STUDIES REGARDING WHEAT LEAVES INVESTMENTS IN RELATION WITH NITROGEN SUPPLY

Adina-Daniela DATCU¹, Florin SALA²

¹West University of Timișoara, Chemistry, Biology, Geography Faculty, Biology-Chemistry Department, 16 Pestalozzi Street, 300115, Timișoara, Romania
²Banat University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timișoara, Timișoara, 300645, Romania

Corresponding author: dana_datcu19@yahoo.com

Abstract. The purpose of this research was to describe the behavior of some determined and calculated indices for wheat leaves samples in relation with ammonium nitrate fertilizing dose. Also, relations between these indices were done. Wheat, *Triticum aestivum*, Ciprian cultivar, was grown within the Didactic Station of Banat’s University of Agricultural Sciences and Veterinary Medicine “King Michael I of Romania” from Timișoara in 2018-2019 agricultural year. Here the soil can be described as cambic chernozem, partially gleized. Wheat plants were fertilized with ammonium nitrate in five experimental doses, 0, 50, 100, 150 and 200 kg ha⁻¹, respectively. Healthy, intact, with no visible damage leaves were harvested (BBCH Code 45) and taken into a laboratory. The samples were scanned, dried in an oven, incinerated and organic matter per surface (OMPS) was determined. This index described the realized investments in organic substances in the case of leaves and had bigger values for doses between 100 and 200 kg ha⁻¹. Moreover, classical gravimetric indices, LDW – leaf dry weight, LAC – leaf ash content, OM – organic matter, were determined in order to realize correlation analysis between all parameters. Also, foliar areas, perimeters and lengths were determined. Mathematical models for foliar area, leaf dry weight and organic matter determination in relation with other indices were developed. Positive correlation were noticed between the majority of the studied indices. Also, PCA and Cluster analysis were realized, resulting in two major groups of plants: those fertilized with N0 and N50, and another group formed by N100, N150 and N200 group.

Keywords: leaves, organic matter, physiological traits, wheat crop

INTRODUCTION

Wheat (*Triticum aestivum* L.), is a member of the Poaceae family and represents the second main grain crop globally. It is one of the important cereals of the world, which meets most of the nutrients requirements for people. In 2017/2018 agricultural year, wheat output was over 761.7 million tons, and in 2019/2020 agricultural year, global demand was around 762.4 million tons (FAO, 2020). It is cultivated in environments ranging from very favorable ones in Western Europe to severely stressed ones in parts of Asia, Africa, and Australia, thereby facing various biotic and abiotic stresses. Nowadays, a major problem for wheat crops is heat stress (Shokat et al., 2020). It is estimated that a 1°C increase in global temperature can cause decrease in global wheat production by 6% (Asseng et al., 2011).

The application of chemical fertilizers to wheat crop in a timely manner consequently increases the production (Pandey et al., 2020). The growth traits of wheat are evolving dramatically when an inoculation of biological fertilizers appears for wheat (Ahmed et al., 2011). Studies have shown that the optimal use of mineral fertilizers and organic manures increases plant inputs, water use efficiency and crop field yield (Zhang et al., 2016).

Nitrogen is the main nutrient involved in growth and development, being part of various chemical compounds, including amino acids, proteins, nucleic acids, photosynthetic pigments, and enzymes (Bungard et al., 1999). A proper use of nitrogen fertilizers enhances photosynthetic rate (Lawlor et al., 1989). The amount of nitrogen necessary for cereals is dependent on the growth phase of the crop (Tranaviciene et al., 2008; Akhter et al., 2016).

Proper nitrogen and water quantities applied to crops improve biomass production (MON et al. 2016; Datcu and Sala, 2018; Datcu et al., 2019), photosynthesis (Zhang, 2018) and grain yield for wheat (Albrizio et al., 2010; Li et al., 2010; Sala et al., 2015; Dar et al., 2018). Nitrogen nutrition is largely considered as the main factor which affects storage proteins, as well as the technological quality of grain (Wieser and Seilmeier, 1999). Applications of nitrogen fertilizer can promote the absorption of soil moisture under dry-period irrigation (Li et al., 2004). Nitrogen application associated to reproductive development leads to a bigger protein synthesis and storage in grains, but a delayed fertilization can lead to restrictions in the amount of nitrogen that can be converted into quality protein (Brown and Petrie, 2006).

Leaves chlorophyll content is associated with the required nitrogen due to the fact that the amount of chlorophyll was significantly related with the nitrogen concentration in wheat plants and leaves, and thus using an appropriate nitrogen dose become an important tool for estimating wheat production (Schlichting et al., 2015; Akhter et al., 2016). Also, an index which describes the interconnection between specific leaf weight and...
ammonium nitrate dose (NSI – Nitrogen supply index), was previously described (Datcu et al., 2020). Thus, dividing this index, more exactly specific leaf weight, in organic and inorganic parts, resulting in a parameter named organic matter per surface (OMPS) became of interest.

The aim of this study was to describe the relations between leaf organic matter per foliar area and ammonium nitrate dose, and between OMPS and other physiological indices, for wheat, Ciprian cv.

