NITROGEN VARIABLE RATE INFLUENCE ON PHOTOSYNTHETIC PIGMENTS IN WHEAT FLAG LEAF

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Abstract. The study evaluated the variation of photosynthetic pigments (chlorophyll, carotenoids) in wheat flag leaves in relation to the nitrogen variation rate. The PG102 winter wheat variety was cultivated, in non-irrigated conditions, on a chernozem type soil. Nitrogen was applied in doses of 0 - 280 kg ha⁻¹ a.s., in a variation rate of 40 kg ha⁻¹ a.s. (active substances). The field experiments took place in ULS "King Mihai I" from Timisoara, Romania. At the time of flowering (BBCH code 6), random samples of flag leaves were taken on each experimental variant. The chlorophyll content (Chl) varied between Chl = 43.73±1.04 (V1) and Chl = 58.92±1.21 (V6). The carotenoid content (Car) varied between Car = 10.12±0.59 (V1) and Car = 21.56±0.49 (V6). Based on the Chl and Car values, the Chl/Car and Car/Chl ratios were calculated for each experimental variant. According to Kruskal-Wallis test, resulted significant difference between sample medians (p (same) = 1.56E-05 in case of Chl data series; p (same) = 2.616E-09 in case of Car data series). The variation of Chl and Car pigments in relation to nitrogen (N) was described by polynomial equations of the 3rd degree, under statistical safety conditions (R² = 0.981 in the case of Chl; R² = 0.986 in the case of Car; p<0.001). The variation of Car in relation to Chl was described by a polynomial equation of degree 3 (R² = 0.978, p<0.001).

Keywords: flag leaf, models, nitrogen, photosynthetic pigments, wheat

INTRODUCTION

Photosynthetic pigments have an important role in the photosynthesis process of plants, through the specific relationship with light energy (absorption, transfer, transformation) and the contribution to the metabolic processes, growth and development of plants (Zeng et al., 2021). The arrangement of pigments in leaves is variable in relation to the plant organism (species, genotype), environmental conditions, culture technologies (in the case of cultivated plants), stress factors, etc. and can be considered a biochemical indicator of photosynthesis (Zeng et al., 2021; Petibon, Wiesenberg, 2022).

The non-destructive methods for determining the content of photosynthetic pigments (e.g. chlorophyll, carotenoids) are increasingly accepted and promoted, as a result of sufficiently high precision, low costs, and especially the facility to determine a large number of samples (Netto et al., 2005).

Netto et al. (2005) determined the chlorophyll content by non-destructive methods (SPAD-502) in coffee plants. The authors concluded that the analysis of chlorophyll by the respective method, correlated with the nitrogen content, can contribute to the understanding and interpretation of the photochemical processes in plants.

Some studies on grass cereals have correlated chlorophyll content (determined by non-destructive methods) and fresh biomass with aerial images (UAV images) to characterize different plant genotypes and generate prediction models through image analysis (Constantinescu et al. 2018).

Non-destructive methods were promoted to determine the distribution of photosynthetic pigments in leaves, based on spectrometers, imaging analysis and calibration and validation models (Zeng et al., 2021). Liao et al. (2023) used techniques based on UAV images (multispectral images) to evaluate the content of photosynthetic pigments in wheat. Through different methods of statistical analysis, they obtained models for estimating the physiological indices under conditions of statistical safety, assessed on the basis of R², RMSE, MAE.

The chlorophyll content was studied in relation to different nitrogen sources, with biometric parameters and the yield in corn culture (Fornari et al. 2020). The authors of the study recorded positive correlations between chlorophyll and yield, and nitrogen from mineral sources generated superior effects on the analyzed plant parameters.

Photosynthetic pigments were analyzed from the perspective of potential strategies for increasing crop yields by developing new directions within molecular biotechnologies (Simkin et al., 2021).

Peng et al. (2021) analyzed the variation of photosynthetic pigments and photochemical processes in relation to the rate of nitrogen application to rice. The authors of the study recorded the increase in the content of photosynthetic pigments with the increase in the nitrogen rate. Also, the authors obtained parabolic relations that described the rice yield in relation to the applied nitrogen rate. Zhang et al. (2021) studied the variation in the content of photosynthetic pigments, and some physiological processes in relation to the degree of shading of the wheat crop in intercropped cultivation systems. The authors of the study found that in the case of intercrops, but
different degrees of shading depending on the fruit tree species, adequate fertilization with nitrogen can contribute to the regulation of photosynthetic capacity.

