VARIATION OF PHOTOSYNTHETIC PIGMENTS AND Chl/Car RATIO IN WHEAT IN RELATION TO FERTILIZATION RATE

Florin SALA

Banat University of Agricultural Sciences and Veterinary Medicine “King Michael I of Romania” from Timisoara, Faculty of Soil Science and Plant Nutrition, 119 Calea Aradului, 300645, Timisoara, Romania

Corresponding author email: florin_sala@usab-tm.ro

Abstract

The study evaluated the content of photosynthetic pigments (Chl, Car) and the Chl/Car ratio in wheat leaves, Alex cultivar, in relation to fertilization rate. Mineral fertilizers with N (0-200 kg ha⁻¹) and P, respectively K (0-150 kg ha⁻¹) generated 11 experimental variants. The tests were performed on the flag leaf (BBCH 7 stage). The chlorophyll content (Chl) varied between 34.73 ± 1.14 units in the control variant (V1) and 60.52 ± 1.17 units in the V8 variant. The carotenoid content (Car) varied between 4.51 ± 0.18 units in the control variant (V1) and 11.41 ± 0.27 units in the V11 variant. The Chl/Car ratio was calculated, which recorded values between 7.70 ± 0.15 in the control variant (V1) and 5.27 ± 0.08 in the V11 variant. Different correlations were found between photosynthetic pigments and N, respectively P, K (eg. r = 0.832 between Chl and N; r = 0.828 between Car and N; r = 0.717 between Car and P, K; r = -0.770 Chl/Car and N; r = -0.728 between Chl/Car and P, K). The regression analysis facilitated the obtaining of some variation models of Chl, Car and the Chl/Car ratio in relation to N and P, respectively K, in statistical safety conditions.

Key words: Chl/Car ratio, fertilizers, flag leaf, photosynthetic pigments, wheat.

INTRODUCTION

Chlorophyll (a,b) and several types of carotenoids are present as pigments in the leaves of all plants (Esteban and García-Plazaola, 2016; Fernández-Marín et al., 2018). Chlorophyll (Chl) is the pigment with high significance and important role in the process of photosynthesis of plants (Li et al., 2018). Concentration values of photosynthetic pigments in leaves have specific and physiological determinations and constraints (Niinemets, 2007).

A series of studies evaluated chlorophyll but also other plant pigments in relation to different conditions and influencing factors, given the importance of photosynthetic pigments for the plant organisms functioning, and for plants farming systems productivity. The content of chlorophyll pigments in plant leaves varies according to a number of internal and external factors (Esteban et al., 2015). Chlorophyll content varies with plant species, genotypes (natural or cultivated), growth conditions, stressors and others (He et al., 2019; Motyka et al., 2020).

The content of photosynthetic pigments for non-stressed plants was evaluated from various databases, which included over 800 species (Esteban et al., 2015; Fernández-Marín et al., 2017). Ivanov et al. (2013) studied chlorophyll pigments in leaves in relation to the longitudinal gradient and found the variation of the chlorophyll/carotenoid ratio from 5.6 to 3.5.

The variation of chlorophyll and carotenoid pigments in some plant species was recorded depending on the edaphic variable condition (Zielewicz et al., 2020), different light conditions (Lima et al., 2017). The distribution of photosynthetic pigments in leaves is influenced by stress factors (environmental stress, eg. diseases, pests, heavy metal) and is considered an indicator of stress levels (Zeng et al., 2021). Leaf pigment has been studied in relation to common bean pathogens (Lobato et al., 2010). Some studies have taken into account and analyzed the size of the leaves, the total leaf area, the one affected by pathogens and the functional photosynthetic one (Drienovsky et al., 2017a, 2017b).
Non-destructive methods for determining photosynthetic pigments have been used in various studies (Cassol et al., 2008; Brito et al., 2011; Liang et al., 2017; Wood et al., 2020), with the advantages that they are practical, fast, precise and ensures the obtaining of information in the conditions of keeping the samples, the dynamic analysis of the samples, etc. The study of chlorophyll and carotenoid pigments and their ratio were evaluated by hyperspectral reflectance in Japanese horseradish (Sonobe et al., 2020). Chlorophyll and carotenoids have been studied in relation to different proteic systems protection (e.g. OHP1/2, LHC/LIL), as mechanisms for the protection of the plant photosynthetic apparatus (Psencik et al., 2020).

