Comparative Study on the Effect of Soil Water Stress on Photosynthetic Function of Triticale, Bread Wheat, and Barley

E. Roohi1, Z. Tahmasebi-Sarvestani1*, S. A. M. Modarres-Sanavy1, and A. Siosemardeh2

ABSTRACT

In order to compare photosynthetic features of triticale, bread wheat, and barley under water deficit conditions, this experiment was carried out in Kurdistan province in western Iran. Four genotypes of triticale, three cultivars of bread wheat, and a new variety of barley were compared under well watered (Ψsoil = -3 bars) and soil water deficit (Ψsoil = -12 bars) conditions in a randomized complete block design (RCBD) with a split-plot arrangement. Biomass production at anthesis accompanied with gas exchanges and chlorophyll fluorescence of flag leaves, as indicators of photosynthetic function of plant, were determined in each treatment. The results showed that by imposing water deficit, photosynthetic rate (Pn) was reduced in all studied genotypes irrespective of plant species. Under water deficit conditions, the highest Pn (6.86 and 5.90 µmol m$^{-2}$ s$^{-1}$) was found in bread wheat variety Pishgam and, triticale genotype No. 1, while the lowest value (3.63 µmol m$^{-2}$ s$^{-1}$) was found in barley variety Bahman. Similar trend was observed for the maximum quantum yield of primary photochemistry ($F_{v}/F_{m}$) and performance index (PI).

Significant positive correlations were found between biomass production at anthesis and both $P_{n}$ ($r = 0.83^{**}$) and $F_{v}/F_{m}$ ($r = 0.77^{*}$). Our data revealed the better performance of Pishgam than Alvand and Zarrin in the reduction rate of biomass at anthesis and photosynthetic features against soil water deficit conditions. Overall, triticale was less affected by water deficit in comparison with wheat and barley in terms of photosynthetic function as indicated by less reduction in $P_{n}$, PI, and $F_{v}/F_{m}$.

Keywords: Barley, Bread wheat, Chlorophyll fluorescence, Gas exchanges, Triticale.

INTRODUCTION

Long-term dehydration in C3 plants impairs various physiological processes and, especially, inhibits photosynthesis (Chaves, 1991; Hura et al., 2007) through decreased CO₂ availability by limiting both stomatal and non-stomatal factors (Flexas et al., 2004; Subrahmanyam et al., 2006; Chaves et al., 2009). These effects vary with the duration and intensity of drought, as well as by plant species (Lawlor and Cornic, 2002; Munns, 2002; Flexas et al., 2004; Galmé’s et al., 2007). Stomata close in response to drought, therefore, the supply of CO₂ is limited, and the photosynthetic apparatus becomes predisposed to increase energy dissipation and down-regulation of photosynthesis when the plant is subjected to high light and temperature (Chaves et al., 2009). Although triticale has been shown to have higher dry matter accumulation and photosynthetic rate than wheat (Winzeler et al., 1987; Bobodzhanov et al., 1990) some studies have suggested that tetraploid wheat had higher water use efficiency than triticale (T300) under drought (Morant-Avice et al., 1994).

Chlorophyll fluorescence has been used as a rapid technique to estimate the operating quantum efficiency of electron transport throughout PS II in leaves (Genty et al., 1989) and its relationship with CO₂ assimilation (Siebke et al., 1997). The genetic

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variability of the net photosynthetic rate, its relative parameters, and chlorophyll fluorescence have been reported previously for small grain cereals (Sarrafi et al., 1989; Grzesiak et al., 2003; Jiang et al., 2006; Hura et al., 2007; Grzesiak et al., 2007). Although these three cereals have been separately investigated in terms of their chlorophyll fluorescence (Lu and Zhang, 1998; Hura et al., 2007; Guo et al., 2009), comparative studies have not been designed clearly for the assessment of any advantages of triticale under conditions of considerable water limitation.

A better understanding of the physiological traits that enable triticale plant to adapt to drought stress and maintain growth, development, and productivity during stress periods would help in breeding for drought resistance. We hypothesized that there were differences in physiological responses among triticale, bread wheat, and barley species under long-term drought stress. Therefore, the comparative study presented here was carried out under limiting water conditions in order to (i) compare the biomass performance and the changes in gas exchange and chlorophyll fluorescence, as methods to study both stomatal and non-stomatal limitations effects on photosynthesis and (ii) determine if any of these parameters may be useful as selection criteria for the breeding of different types of cereals for tolerance to water stress.

