Photosynthetic efficiency and quantitative reaction of bread winter wheat to mild short-term drought conditions

Josip KOVAČEVIĆ1,*, Maja KOVAČEVIĆ1, Vera CESAR2, Georg DREZNER1, Alojzije LALIĆ1, Hrvoje LEPEDUŠ1, Zvonimir ZDUNIĆ1, Zorica JURKOVIĆ1, Krešimir DVOJKOVIĆ2, Zorana KATANIĆ1, Vlado KOVAČEVIĆ3

1Agricultural Institute Osijek, Osijek, Croatia
2Department of Biology, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia
3Faculty of Agriculture in Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

* Correspondence: josip.kovacevic@poljinos.hr

Abstract: The effect of water reduction stress on photosynthetic efficiency and the agronomic traits of 10 bread winter wheat cultivars were investigated, with an aim to develop breeding methods for improving wheat drought tolerance. In the 2008 and 2009 crop seasons, the trial was grown in vegetative pots according to the method of a 2-factorial experiment (cultivar and treatment) as a randomized complete block with 3 replications. The stressed version of the treatment was exposed to mild water reduction short-term stress 3 times (at the end of tillering stage, at the flag leaf to beginning of heading stage, and at the grain filling stage). Three photosynthetic efficiency parameters (Fv/Fm, ET0/ABS, and PIABS) were investigated at the end of the tillering stage (water content of soil: 22.4%–28.8% volume for the stressed variant and 29.8%–37.9% volume for the control), and 6 agronomic traits were estimated before and after winter wheat harvest. Significant differences were detected among cultivars for all investigated traits. The investigation revealed that water reduction stress decreased all examined agronomic traits. However, photosynthetic parameters mostly had higher values during mild short-term drought stress conditions. In addition, a significant interaction between cultivars and treatments was detected for 1000-grain weight and grain number per spike, but there was not any significant interaction for harvest index, number of spikes per pot, grain yield, and biomass per pot, nor for photosynthetic efficiency parameters.

Key words: Agronomic traits, drought stress, grain yield, photosynthetic efficiency, stability index, winter wheat cultivar

1. Introduction

Quantity of crop production per area unit and its stability depend on abiotic and biotic factors (drought, hot and cold weather conditions, chemical and mechanical soil traits, different diseases, etc.). The unfavorable abiotic factors, such as high temperature and drought conditions, cause abiotic stress; the measurable response of the plant to abiotic stress is the quantitative trait (Shao et al. 2005a; Araus et al. 2008; Collins et al. 2008).

The climate changes and its impact on the crop production are a real problem today (Vasil 2003). Due to the climate's inclination to higher temperatures with very frequent hot and drought-stress conditions, there is an urgent need for adaptation to this change in the world's wheat production regions (Shao et al. 2005a, 2005b; Reynolds et al. 2007; Araus et al. 2008). Consequently, it is important to improve drought and heat stress tolerance in wheat breeding programs (Reynolds et al. 2007; Shao et al. 2008; İlker et al. 2011). Reynolds et al. (2007) reported that, in drought environments, a number of mechanisms may be useful based on recent knowledge, such as osmotic adjustment, accumulation and remobilization of stem reserves, superior spike photosynthesis, heat and desiccation tolerant enzymes, and anatomical adaptations to conserve moisture, such as leaf rolling or waxiness. Many authors investigated water deficit or drought stress impact on agricultural plants. They reported that the water deficit has an impact through the disruption of all or some of the physiological and biochemical processes of plants, which have an effect on the rate of plant growth and yield (Ceccarelli et al. 1998, 2000; Denčić et al. 2000; Shao et al. 2005a, 2005b; Ni et al. 2009; Akhkha et al. 2011), as well as the rate of photosynthesis (Lawlor and Uprety 1991; Tezara et al. 1999; Shao et al. 2005a; Araus et al. 2008; Ni et al. 2009; Akhkha et al. 2011). Different methods have estimated that it is possible to find and select drought and heat stress tolerant genotypes (cultivars) in the early growth stage (Blum 1989; Martin and Ruiz-Torres 1992; Reynolds et al. 1994, 2007; Loggini et al. 1999; Rekika et al. 2000; Siddique et al. 2000; Dash and Mohanty 2002;
Dhanda et al. 2002, 2004; Shao et al. 2005a, 2005b; Tas and Tas 2007; Araus et al. 2008; Guóth et al. 2008; Guóth 2009; Akhkha et al. 2011; Lalić et al. 2011; Kovačević et al. 2011; Grzesiak et al. 2012). Grzesiak et al. (2012) reported a significantly positive correlation between determining changes by water deficit in the dry weight of seedlings in the greenhouse and drought susceptibility index given in field experiments for hybrids of maize (R² = 0.614) and lines of triticale (R² = 0.535). In addition, Dhanda et al. (2002, 2004) reported that the seedling stage of growth could better reflect the physiological traits of different wheat genotypes, being probable to connect with the leaf senescence (Al-Khatib and Paulsen 1984; Harding et al. 1990).