High importance present the content of photosynthetic pigments in agricultural crops, from the ecophysiological perspective, agro-ecosystems functionality and agricultural yields (Rawashdeh and Sala, 2013; Esteban et al., 2015; Shafiq et al., 2021). The photosynthetic pigments content has been studied in relation to the senescence of the flag leaf in wheat plants in field conditions (Lu et al., 2001). The chlorophyll content in vegetables was studied in intercropping conditions (tomato/lettuce) and differentiated use of light (da Cunha-Chiamolera et al., 2017).

Variation in chlorophyll content in some crop plant species has been studied in relation to different types and doses of fertilizers and soil management systems (Kulsum et al., 2007; Bojović and Marković, 2009; Wang et al., 2009; Davaran-Hagn et al., 2015; Fiorentini et al., 2019; Purbajanti et al., 2019). For the characterization of some species and varieties of agricultural interest, and the management of agricultural crops, some studies evaluated the chlorophyll content based on satellite or aerial images, which facilitated the creation of models for crop evaluation and estimation of agricultural production (Herbei and Sala, 2016; Constantinescu et al., 2018; Croft et al., 2019).

Photosynthetic pigments have also been studied in relation to stressors and protective mechanisms of cultivated plants. Chlorophyll and carotenoids in corn leaves have been evaluated in relation to exposure to heavy metals, e.g. cadmium (Drazkiewicz and Baszyński, 2005; Alpha et al., 2009), and in some roadside plant communities in relation to different heavy metals (Popova, 2017). The present study evaluated the variation in the content of photosynthetic pigments in wheat plants in relation to mineral fertilization.

**MATERIALS AND METHODS**

The study evaluated the content of photosynthetic pigments (chlorophyll a and b, Chl; carotenoids, Car) and the Chl/Car ratio in wheat leaves in relation to different fertilization rate with N and P, respectively K. The Alex wheat cultivar was used as a biological material.

Different levels of fertilization were performed with P, K (0, 50, 100 and 150 kg ha⁻¹) and N (0, 50, 100, 150 and 200 kg ha⁻¹), associated in 11 experimental variants (V1-V11). The fertilizers used were complex type (NPK; 15:15:15), and ammonium nitrate.

The content of photosynthetic pigments in the leaves (Chl and Car) was determined by non-destructive methods. The chlorophyll content was determined with a portable chlorophyllmeter SPAD 502Plus (KONICA MINOLTA), and the carotenoid content was determined with a portable ACM-200 Plus device (OPTI-SCIENCES).

Photosynthetic pigments (Chl and Car) were determined on the flag leaf, in the BBCH 7 stage - Development of fruit (Meier, 2001). Determinations were made on 25 leaves in each experimental variant, in three repetitions, randomized in each plot. Based on the values of the content of chlorophyll (Chl) and carotenoids (Car), the Chl/Car ratio was calculated on each leaf sample, respectively the experimental variant. The distribution of the experimental data series for the Chl, Car indices and the Chl/Car ratio was analyzed. The experimental data were analyzed by ANOVA test, correlation analysis, regression analysis, Cluster analysis. For the statistical interpretation of the results were used the parameter p (p<0.05), the correlation and regression coefficients (r, R²), the cophenic coefficient (Coph.corr.), similarity and distance indices (SDI), and RMSEP, equation (1).
\[ \text{RMSEP} = \sqrt{\frac{1}{n} \sum_{j=1}^{a} (y_j - \hat{y}_j)^2} \]  

PAST software (Hammer et al., 2001) and Wolfram Alpha (2020) were used for statistical processing and analysis of experimental data.

RESULTS AND DISCUSSIONS

Through differentiated fertilization with N and P, respectively K, wheat plants, Alex cultivar, benefited from various levels of nutrition. This was reflected and quantified in the flag leaf, in the content of photosynthetic pigments, chlorophyll (Chl) and carotenoids (Car) (Table 1). The chlorophyll content varied between 34.73 ± 1.14 SPAD units in the control variant (V1) and 60.52 ± 1.17 SPAD units, in the case of the V8 variant. The carotenoid content (Car) varied between 4.51 ± 0.18 units in the control variant (V1) and 11.41 ± 0.27 units in the V11 variant. Based on the Chl and Car values recorded, the Chl/Car ratio was calculated, which varied between 7.70 ± 0.15 in the control variant (V1) and 5.27 ± 0.08 in the V11 variant. The statistical analysis of the experimental data showed a normal distribution of the values of the studied photosynthetic pigments (Chl, Car) and of the Chl/Car ratio (Figure 1).