The variability of photosynthetic pigments was studied in the leaves of different plant species (herbaceous and arboreal) by chromatographic separation, sequential extraction and simultaneous quantification of the pigments, under very high precision conditions (Petibon, Wiesenberg, 2022).

Photosynthetic pigments in cultivated plants (e.g. zucchini) were studied in relation to treatments with biostimulators, associated with the biochemical composition of the plants (Abd-Elkader et al., 2022). The authors of the study found out the biopreparations and products with favorable effects on plants, yield and some quality elements.

In order to improve the photosynthetic attributes of plants, photosynthetic pigments, represented by chlorophyll (a, b), carotenoids, anthocyanins, were studied in relation to different priming treatments (Sherin et al. 2022).

Korotkova et al. (2022) evaluated the content of chlorophyll and carotenoids, as an effect of the treatments of wheat seeds, grown in traditional and ecological technologies. Through appropriate statistical analyses, the authors managed to differentiate the recorded results, as an effect of seed treatments, under statistical safety conditions (LSD05).

Kubar et al. (2022) analyzed the influence of the variable rate of nitrogen fertilization on photosynthetic pigments and other physiological processes in wheat and identified the fertilization rate with beneficial effects, under the study conditions.

Zhao et al. (2023) studied photosynthetic pigments and their distribution in leaves in relation to ten different applications of nitrogen, and based on experimental data, through partial regression analysis, they obtained quantitative models with statistical certainty.

This study analyzed the variation of photosynthetic pigments, represented by chlorophyll (Chl) and carotenoids (Car) in wheat leaves, PG102 variety, and the calculated ratios (Chl/Car, Car/Chl), in relation to mineral fertilization with nitrogen, and formulated models of pigment content variation depending on the growth rate of nitrogen doses.

**MATERIAL AND METHODS**

The study was organized and carried out at ULS "King Mihai I" in Timisoara, Romania. The biological material was represented by the PG102 wheat variety. The wheat culture was carried out on a chernozem type soil, under non-irrigated culture conditions, figure 1. Mineral fertilization with nitrogen was applied, in variable doses between 0 – 280 kg a.s. ha⁻¹ (a.s. – active substances). Granulated ammonium nitrogen fertilizer was used, with a content of 33.5% N a.s. The climatic conditions during the vegetation period, agricultural year 2021-2022, are presented in figure 2.

The determination of the content of photosynthetic pigments was made on the standard leaf, at the time of flowering, BBCH code 6 - Flowering, anthesis (Meier, 2001). Leaf samples were taken randomly, on which the determinations of the two photosynthetic pigments, chlorophyll (Chl) and carotenoids (Car) were made.

Chlorophyll content was determined with the SPAD-502Plus chlorophyll meter (KONICA MINOLTA). The carotenoid content was determined with the ACM-200 Plus apparatus (OPTI-SCIENCES). Based on the recorded data, the ratio between the values of the photosynthetic pigments (Chl/Car and Car/Chl) was calculated.

Figure 1. Aspect from the experimental field
The experimental data were analyzed under the aspect of statistical safety and the presence of the variant (Anova test). The distribution of the experimental values and the difference between the data series on experimental variants was analyzed, independently for Chl and Car. The variation of photosynthetic pigment values in relation to nitrogen doses was analyzed. For the analysis of experimental data, determinations and the generation of graphs, the calculation module in EXCEL and dedicated applications were used (Hammer et al., 2001).