Our hypothesis is that an analysis of photosynthetic efficiency parameters in the juvenile stage of winter wheat growth may be used successfully for predicting drought tolerance, which could be useful in winter wheat breeding programs to improve grain yield and stability. The aim of this study was to compare some widely used parameters of photosynthetic performance (Fv/Fm, ET0/ABS, and PI ABS) at the end of the tillering stage.

2. Materials and methods

Ten bread winter wheat cultivars were evaluated through 2 different treatments in 60 vegetative pots in the 2008 and 2009 crop seasons. The tested bread winter wheat cultivars have commercial importance for wheat production in the Republic of Croatia. Eight of them were developed in the Agricultural Institute of Osijek (Croatia), and 2 are of foreign origin. Croatian cultivars were Super Žitarka (A1), Katarina (A2), Lucija (A3), Žitarka (A4), Alka (A5), Srpanjka (A6), Golubica (A7), and Renata (A8), while the 2 foreign cultivars were designated simply as A9 and A10. The trial plants were grown in vegetative pots applying the 2-factorial experiment method (cultivar and treatment) in a randomized complete block with 3 repetitions. The vegetative pots were filled with an upper layer (depth up to 30 cm) of soil from the experimental field. The soil had good fertility and had the same mechanical, physical, and chemical composition in every pot. Pore volume was 49%, water capacity was 39%, and air capacity was 10%. The pots were filled with the mentioned soil and saturated with water to 39% volume of soil (100% field capacity [FC] approximately, or absolute water capacity). Soil volume was 9800 cm³ per vegetative pot, and it was measured 10 days after the initial filling and saturation of soil with water. Sowing of trial material was carried out 7 days after the filling and saturation of soil with water on 20 December 2008 by sowing 32 seeds per vegetative pot. Seeds were arranged in a circle of 20 cm in diameter in 16 hills with distance of 3.9 cm between hills at a depth of 3.5 cm. The stressed version (B2) of the treatment was exposed to mild water reduction short-term stress 3 times:

a) At the end of the tillering stage (EC 29 – Eucarpia Code) (Reiner et al. 1992). The soil moisture content was kept from 29.8% to 37.9% of soil volume (76.4% to 97.18% FC) for the control (B1) and from 22.4% to 28.8% of soil volume (57.4% to 73.8% FC) for the water stress condition treatment (B2) (Figure 1).

b) During the flag leaf and the beginning of the heading stage (EC 49/51). The soil moisture content was kept from 26.5% to 35.4% (67.9% to 90.8% FC) of soil volume for the control (B1) and from 16.6% to 20.1% of soil volume (42.6% to 51.5% FC) for the water stress condition treatment (B2) (Figure 1).

c) During the grain filling period (EC 75/85). The soil moisture content was kept from 24.3% to 28.8% of soil volume (62.3% to 73.8% FC) for the control (B1) and from 20.9% to 25.0% of soil volume (53.6% to 64.1% FC) for the water stress condition treatment (B2) (Figure 1).