Table 1. Average values of photosynthetic pigments depending on fertilization rate, flag leaf, Alex wheat cultivar

<table>
<thead>
<tr>
<th>Trial</th>
<th>N</th>
<th>P, K</th>
<th>Chl</th>
<th>Car</th>
<th>Chl/Car</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>0</td>
<td>0</td>
<td>34.73±1.14</td>
<td>4.51±0.18</td>
<td>7.70±0.15</td>
</tr>
<tr>
<td>V2</td>
<td>100</td>
<td>0</td>
<td>46.17±1.15</td>
<td>6.26±0.25</td>
<td>7.38±0.19</td>
</tr>
<tr>
<td>V3</td>
<td>200</td>
<td>0</td>
<td>57.29±1.02</td>
<td>9.40±0.31</td>
<td>6.09±0.18</td>
</tr>
<tr>
<td>V4</td>
<td>50</td>
<td>50</td>
<td>45.83±0.81</td>
<td>6.94±0.21</td>
<td>6.61±0.14</td>
</tr>
<tr>
<td>V5</td>
<td>100</td>
<td>50</td>
<td>54.94±0.85</td>
<td>9.30±0.24</td>
<td>5.91±0.13</td>
</tr>
<tr>
<td>V6</td>
<td>200</td>
<td>50</td>
<td>57.16±1.33</td>
<td>10.72±0.44</td>
<td>5.33±0.13</td>
</tr>
<tr>
<td>V7</td>
<td>100</td>
<td>100</td>
<td>55.68±0.76</td>
<td>9.43±0.36</td>
<td>5.90±0.13</td>
</tr>
<tr>
<td>V8</td>
<td>150</td>
<td>100</td>
<td>60.52±1.17</td>
<td>10.87±0.35</td>
<td>5.57±0.11</td>
</tr>
<tr>
<td>V9</td>
<td>200</td>
<td>100</td>
<td>59.51±1.05</td>
<td>10.81±0.32</td>
<td>5.51±0.12</td>
</tr>
<tr>
<td>V10</td>
<td>150</td>
<td>150</td>
<td>57.91±0.59</td>
<td>10.18±0.29</td>
<td>5.69±0.12</td>
</tr>
<tr>
<td>V11</td>
<td>200</td>
<td>150</td>
<td>60.12±0.76</td>
<td>11.41±0.27</td>
<td>5.27±0.08</td>
</tr>
</tbody>
</table>

Figure 1. Distribution histograms of data series for studied physiological indices, wheat Alex cultivar (Chl data values - green colour; Car data values - red colour; Chl/Car ratio values - blue colour)
Statistical analysis on the distribution of values in each set of experimental data, led to abundance distribution models, as geometric model, for each index studied, Figure 2; k: 0.002264, \( \chi^2: 68.8, p \text{ (same): } 1 \), for Chl, figure 2 (a); k: 0.004015, \( \chi^2: 18.54, p \text{ (same): } 1 \) for Car, figure 2 (b); k: 0.002048, \( \chi^2: 3.027, p \text{ (same): } 1 \) for Chl/Car ratio, Figure 2 (c).

There was a high variation of chlorophyll content under the influence of N, on the levels of P and K respectively. Thus, at the level of PK 0, the chlorophyll content varied between 34.73 ± 1.14 and 57.29 ± 1.02 SPAD units, related to the variation N between 0 - 200 kg ha\(^{-1}\). At the PK 50 level, the chlorophyll content varied between 45.83 ± 0.81 and 57.16 ± 1.33 SPAD units, related to the N variation between 50 - 200 kg ha\(^{-1}\). At the level of PK 100, Chl varied between 55.68 ± 0.76 and 60.52 ± 1.17 SPAD units, corresponding to the variation N between 100 and 200 kg ha\(^{-1}\). At the level of PK 150, Chl varied between 57.91 ± 0.59 and 60.12 ± 0.76 SPAD units, afferent N 150 - 200 kg ha\(^{-1}\). Similarly, the variation of the carotenoid content was registered, but on a small scale of values, on the 4 levels of PK provided, and depending on the doses of N.

Different levels of correlation were found between the values of photosynthetic pigments and fertilizers (N, respectively P, K). Strong positive correlations were found between Chl and N (\( r = 0.832 \)) and between Car and N (\( r = 0.828 \)). Moderate correlations were found between Car and P (\( r = 0.717 \)), between the Chl/Car ratio and N (\( r = -0.770 \)), respectively between Chl/Car ratio and P (\( r = -0.728 \)). A weak correlation was found between Chl and P (\( r = 0.655 \)).

Also, there were very strong, positive correlations between Chl and Car (\( r = 0.977 \)), and very strong negative correlations between the values of the Chl/Car ratio and Chl (\( r = -0.939 \)), respectively between Chl/Car ratio and Car (\( r = -0.984 \)).