RESULTS AND DISCUSSIONS

The determination of the photosynthetic pigments in the wheat leaves, variety PG102, led to the values presented in table 1 (average values and standard error calculated for each variant). Based on the values of chlorophyll content (Chl) and carotenoid content (Car), the ratios Chl/Car and Car/Chl were calculated, table 1.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Chl</th>
<th>Car</th>
<th>Chl/Car</th>
<th>Car/Chl</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>43.73±1.04</td>
<td>10.12±0.59</td>
<td>4.320±0.326</td>
<td>0.232±0.016</td>
</tr>
<tr>
<td>V2</td>
<td>51.64±1.59</td>
<td>12.71±0.78</td>
<td>4.063±0.307</td>
<td>0.246±/-0.17</td>
</tr>
<tr>
<td>V3</td>
<td>56.06±1.02</td>
<td>15.16±0.67</td>
<td>3.699±0.155</td>
<td>0.270±0.012</td>
</tr>
<tr>
<td>V4</td>
<td>56.59±1.47</td>
<td>16.89±1.03</td>
<td>3.351±0.194</td>
<td>0.298±0.016</td>
</tr>
<tr>
<td>V5</td>
<td>57.77±1.01</td>
<td>20.01±0.79</td>
<td>2.887±0.097</td>
<td>0.346±0.012</td>
</tr>
<tr>
<td>V6</td>
<td>58.92±1.21</td>
<td>21.56±0.49</td>
<td>2.734±0.044</td>
<td>0.366±0.006</td>
</tr>
<tr>
<td>V7</td>
<td>57.73±1.21</td>
<td>19.81±0.82</td>
<td>2.914±0.082</td>
<td>0.343±0.010</td>
</tr>
<tr>
<td>V8</td>
<td>56.40±1.41</td>
<td>16.71±0.54</td>
<td>3.375±0.044</td>
<td>0.396±0.004</td>
</tr>
</tbody>
</table>

The data series for chlorophyll (Chl) and carotenoids (Car) showed normal distribution, with graphic representation in figure 3 (a) for chlorophyll (Chl), respectively in figure 3 (b) for carotenoids (Car). According to the analysis, the values of the correlation coefficient for chlorophyll (Chl) on experimental variants were: r=0.975 (N0), r=0.945 (N40), r=0.983 (N80), r=0.973 (N120), r=0.873 (N160), r=0.913 (N200), r=0.978 (N240), r=0.899 (N280).

In the case of the carotenoid content (Car), the values of the correlation coefficient with the former:
r=0.971 (N0), r=0.903 (N40), r=0.993 (N80), r=0.977 (N120), r=0.987 (N160), r=0.981 (N200), r=0.906 (N240),
r=0.913 (N280).

![Graphical representation for Chl and Car, as normal probability plot, in relation to the experimental variants, wheat variety PG102](image)

The ANOVA test (one-way ANOVA) was used to analyze the data series of the photosynthetic pigments, CHl and Car. From the analysis of the CHl data series, test for equal means, the values in table 2 resulted.

![Graphical representation for Chl and Car, as normal probability plot, in relation to the experimental variants, wheat variety PG102](image)

**Figure 3.** Graphical representation for Chl and Car, as normal probability plot, in relation to the experimental variants, wheat variety PG102

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sum of sqrs</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>p (same)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>1567.63</td>
<td>7</td>
<td>223.948</td>
<td>15.64</td>
<td>9.08E-12</td>
</tr>
<tr>
<td>Within groups</td>
<td>916.167</td>
<td>64</td>
<td>14.3151</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2483.8</td>
<td>71</td>
<td>1.00E-05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

According to Kruskal-Wallis test, for equal medians (CHl data series), resulted H (chi^2) = 34.21, Hc (tie corrected) = 34.23, p (same) = 1.56E-05, and there is significant difference between sample medians. Residual values were represented according to the diagram in figure 4, normal fit.

![Graphical representation for Chl and Car, as normal probability plot, in relation to the experimental variants, wheat variety PG102](image)

**Figure 4.** The distribution of the residual values, in the analysis of the CHl data series
From the analysis of the Car data series, test for equal means, the values from table 3 resulted. According to Kruskal-Wallis test, for equal medians (Car data series), resulted \( H (\text{chi}^2) = 53.76, \ H_c (\text{tie corrected}) = 53.77, \ p \ (\text{same}) = 2.616E-09, \) and there is significant difference between sample medians. Residuals for Car, was represented according to the diagram in figure 5, normal fit.