### MATERIALS AND METHODS

#### Plant Materials and Growth Conditions

The experiment was conducted during the growing season of 2009-2010 at the Experimental Field of Grizeh Station in Kurdistan Province, west of Iran. The experimental design was a randomized complete block design (RCBD) with a split-plot arrangement. Two water regimes were used for the main plots, which comprised sub-plots with eight genotypes, including four spring triticale lines, three bread wheat cultivars, and one barley variety (Table 1), as follows: irrigation to achieve a soil water potential of -3 bars ($\Psi_{soil} = -3$ bars) i.e. well watered treatment (WW), and irrigation to achieve a soil water potential of -12 bars ($\Psi_{soil} = -12$ bars) i.e. water deficit treatment (WD). Plots were replicated three times. Irrigation was set according to the moisture retention curve of the soil. During rainy days, a mobile rain shelter for each block ($6 \times 10 \times 2.5$ m) was used in the WD treatment to prevent infiltration of the rain, consequently, the soil moisture was retained at a level of -12 bars. To limit the lateral penetration into the soil, a drainage ditch was dug around the rain shelter. A drip irrigation system with drip lines between the crop rows was used. Irrigation was applied from the developmental stage of

<table>
<thead>
<tr>
<th>Crop</th>
<th>No</th>
<th>Variety/Pedigree</th>
<th>Growth habit</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triticale</td>
<td>No. 1</td>
<td>ET-79-17, ARDI-1/TOPO 1419//ERIZO-9 cty 87352</td>
<td>Spring</td>
<td>CIMMYT a</td>
</tr>
<tr>
<td></td>
<td>No. 2</td>
<td>Juanillo-092</td>
<td>Spring</td>
<td>CIMMYT</td>
</tr>
<tr>
<td></td>
<td>No. 3</td>
<td>ET-82-15, RONDO/BANT_5//ANOAS_2/3/VICUNA_4</td>
<td>Spring</td>
<td>CIMMYT</td>
</tr>
<tr>
<td></td>
<td>No. 4</td>
<td>ET-82-18, SRIER-29/FARS-1//MANATI-1</td>
<td>Spring</td>
<td>CIMMYT</td>
</tr>
<tr>
<td></td>
<td>No. 5</td>
<td>Alvand</td>
<td>Winter</td>
<td>SPII b</td>
</tr>
<tr>
<td>Bread</td>
<td>No. 6</td>
<td>Pishgam</td>
<td>Winter</td>
<td>SPII</td>
</tr>
<tr>
<td>Wheat</td>
<td>No. 7</td>
<td>Zarrin</td>
<td>Winter</td>
<td>SPII</td>
</tr>
<tr>
<td>Barley</td>
<td>No. 8</td>
<td>Bahman</td>
<td>Winter</td>
<td>SPII</td>
</tr>
</tbody>
</table>

a International Maize and Wheat Improvement Center.
b Seed and Plant Improvement Institute of Iran.

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Table 1. The list of crop species and genotypes with their growth habit and source.
Soil Water Stress and Photosynthetic Function

stem elongation onwards. A water flow meter and a Time-Domain Reflectometry (TDR) were used to measure the amount of applied irrigation water and soil water content, respectively. Conventional cultural practices were implemented, including the usual sowing rate, fertilizer application, and post-emergence herbicide application, followed by hand-hoeing where necessary to control the weeds. Planting was performed on 23 October, and ample rainfall occurred on 28 October, which helped crop emergence. Each plot consisted of 6 rows, 20 cm apart.

**Measurements**

The date of anthesis was recorded for each plot when 50% of the spikes of the main shoots had either visibly exerted anthers or when the anthers that had dehisced were observed through the palea (Estrada–Compuzano et al., 2008). Three flag leaves were labeled and representative physiological traits, including gas exchanges, chlorophyll fluorescence parameters, water loss and relative water content were measured. To determine the biomass yield at anthesis (BYAA), 50 plants were harvested and oven dried at 80 °C for 48 hours and then weighed.