MATERIAL AND METHODS
The studied plant species was *Triticum aestivum* ssp. vulgare, Ciprian cv and the experimental field was within the Didactic Station of Banat University of Agricultural Sciences and Veterinary Medicine from Timisoara.

The research was conducted in the agricultural year 2018-2019 on a slightly gleized cambic chernozem, which presented a neutral reaction (pH = 6.7-6.8), but also good humus supply (H = 3.2%) and medium fertility (Sala et al., 2016). Regarding the texture, the soil was loamy.

Didactic Station of BUASVM Timisoara has moderate continental climate and according to National Meteorology Administration data, monthly precipitations ranged between 33.7 and 78.1 l m$^{-2}$ in the last years and monthly temperature ranged was 10.85 °C.

Wheat crop was fertilized with ammonium nitrate in five doses: 0, 50, 100, 150 and 200 kg active substance ha$^{-1}$. Wheat samples were harvested in May 2019 (BBCH scale 45). Intact leaves were taken to laboratory and scanned. The obtained images were analyzed with Digimizer software, foliar area (FA), leaf perimeter (P) and leaf length (L) being obtained. Next, in order to calculate organic matter per surface and to complete correlation analysis, the samples were placed into an oven (Sauter Model), at 85 °C for 24 hours. Then, using an analytical balance (Kern model), leaf dry weight (LDW) of the samples was obtained. After this, the probes were introduced in porcelain recipients and in a calcinator (Nabertherm Model) for 2 hours, at 500 °C, with the purpose to obtain leaf ash content (LAC). Organic matter (OM) is thus the difference between LDW and LAC.

Organic matter per surface (OMPS) can be calculated using (1):

\[
\text{OMPS (mg cm}^{-2}\text{)} = \frac{\text{OM}}{\text{FA}} \times 1000
\]

(1)

The schematic diagram of methodology can be observed in Figure 1.

Data processing was realized using Microsoft Excel 2013, PAST v. 3.20 (Hammer et al., 2001), and Wolfram Alpha (2020).
RESULTS AND DISCUSSIONS

Organic matter per surface clearly varied depending on ammonium nitrate dose (Table 1). The lowest values were obtained for N 0 and N 50 experimental variants. N 200 variant presented a medium value, and the highest values were determined for N 100 and N 150 samples. Thus, between the last variants, organic depositions per surface are made more efficiently. This index presented the same trend as nitrogen supply index, described in DATCU et al. (2020). NSI, and possible OMPS, are interconnected with photosynthesis efficiency and this process is increased with a proper nitrogen nutrition, in growth studies before anthesis and fruit development.

Table 1. Variation of OMPS depending on N dose

<table>
<thead>
<tr>
<th>Experimental variant</th>
<th>N dose</th>
<th>OMPS (mg cm(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>N0</td>
<td>0</td>
<td>3.61</td>
</tr>
<tr>
<td>N50</td>
<td>50</td>
<td>3.78</td>
</tr>
<tr>
<td>N100</td>
<td>100</td>
<td>5.11</td>
</tr>
<tr>
<td>N150</td>
<td>150</td>
<td>4.80</td>
</tr>
<tr>
<td>N200</td>
<td>200</td>
<td>4.17</td>
</tr>
</tbody>
</table>

OMPS – organic mass per surface

After the completion of correlation analysis, the correlation matrix between physiological indices resulted (Table 2). FA was very high positively correlated with L (r=0.958), LDW (r=0.961), LAC (r=0.934), OM (r=0.959). Also, LAC presented the same tendencies in relation with LDW (r=0.957). OMPS presented positive, but not very high correlations with the other studied parameters. Thus, this index is very strong related only with NSI (DATCU et al., 2020), although is calculated using FA and LDW.

Table 2. Correlation matrix between the analyzed indices

<table>
<thead>
<tr>
<th></th>
<th>FA (cm(^2))</th>
<th>P (cm)</th>
<th>L (cm)</th>
<th>LDW (g)</th>
<th>LAC (g)</th>
<th>OM (g)</th>
<th>OMPS (mg cm(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA (cm(^2))</td>
<td>9.14E-09</td>
<td>6.52E-14</td>
<td>2.29E-14</td>
<td>9.26E-12</td>
<td>4.02E-14</td>
<td>0.029</td>
<td></td>
</tr>
<tr>
<td>P (cm)</td>
<td>0.877</td>
<td>1.27E-09</td>
<td>1.64E-07</td>
<td>9.79E-07</td>
<td>1.76E-07</td>
<td>0.051</td>
<td></td>
</tr>
<tr>
<td>L (cm)</td>
<td>0.958</td>
<td>0.897</td>
<td>4.23E-09</td>
<td>7.99E-08</td>
<td>4.51E-09</td>
<td>0.099</td>
<td></td>
</tr>
<tr>
<td>LDW (g)</td>
<td>0.961</td>
<td>0.839</td>
<td>0.885</td>
<td>7.58E-14</td>
<td>3.82E-36</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>LAC (g)</td>
<td>0.934</td>
<td>0.809</td>
<td>0.849</td>
<td>0.957</td>
<td>7.20E-13</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>OM (g)</td>
<td>0.959</td>
<td>0.838</td>
<td>0.884</td>
<td>1.000</td>
<td>0.947</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>OMPS (mg cm(^{-2}))</td>
<td>0.437</td>
<td>0.394</td>
<td>0.337</td>
<td>0.650</td>
<td>0.533</td>
<td>0.659</td>
<td></td>
</tr>
</tbody>
</table>


Based on the identified correlation levels, regression analysis was used to evaluate the variation of some physiological indices in wheat leaves. For a high precision, 16 decimals where used in equations (2), (3) and (4). Thus, the variation of FA in relation to P and L was described by equation (2), with statistical safety being assured R\(^2\)=0.998, p<0.001.