The interdependence relationship between the content of photosynthetic pigments (Chl and Car), under the influence of N and P, respectively K, under experimental conditions, was described by a polynomial equation of degree 2, equation (2), under conditions of...
The variation in chlorophyll (Chl) content was described by equation (7). The graphical distribution in 3D format and in the form of isoquants of Chl in relation to N (x-axis) and P, respectively K (y-axis) is shown in Figures 3 and 4. Based on the values of the equation (7) coefficients, the optimal values for N and P (respectively K) in relation to Chl in the flag leaf in wheat plants were calculated. Thus, resulted the values $x_{opt} = 142.37$ kg a.s. ha$^{-1}$ N, and $y_{opt} = 113.72$ kg a.s. ha$^{-1}$ P, respectively K.

$$\text{Chl} = ax^2 + by^2 + cx + dy + exy + f$$

where: Chl - Chlorophyll content in wheat leaves (SPAD units); x - N doses (kg a.s. ha$^{-1}$); y - P,K doses (kg a.s. ha$^{-1}$); a, b, c, d, e, f - coefficients of the equation (7); $a = -0.0016510$; $b = -0.0007375$; $c = 0.6127788$; $d = 0.3463489$; $e = -0.0012544$; $f = 0$.

From the regression analysis was obtained the equation (8) which described the variation of the carotenoid content (Car) depending on N and P, respectively K (y-axis):

$$\text{Car} = a'x^2 + b'y^2 + c'x + d'y + e'xy + f'$$

where: Car - Carotenoid content in wheat leaves (SPAD units); a', b', c', d', e', f' - coefficients of the equation (8).
and P (respectively K), as a direct and interaction effect, in conditions of statistical safety, p <0.001. The graphical distribution of Car values in relation to N and P, respectively K, is rendered in 3D and in isoquants format in Figures 5 and 6.

\[
\text{Car} = ax^2 + by^2 + cx + dy + exy + f
\]  

(8)

where: Car - Carotenoid content in wheat leaves;

- x - N doses (kg a.s. ha\(^{-1}\));
- y - P, K doses (kg a.s. ha\(^{-1}\));
- a, b, c, d, e, f - coefficients of the equation (8);

- a= -0.0001489;
- b= -0.0001960;
- c= 0.0767617;
- d= 0.0831694;
- e= -0.0002369;
- f= 0.

Based on the values of the equation (8) coefficients, the optimal values for N and P (respectively K) were calculated in relation to Car. Thus, the values \(x_{opt} = 171.39\) kg a.s. ha\(^{-1}\) N, respectively \(y_{opt} = 108.52\) kg a.s. ha\(^{-1}\) P, respectively K were obtained.

From the analysis of the optimal values of fertilizers in relation to Chl and Car, a large difference was found regarding nitrogen N, and close values for P, respectively K. Thus, in relation to Chl, the value of 142.37 kg a.s. ha\(^{-1}\) N, and in relation to Car the value of 171.39 kg a.s. ha\(^{-1}\) N, were found, with a difference of 29.02 kg a.s. N. Regarding P, respectively K, in relation to Chl the value 113.72 kg a.s. ha\(^{-1}\) (P, respectively K), and in relation to Car the value of 108.52 kg a.s. ha\(^{-1}\) (P, respectively K) was obtained, with a difference of 5.20 kg a.s.

This shows that N was determinant in the variation of the differences in the Chl/Car ratio in wheat leaves, while P and K respectively generated minor differences in the experimental conditions.

The regression analysis was also used to analyze the variation of the Chl/Car ratio in relation to N and P, respectively K, and equation (9) was obtained, in statistical safety conditions, \(R^2 = 0.827, p <0.001\).

\[
\text{Chl/Car} = ax^2 + by^2 + cx + dy + exy + f
\]  

(9)

where: Chl/Car - Chlorophyll/Carotenoids ratio in wheat, flag leaves;

- x - N doses (kg a.s. ha\(^{-1}\));
- y - P, K doses (kg a.s. ha\(^{-1}\));
- a, b, c, d, e, f - coefficients of the equation (9);

- a= -0.0003855;
- b= -0.0000391;
- c= 0.1079403;
- d= 0.0014652;
- e= -0.0000321;
- f= 0.

The graphical distribution of the values of the Chl/Car ratio in relation to N and P, respectively K, is shown in 3D form and in the form of isoquants in Figures 7 and 8.

