohydrate content in the two winter wheat varieties. *Agronomy Research*, *6*(2), 555–561.

Tucker, M. (2004). *Primary Nutrients and Plant Growth*. In: Essential Plant Nutrients (SCRIBD, Ed.). North Carolina Department of Agriculturae.

Wang, X. J. (2019). *Plant physiology*. Higher Education Press: Beijing, China, 71-75.

Zhang, X. C., Shangguan, Z. P. (2007). Effect of nitrogen fertilization on photosynthetic pigment and fluorescence characteristics in leaves of winter wheat cultivars on dryland. *J. Nuclear Agric*. Sci*., 21*, 299-304.