Figure 1. Water content in the soil in each vegetative pot with winter wheat cultivars in the period from 1 to 16 March, from 16 to 30 April, and from 16 to 31 May with different regimes of soil moisture (B1 – soil water content near a “well-watered” level; B2 – short-term stress condition group, 3 pauses in the adding of water).
The moisture level of the soil in the pots was checked every day using the portable Watermark soil moisture sensor by weighing every pot at 39% volume water content (100% absolute water capacity of soil). All the pots of both the control treatment (B1) and the drought stress treatment (B2) were weighed immediately after inducing maximum drought stress in B2 pots. Water content was calculated as the difference between the water weight at 39% volume (100% FC) in the soil and the volume of the water deficit in each vegetative pot of both treatments. It varied from 16.6% to 39% volume of soil. During the crop seasons, the temperature of the air ranged from −3.9 °C to 32.9 °C, and relative air humidity ranged from 25.8% to 99%.

The photosynthetic efficiency parameters were observed at the end of tillering stage (EC 29) at the same time for B1 and B2 treatments shortly after causing maximum drought stress conditions (12 March) (Figure 1). The measuring of these parameters was performed on the second leaf from the top (n = 3 plants per vegetative pot, 180 plants in total for both treatments) by portable fluorometer Handy Plant Efficiency Analyser (Handy PEA, Hansatech Instruments Limited, King’s Lynn, Norfolk, UK) according to the method of Strasser et al. (1995), as follows: maximum quantum yield of primary photochemistry II (Fv/Fm), quantum yield of electron transport (ET0/ABS), and photosynthetic performance index (PIABS).

Strasser et al. (1995, 2004), Oukarroum et al. (2007), and Lin et al. (2009) described in detail the photochemical and physiological aspects and the methods of the monitoring and calculation of the photosynthetic efficiency parameters Fν/Fm, ET0/ABS, and PIABS by using the values at the steps OJIP of the polyphasic rising transient (JIP-test).

Six agronomic traits were analyzed before and after winter wheat harvest:

- Analysis based on 10 randomly chosen plants in each pot (n = 10 plants per pot, 600 plants in total for both treatments): grain number per primary spike (GNS).
- Analysis based on vegetative pot (n = 1 per pot, 60 pots in total for both treatments): spike number (SNP), 1000-grain weight (TGW), biomass weight (the total weight of air-dried plants without root) (BWP), harvest index (ratio between grain weight per pot and biomass weight per pot; HI), and grain yield (GYP).

Indices of stress tolerance of the winter wheat cultivar were calculated as the yield stability index (YSI) of the cultivars (Bouslama and Schapaught 1984; Talebi et al. 2009) with reference to the stability indices (SIs) for all the estimated agronomic traits and the photosynthetic parameters, in the same way as was done for grain yield by using the following formula:

\[
\text{YSI} = \frac{\text{YiB2}}{\text{YiB1}}, \quad \text{or} \quad \text{SI} = \frac{\text{SI}}{\text{mean value of each cultivar in B1 treatment}}
\]

\[
\text{YiB1} = \text{grain yield of the “i” winter wheat cultivar in B1 treatment (control)},
\]

\[
\text{YiB2} = \text{grain yield of the “i” winter wheat cultivar in B2 treatment,}
\]

\[
i = \text{from 1 to 10.}
\]

All biometrical analyses were performed using SAS 9.1 statistical software (SAS Institute 2003).

3. Results

The analysis of variance showed that the differences between cultivars were highly significant (Tables 1 and 2) for all examined photosynthetic parameters: Fν/Fm (Figure 2a), ET0/ABS (Figure 2b), and PIABS (Figure 2c). The analysis of variance also showed a highly significant difference between cultivars for the examined agronomic characters (Tables 1 and 2): GNS (Figure 2d), SNP (Figure 2e), TGW (Figure 2f), BWP (Figure 3a), HI (Figure 3b), and GYP (Figure 3c). The treatment produced significant effects on ET0/ABS (Figure 2b; Tables 1 and 2) and PIABS (Figure 2c; Tables 1 and 2), as well as on all of the examined agronomic characters (Tables 1 and 2; Figures 2d–2f and 3a–3c). In addition, the treatment did not produce significant effects for only Fν/Fm (Table 1; Figure 2a). Interactions between cultivars and treatments were significant for photosynthetic efficiency parameters ET0/ABS (Figure 2b) and PIABS (Figure 2c), as well as for GNS (Figure 2d) and TGW (Figure 2f; Tables 1 and 2).