From the analysis of the graphical distribution, figure 7, it was found that the variation of the Chl/Car ratio was significantly determined by N, and the contribution of P, respectively K was minor, in the conditions of the present study.
Cluster analysis facilitated the grouping of variants according to the degree of similarity in relation to Chl and Car, Figure 9, in statistical safety conditions (Coph. Corr = 0.868). Resulted two distinct clusters, with several subclusters. A C1 cluster comprised variants V1 (with the lowest values Chl and Car) and variants (V2, V4). Cluster C2 comprised the other 8 variants, grouped into two subclusters C2-1 and C2-2. Subcluster C2-2 comprised variants [(V8, V11) V9] with the best results for the studied photosynthetic pigments. Subcluster C2-1 included the other variants, with intermediate values for Chl and Car [(V5, V7), and V3 (V6, V10)].

From the analysis of similarity and distances indices (SDI), Table 2, a very high level of similarity was found between variants V8 and V11 (SDI = 0.73593), followed by variants V5 and V7 (SDI = 0.7514), variants V9 and V11 (SDI = 0.88865), and variants V6 and V10 (SDI = 0.99182). At the level of the other variants, the SDI values registered supraunitary values, gradually increasing, between SDI = 1.0136 at the level of variants V8 and V9, up to SDI = 26.423 which indicated the maximum distance of similarity in the case of the study (V1 and V11).
Table 2. SDI values in wheat fertilized variants, Alex cultivar, in relation to Chl and Car

<table>
<thead>
<tr>
<th></th>
<th>V1</th>
<th>V2</th>
<th>V3</th>
<th>V4</th>
<th>V5</th>
<th>V6</th>
<th>V7</th>
<th>V8</th>
<th>V9</th>
<th>V10</th>
<th>V11</th>
</tr>
</thead>
<tbody>
<tr>
<td>V11</td>
<td>26.423</td>
<td>15.019</td>
<td>3.567</td>
<td>15.033</td>
<td>5.6298</td>
<td>3.04</td>
<td>4.9021</td>
<td>0.73593</td>
<td>0.88865</td>
<td>2.5639</td>
<td>2.5639</td>
</tr>
</tbody>
</table>

The estimation of the variation of the pigments Chl and Car, as well as of the Chl/Car ratio in the wheat flag leaf, Alex cultivar, in relation to the mineral elements N and P (respectively K) was possible in statistical safety conditions. The prediction of photosynthetic pigments based on N and P (respectively K) was possible in conditions of RMSEP = 12.14465 for Chl, in conditions of RMSEP = 2.10540 for Car and respectively in conditions of RMSEP = 2.60288 for the Chl/Car ratio. The models that best described the variation of Chl, Car and the Chl/Car ratio according to the fertilizing elements were of the polynomial type of degree 2, in statistical safety conditions. The regression analysis facilitated the obtaining of equations that described the variation of the Chl, Car pigments and of the Chl/Car ratio, as a direct effect and of the interaction of the fertilizing elements, in conditions of statistical safety. Also, based on the equations resulting from the regression analysis, it was possible to find the optimal values of N and P (respectively K) in relation to Chl, Car and Chl/Car ratio.

Kulsum et al. (2007) reported linear models of variation of Chl in the species *Vigna mungo* L. in relation to N doses, in the range 0 - 100 kg N ha⁻¹, in statistical safety conditions (R² = 0.7631 to R² = 0.8599). Rawashdeh and Sala (2015) reported a significant increase (p = 0.05) in the content of chlorophyll in wheat leaves, Alex cultivar, in relation to foliar fertilization with Fe and B. Zhang et al. (2016) found the differentiated influence of the thermal factor, respectively N on Chl and Car in the species *Phragmites communis* and *Leymus chinensis*, in Temperate Meadow Ecosystem conditions. Constantinescu et al. (2018) evaluated the Chl variation in relation to different cereals genotypes and obtained a model of interdependence between fresh biomass and Chl, in the characterization of the studied genotypes.

CONCLUSIONS

The photosynthetic pigments Chl and Car showed a close variation in relation to the fertilizing elements N and P (respectively K), highlighted at the level of correlation coefficients, in conditions of statistical safety. Polynomial models, of the grade 2, described the variation Chl and Car in direct relation to N, and P, respectively K. The Chl/Car ratio showed a decreasing trend in relation to the increase of fertilizer doses (N, P and K, respectively), in the studied conditions. The regression analysis facilitated the obtaining of equations that described the variation Chl and Car under the influence of N and P (respectively K), as a direct and interaction effect. 3D and isoquants models described the variation of Chl and Car in relation to N and P (respectively K) and the optimal values of N and P (respectively K) in relation to Chl and Car were found.

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