The graphical distribution of FA variance in regard to P (x axis) and L (y axis) is presented in 3D form in figure 2, and in figure 3 as an isoquant.

\[ FA = aP^2 + bL^2 + cx + dy + exy + f \]  (2)

where:  
FA - foliar area;  
\(x\) – leaf perimeter (P);  
\(y\) – leaf length (L);  
\(a, b, c, d, e, f\) – coefficients of the equation (2);  
\(a=0.2326894\);  
\(b=1.4636471\);  
\(c=-1.2513430\);  
\(d=3.1750442\);  
\(e=-1.1451296\);  
\(f=0\).
LDW variation in regard to LAC and FA was described by equation (3), statistical safety being assured, $R^2=0.996$, $p<0.001$. The graphical distribution of LDW in regard to LAC (x axis) and FA (y axis) is presented in 3D form in figure 4, and in as an isoquant in figure 5.

From the analysis of equation’s (3) coefficients and graphical distributions (figures 4 and 5) it was found that the LDW variation was significantly determined by the values of LAC (x axis), while FA index (y axis) had a negligible direct contribution.

$$LDW = ax^2 + by^2 + cx + dy + exy + f$$  \(3\)

where:  
LDW - foliar area;  
x – leaf ash content (LAC);  
y – foliar area (FA);  
a, b, c, d, e, f – coefficients of the equation (3);  
a= 0;  
b= -0.0003440;  
c= -4.1235717;  
d= 0.0068592;  
e= 0.6988720;  
f= 0.
Figure 4. Distribution of LDW (leaf dry weight) values, 3D plotted, in regard to LAC (x axis) and FA (y axis), Ciprian wheat cv.

Figure 5. Distribution of LDW (leaf dry weight) values, as isoquant, in regard to LAC (x axis) and FA (y axis), Ciprian wheat cv.

The variation of OM in regard to LAC and LDW was described by equation (4), statistical safety being assured, $R^2=0.999$, $p<0.001$. Graphical distribution of OM variation in regard to LAC (x axis) and LDW (y axis) is 3D plotted in figure 6, and as isoquant in figure 7.

$$OM = ax^2 + by^2 + cx + dy + exy + f$$  \tag{4}$$

where: OM - organic matter,
x – leaf ash content (LAC);
y – leaf dry weight (LDW);
a, b, c, d, e, f – coefficients of the equation (4);
a= 0;
b= -6.18618774513104E-14;
c= -1.00000000000005;
d= 1.00000000000001;
e= 5.79610370533726E-13;
f= 0.
Figure 6. Distribution of OM (organic matter) values, plotted in 3D, in regard to LAC (x axis) and LDW (y axis), Ciprian wheat cv.

Figure 7. Distribution of OM (organic matter) values, plotted as isoquant, in regard to LAC (x axis) and LDW (y axis), Ciprian wheat cv.

PCA analysis resulted in the diagram from figure 8. PC1 explained 91.206% of variance, while PC2 explained 8.1122% of variance.

Figure 8. PCA distribution diagram of variants in regard to studied indices
Cluster analysis resulted in dendrogram from figure 9. Variants grouping based on similarity, in regard the studied indices values, while statistical safety being assured (Coph. corr=0.830).

**Figure 9. Cluster grouping based on Euclidean distances**

**CONCLUSIONS**

This study presented data, on the one hand, about some physiological modifications, which appear in ammonium nitrate fertilized wheat samples. Five treatments were applied, between 0 and 200 kg active substance per hectare. The analyzed indices were obtained through scanning, drying and calcinating leaf probes. Organic matter per surface was high for N 100 and N 150 probes, suggesting that organic deposition, and thus photosynthesis, are efficient in this range. On the other hand, correlation analysis between OMPS and other gravimetric and physiological indices was accomplished, resulting not very high, but positive correlations. The rest of the parameters were strongly interconnected. Cluster analysis conducted to two groups, one consisting of no fertilized and low fertilized variants (N 0 and N 50), and the second one of N 100, N 150 and N 200 variants.

**ACKNOWLEDGMENTS**

The authors wish to thank the Didactic Station of Banat University of Agricultural Sciences and Veterinary Medicine „King Michael I of Romania” from Timisoara, but also to West University of Timisoara, Faculty of Chemistry, Biology, Geography for the equipment and the Agricultural Research and Development Station Lovrin for the wheat seed.

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