Cultivars A2, A3, and A5 had lower values of photosynthetic parameters Fν/Fm (Figure 2a), ET0/ABS (Figure 2b), and PIABS (Figure 2c) in the control treatment (B1) than in short-term drought stress treatment (B2). The differences between the treatment groups for cultivars A2, A3, and A5 were significant at the level P < 0.01 for the parameters ET0/ABS (Figure 2b) and PIABS (Figure 2c) (Table 2). Consequently, cultivars A2, A3, and A5 had the best stability index values for parameter ET0/ABS (1.105, 1.092, and 1.074, respectively) (Figure 4). The values of photosynthetic efficiency parameters ET0/ABS (Figure 2b) and PIABS (Figure 2c) were also lower in the control treatment (B1) for cultivars A6 and A7, which was significant at level P < 0.05 (Table 2) and which caused the respective values of the stability indices of the mentioned parameters (Figure 4).

The influence of the short-term drought stress on winter wheat cultivars (B2 treatment) caused a significant difference in terms of decreasing values for all investigated agronomic traits (Figures 2d–2f and Figures 3a–3c; Table 2), except for GNS in cultivar A6 (Figure 2d) and TGW in cultivar A7 (Figure 2f). These responses in the opposite
Parameters ET$_0$/ABS (Figure 2b) and PI$_{ABS}$ (Figure 2c) had significantly higher values on average for mild short-term drought stress treatment (B2) compared to the control (B1), while parameter F$_v$/F$_m$ (Figure 2a) mainly had the same values in the drought stress treatment (B2) and control (B1) (Table 1).

Cultivar A5 had the highest values of GYP (Figure 3c) and the best stability index of HI (1.045) (Figure 4). Cultivar A5 also had better stability indices of the photosynthetic parameters F$_v$/F$_m$, ET$_0$/ABS, and PI$_{ABS}$ (Figure 4).

Accordingly, winter wheat cultivar A3, with higher values of GYP (Figure 3c), had the best stability indices of SNP (1.045), BWP (0.913), and GYP (0.893) (Figure 4). The highest stability index of GNS (1.190) was that of cultivar A7 (Figure 4). It is clear that the examined winter wheat cultivars A2, A3, A5, and A6, with better stability indices of the photosynthetic parameters F$_v$/F$_m$, ET$_0$/ABS, and PI$_{ABS}$ (Figure 4), had higher values of GNS (Figure 2d), HI (Figure 3b), and GYP (Figure 3c), and better stability indices for the mentioned agronomic characters (Figure 4).

### Table 1. The variance and F-test results for winter wheat cultivars, treatments, and interactions in the trial under control (B1) and in short-term drought stress conditions (B2).