The reduction in value of each variable by water deficit treatment, WD, which was taken as an approximate representation of its sensitivity to water stress, was calculated according to Blum et al. (1983), as follows:

\[
\% \text{ reduction} = 100 \times \frac{\text{WW} - \text{WD}}{\text{WW}} \quad (1)
\]

Where, WW and WD are the values of the variables under the control and stress conditions, respectively

**Leaf Gas Exchange**

The leaf gas exchanges were measured on three labeled, fully expanded, flag leaves using a gas analyzer device (IRGA,ADC, LCA4.UK). The net photosynthesis rate (Pn), transpiration rate per leaf area (E), stomatal conductance (gs), internal concentration of CO₂ (Ci), and apparent carboxylation efficiency (Pn/Ci) were determined at a light intensity of 1,200–1,600 µmol (photon) m⁻² s⁻¹ and ambient CO₂ concentration of 380–400 ppm.

**Chlorophyll Fluorescence Parameters**

The same three flag leaves labeled to measure the gas exchanges were fully dark-adapted to determine chlorophyll fluorescence parameters using a plant stress meter (Handy PEA V1.3, U.K). Based on definitions of Yusuf et al. (2010) and Han et al. (2009), the following parameters were determined:

- \( F_{V/Fm} \): Maximum quantum yield of primary photochemistry, PS II;
- \( F_{V/Fo} \): Ratio of the variable fluorescence to the ground fluorescence (Maxwell and Johnson, 2000);
- \( PI \): Performance index (potential) for energy conservation from excitation to the reduction of PSI end acceptors;
- \( Dl0/CSo \): Dissipated energy flux per excited cross section at \( t = 0 \);
- \( RC/CSm \): Maximum number of active reaction centers.

**Water Relations**

Relative water content and water loss were determined to express water relations. Five flag leaves of each genotype from the WW treatments were sampled to determine the water loss (WL). The leaves were cut and weighed (fw₁), allowed to desiccate in a dark room at 25°C and, after 24 hours, reweighed (fw₂). The leaves were then oven dried at 80°C (dw) for 24 hours, and the water loss was calculated by the following formula (Grzesiak et al., 2003):

\[
WL = (fw_1 - fw_2) \times dw^{-1} \quad (2)
\]

**Relative Water Content**

Relative water contents (RWC) were determined for detached leaves. The relative water contents (RWC) were calculated from
flag leaf blades using the method devised by Mata and Lamattina (2001) with the help of the following equation: 

$$RWC(\%) = \frac{(FW - DW)}{(TW - DW)} \times 100$$

(3)

The fresh weight (FW) was measured immediately after excision, the full turgid weight (TW) was determined after rehydration of the leaves placing them in a test tube containing distilled water for 24 hours at 4°C in darkness, and the dry weight (DW) was determined after oven drying at 80°C for 48 hours.

**Statistical Analysis**

All of the data were analyzed using one-way analysis of variance. The means±SE were used to compare the data. Simple correlation analyses were used to determine the relationships between the measured parameters.

**RESULTS**

The total rainfall in the 2009-2010 growing season was 455 mm, compared to the long-term average of 450 mm. However, the rainfall pattern showed a normal distribution during the growth season. Approximately 40% of the seasonal rain occurred during autumn and 29% during winter, whereas 31% of the rain fell during spring, from the end of March to the middle of May. The rainfall was normally distributed, where no increase in competition for the limited resources (mainly water) occurred during the vegetative and early reproductive growth stages, except in the stress treatment, in which the plants were grown under a rain shelter.

**Biomass Production at Anthesis**

Water stress ($\Psi_{soil} = -12$ bars) significantly decreased the biomass at anthesis in our tested genotypes (Table 2). This reduction rate ranged from 17% in triticale No. 1 to 38% in barley variety Bahman. However, genotypes did not differ much in the dry matter production under soil water deficit, with the exception of Bahman. We compared the studied species based on their response to water stress using the parameter of the percent reduction for each trait. The mean biomass yield reductions due to water deficit were 26, 29, and 38% for, respectively, triticale, wheat, and barley.

**Water Loss and Relative Water Content**

A significant variation ($P \leq 0.05$) was found among the genotypes for the parameter of water loss (WL). Generally, the amount of WL in triticale ($0.259 \text{ g g}^{-1} \text{ dw}$) was less than wheat ($0.305 \text{ g g}^{-1} \text{ dw}$) and barley ($0.557 \text{ g g}^{-1} \text{ dw}$) (Table 2), suggesting more relative water absorption or water maintenance in triticale when faced with drought. In terms of relative water content (RWC), a decrease occurred in all the genotypes under water stress, a sharp decline in barley (23%) was detected, which led to the lowest values for gas exchange and fluorescence, as described below. The rate of decline in Pishgam was less than the other two wheat varieties (Table 2). Under water deficit a significant relationship was found between $RWC$ and $P_n$ (Figure 1).