**Table 3. Anova test for the Car data series, wheat variety PG102**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sum of sgrs</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>p (same)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups:</td>
<td>951.733</td>
<td>7</td>
<td>135.962</td>
<td>28.14</td>
<td>2.95E-17</td>
</tr>
<tr>
<td>Within groups:</td>
<td>309.193</td>
<td>64</td>
<td>4.83115</td>
<td>Permutation p (n=99999)</td>
<td></td>
</tr>
<tr>
<td>Total:</td>
<td>1260.93</td>
<td>71</td>
<td>1.00E-05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Figure 5. Distribution of residual values, in Car values analysis](image)

The variation of Chl and Car in relation to N doses was studied by regression analysis. In the case of Chl, equation (1) described the variation of Chl in relation to N, under conditions of \( R^2=0.981, \ p<0.001, \ F=68.647. \)

In the case of carotenoid content, equation (2) described the variation of Car in relation to N, under conditions of \( R^2 = 0.986, \ p=0.00003, \ F=96.697. \) The graphical distribution of the two pigments in wheat flag leaves (Chl, Car) is presented in figure 6.

\[
\text{Chl} = -0.000395x^2 + 0.1492x + 45.05019 \tag{1}
\]

where: Chl – chlorophyll content; \( x \) – nitrogen doses (kg ha\(^{-1}\) a.s.)

\[
\text{Car} = -1.718E - 06x^3 + 0.0004449x^2 + 0.03279x + 10.35 \tag{2}
\]

where: Car – carotenoid content; \( x \) – nitrogen doses (kg ha\(^{-1}\) a.s.)

The variation of Car in relation to Chl was described by equation (3), under conditions of \( R^2=0.978, \ p=0.00084, \ F=61.155. \) The graphic distribution of Car values in relation to Chl is presented in figure 7.

\[
\text{Car} = 0.00945x^3 - 1.391x^2 + 68.35x - 1109 \tag{3}
\]

where: Car – carotenoid content; \( x \) – Chl, chlorophyll content

Variable doses of fertilization were studied for different agricultural crops, in the reference area, from the perspective of optimizing technologies and yields, and imaging analysis methods were used to describe the wheat crop under field conditions (Sala and Boldea, 2011; Sala et al., 2020).
The variation of the chlorophyll content in the leaves was studied in relation to nitrogen in different species of crop plants and in relation to different doses of nitrogen, in order to quantify the effect of different sources of nitrogen, the way to capitalize on the nutritional conditions, the effect on the processes photosynthetic, yield and quality indices in agricultural production (Netto et al., 2005; Fornari et al. 2020; Peng et al., 2021; Kubar et al., 2022; Zhao et al., 2023).

Recent studies on the distribution of Chl and Car along the length of wheat leaves (Jurjescu and Sala, 2023). The authors of the study analyzed and described through mathematical and graphic models the variation in the content of photosynthetic pigments along the length of wheat leaves. At the same time, the authors of the study identified certain areas along the length of the limb where the content of photosynthetic pigments expresses the average value, a fact that can lead to more efficient methods for determining the content of chlorophyll and carotenoids on the limb of wheat leaves.

Similar studies were done on rice Yuan et al. (2016). The authors of the study identified a certain area
along the length of the rice leaf (2/3 from the base of the leaf) where the chlorophyll content by the non-destructive method (SPAD values) presented the average value of the content at the level of the leaves.

CONCLUSIONS
Nitrogen applied in variable doses, in the range 0 - 280 kg ha⁻¹ a.s. determined the differentiated nutrition of wheat plants, variety PG102, a fact expressed by the variable content of photosynthetic pigments at the level of the standard leaf. The data series for chlorophyll (Chl) and carotenoids (Car) showed normal distribution.

The calculated Chl/Car and Car/Chl ratios also showed a specific rate of variation, in relation to fertilization and leaf pigment content.

The variation of Chl and Car in relation to the doses of N was described by polynomial equations of the 3rd degree, under statistical safety conditions (p<0.001). The variation of Car in relation to Chl was also described by models in the form of an equation, under conditions of p<0.001.

The differences in the values of chlorophyll (Chl) and carotenoids (Car) content between the experimental variants was confirmed by the Anova test (p<0.01, significant difference between sample medians).

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