<table>
<thead>
<tr>
<th>Source of variability</th>
<th>Replication</th>
<th>Cultivar</th>
<th>Treatment</th>
<th>Interaction</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSD0.05</td>
<td>2.0000000</td>
<td>9.0000000</td>
<td>1.0000000</td>
<td>9.0000000</td>
<td>159.0000000</td>
</tr>
<tr>
<td>F$_v$/F$_m$</td>
<td>0.000444*</td>
<td>0.000259**</td>
<td>0.000209</td>
<td>0.000094</td>
<td>0.000085</td>
</tr>
<tr>
<td>ET$_0$/ABS</td>
<td>0.002096*</td>
<td>0.003696***</td>
<td>0.015500***</td>
<td>0.001237**</td>
<td>0.000486</td>
</tr>
<tr>
<td>PI$_{ABS}$</td>
<td>0.1782</td>
<td>0.3905***</td>
<td>1.7910***</td>
<td>0.1310*</td>
<td>0.0675</td>
</tr>
<tr>
<td>n – 1</td>
<td>2</td>
<td>9</td>
<td>1</td>
<td>9</td>
<td>578</td>
</tr>
<tr>
<td>GNS</td>
<td>105.97</td>
<td>2436.20***</td>
<td>5298.48***</td>
<td>737.37***</td>
<td>61.31</td>
</tr>
<tr>
<td>n – 1</td>
<td>2</td>
<td>9</td>
<td>1</td>
<td>9</td>
<td>38</td>
</tr>
<tr>
<td>SNP</td>
<td>3.02</td>
<td>67.19***</td>
<td>19.27*</td>
<td>5.42</td>
<td>4.07</td>
</tr>
<tr>
<td>TGW</td>
<td>0.36</td>
<td>91.34***</td>
<td>7.17*</td>
<td>23.09***</td>
<td>1.56</td>
</tr>
<tr>
<td>BWP</td>
<td>14.11</td>
<td>73.51**</td>
<td>3552.78***</td>
<td>29.52</td>
<td>20.44</td>
</tr>
<tr>
<td>HI</td>
<td>0.000080</td>
<td>0.008814***</td>
<td>0.006691***</td>
<td>0.00533</td>
<td>0.00496</td>
</tr>
<tr>
<td>GYP</td>
<td>0.64</td>
<td>75.25***</td>
<td>780.97***</td>
<td>6.56</td>
<td>7.71</td>
</tr>
</tbody>
</table>

F-test is significant: * P < 0.05; ** P < 0.01; *** P < 0.001.

### Table 2. Test of the difference between the average values of traits of 10 winter wheat cultivars for the control treatment (well-watered) (B1) and short-term stress condition treatment (3 pauses in the adding of water) (B2), and the averages of treatments and differences between treatments.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Treatment B1</th>
<th>Treatment B2</th>
<th>Average of treatments</th>
<th>Difference B1–B2</th>
</tr>
</thead>
<tbody>
<tr>
<td>F$_v$/F$_m$</td>
<td>Ns</td>
<td>Ns</td>
<td>0.0030</td>
<td>0.0030</td>
</tr>
<tr>
<td>ET$_0$/ABS</td>
<td>0.0182</td>
<td>0.0243</td>
<td>0.0140</td>
<td>0.0140</td>
</tr>
<tr>
<td>PI$_{ABS}$</td>
<td>0.2290</td>
<td>0.3023</td>
<td>0.2617</td>
<td>0.2617</td>
</tr>
<tr>
<td>GNS</td>
<td>4.39</td>
<td>5.79</td>
<td>5.02</td>
<td>0.63</td>
</tr>
<tr>
<td>SNP</td>
<td>4.04</td>
<td>5.54</td>
<td>5.29</td>
<td>1.55</td>
</tr>
<tr>
<td>TGW, g</td>
<td>2.32</td>
<td>3.19</td>
<td>2.75</td>
<td>0.43</td>
</tr>
<tr>
<td>BWP, g pot$^{-1}$</td>
<td>7.83</td>
<td>10.73</td>
<td>9.27</td>
<td>2.94</td>
</tr>
<tr>
<td>HI</td>
<td>0.027</td>
<td>0.037</td>
<td>0.034</td>
<td>0.007</td>
</tr>
<tr>
<td>GYP, g pot$^{-1}$</td>
<td>4.23</td>
<td>5.79</td>
<td>5.28</td>
<td>1.55</td>
</tr>
</tbody>
</table>
4. Discussion

Results of this paper show that there were relationships between the higher GNS (Figure 2d), lower TGW (Figure 2f), and higher HI (Figure 3b) and the higher GYP (Figure 3c). These were especially observed in winter wheat cultivars A2, A3, and A5. Higher stability indices of the photosynthetic efficiency parameters (SIs of $F_{v}/F_{m}$, $ET_{0}/ABS$, and $PI_{ABS}$) (Figure 4) had wheat cultivars with higher GYP values (A2, A3, and A5) (Figure 3c), which also had a higher stability index for GYP (YSI) (Figure 4). In addition, the same wheat cultivars possessed higher values of water use efficiency (unpublished data). That is why the photosynthetic efficiency parameters can be good for predicting important agronomic traits, such as grain yield and stability, in the juvenile stage of bread winter wheat growth. Similar intentions in the investigation of the photosynthetic efficiency parameters of wheat and barley were reported by Arnau et al. (1997), Lu and Zhang (1999), Rekika et al. (2000), Siddique et al. (2000), Mohanty (2003), Shao et al. (2005a, 2005b), He et al. (2006), Oukarroum et al. (2007), Paknejad et al. (2007), and Parry et al. (2011). Shao et al. (2005a) pointed out that wheat photosynthesis is the basis for wheat yield (at 90%–95%) and quality, mainly attained by physiological regulation in the field and highly linked with growth-developmental phases. Shao and Chu (2005) and Shao et al. (2005a, 2005b, 2006, 2008) pointed out the essential role of plant molecular biology and genetic control of plant physiology, aiming to improve the abiotic stress tolerance and yield by breeding methods. Furthermore, Krouma (2010) pointed out indications of
Figure 3. The average values of 10 winter wheat cultivars for a) biomass weight per pot (BWP), b) harvest index (HI), and c) grain yield per pot (GYP) of control treatment (B1) and short-term stress condition treatment (B2), and the averages of the treatments.

Figure 4. Stability indices for 10 winter wheat cultivars: a- maximum quantum yield of primary photochemistry II (Fv/Fm), b- quantum yield of electron transport (ET0/ABS), c- photosynthetic performance index (PIABS), d- grain number per spike (GNS), e- spike number per pot (SNP), f- 1000-grain weight (TGW), g- biomass weight per pot (BWP), h- harvest index (HI), i- grain yield per pot (GYP).
a strong relationship between photosynthesis and osmotic adjustment and between stomatal conductance and water status under drought stress for chickpea genotypes.

The results of this paper are consistent with those reported by Shao et al. (2005a), Huseynova et al. (2010), and Balouchi (2010). They reported that the difference of photosynthetic parameters between drought stress condition and control (well-watered) was less in drought-tolerant wheat cultivars (Huseynova et al. 2010), whereas Balouchi (2010) reported higher values of Fv/Fm under water stress conditions in relation to controls for Australian wheat genotypes. Shao et al. (2005a) reported different reactions of wheat cultivars to drought conditions, because drought-tolerant cultivars expressed a higher photosynthesis rate at a lower level of soil water capacity (45% FC) monitored during the seedling and tillering stage. Additionally, Shao et al. (2005a, 2005b) detected changes in the activities of antioxidative enzymes (peroxidase, superoxide dismutase, and catalase) and photosynthetic parameters (photosynthesis rate, stomatal conductance, and transpiration rate) depending on genotypes, growth stages, soil water levels, and intensity of stress conditions. Recent studies have reported that stress signals are influential in a signal system, which can control defensive gene expression in plants to resist drought, especially increasing NADP-malic enzyme activity, which compensates the decrease of another enzyme’s activity under drought stress, and is more expressed in C3 plants in relation to C4 plants, closely connected with photosynthesis (Shao et al. 2008; Liu et al. 2010).

In this paper, the stability indices of the photosynthetic parameters ET0/ABS and PIABS showed the same results for cultivars A2, A3, A5, and A6. Furthermore, it is possible to point out the similarity in the grouping results on the basis of genetic similarity of cultivars Srpanjka (A6), Alka (A5), and Lucija (A3) than the grain yield of cultivars Golubica (A7) and Žitarka (A4). The results of the winter wheat grain yield of the mentioned cultivars obtained in the trials during many growth seasons and on many locations were relatively similar to the results of this paper for GYP. Dvojković (2009) reported a high degree of genetic similarity of cultivars Srpanka (A6), Lucija (A3), and Alka (A5); among cultivars Golubica (A7), Žitarka (A1), and Žitarka (A4); and between foreign cultivars A9 and A10.

Based on this research, it is possible to infer the existence of a connection between the photosynthetic parameters (Fv/Fm, ET0/ABS, and PIABS) monitored at the end of the tillering stage and the components of grain yield and stability for the examined winter wheat cultivars, and the relationship with their genetic diversity, as well, which can be useful in wheat breeding.

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KOVÁČEVIĆ et al. / Turk J Agric For


393