![Figure 1](image-url)
Table 2. Mean biomass yield at anthesis (BYAA), relative water content (RWC), leaf temperature (LT), and gaseous exchanges under well watered (WW, $\Psi_{soil} = -3$ bars) and water deficit (WD, $\Psi_{soil} = -12$ bars) conditions. The data are shown as Mean±SE.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Treatment</th>
<th>No. 1</th>
<th>No. 2</th>
<th>No. 3</th>
<th>No. 4</th>
<th>Alvand</th>
<th>Pishgam</th>
<th>Zarrin</th>
<th>Bahman</th>
</tr>
</thead>
<tbody>
<tr>
<td>BYAA (g plant$^{-1}$)</td>
<td>WW</td>
<td>4.1±0.12</td>
<td>5.1±0.15</td>
<td>4.8±0.17</td>
<td>4.8±0.16</td>
<td>4.8±0.11</td>
<td>4.5±1.51</td>
<td>4.7±0.15</td>
<td>3.5±0.08</td>
</tr>
<tr>
<td></td>
<td>WD</td>
<td>3.4±0.19</td>
<td>3.4±0.12</td>
<td>3.6±0.05</td>
<td>3.5±0.27</td>
<td>3.0±0.43</td>
<td>3.7±0.25</td>
<td>3.3±0.05</td>
<td>2.2±0.21</td>
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<td>% Reduction</td>
<td>WW</td>
<td>17</td>
<td>33</td>
<td>25</td>
<td>28</td>
<td>37</td>
<td>19</td>
<td>30</td>
<td>38</td>
</tr>
<tr>
<td>RWC (%)</td>
<td>WD</td>
<td>80.0±6.55</td>
<td>83.5±5.02</td>
<td>82.6±3.99</td>
<td>80.2±4.28</td>
<td>81.0±5.28</td>
<td>79.5±2.92</td>
<td>79.4±1.93</td>
<td>77.4±5.03</td>
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<tr>
<td>% Reduction</td>
<td>WW</td>
<td>11</td>
<td>15</td>
<td>24</td>
<td>9</td>
<td>20</td>
<td>5</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td>E (mmol m$^{-2}$s$^{-1}$)</td>
<td>WW</td>
<td>5.0±0.26</td>
<td>53±0.26</td>
<td>5.1±0.69</td>
<td>5.6±0.95</td>
<td>5.4±0.77</td>
<td>5.9±0.64</td>
<td>6.0±0.58</td>
<td>4.9±0.91</td>
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<tr>
<td></td>
<td>WD</td>
<td>3.5±0.58</td>
<td>3.19±0.38</td>
<td>3.02±0.13</td>
<td>3.32±0.20</td>
<td>3.24±0.45</td>
<td>4.29±0.71</td>
<td>3.51±0.44</td>
<td>2.8±0.41</td>
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<tr>
<td>% Reduction</td>
<td>WW</td>
<td>30</td>
<td>39</td>
<td>41</td>
<td>41</td>
<td>49</td>
<td>27</td>
<td>41</td>
<td>43</td>
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<tr>
<td>Ci (ppm)</td>
<td>WW</td>
<td>250.7±6.6</td>
<td>238.4±12.9</td>
<td>244.8±30.5</td>
<td>231.7±13.5</td>
<td>234.7±11.3</td>
<td>242.2±9.8</td>
<td>260.1±4.3</td>
<td>247.5±19.2</td>
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<tr>
<td></td>
<td>WD</td>
<td>192.7±19.3</td>
<td>210.9±13.1</td>
<td>202.4±12.6</td>
<td>226.2±20.8</td>
<td>217.4±2.2</td>
<td>210.7±10.1</td>
<td>224.1±10.0</td>
<td>242.1±9.4</td>
</tr>
<tr>
<td>% Reduction</td>
<td>WW</td>
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<td>12</td>
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</tr>
<tr>
<td>Ps (μmol m$^{-2}$s$^{-1}$)</td>
<td>WW</td>
<td>7.9±0.87</td>
<td>8.8±0.64</td>
<td>9.5±1.59</td>
<td>10.6±1.55</td>
<td>10.3±0.59</td>
<td>11.8±1.66</td>
<td>9.7±0.02</td>
<td>7.9±2.47</td>
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<tr>
<td></td>
<td>WD</td>
<td>5.9±1.26</td>
<td>5.3±0.70</td>
<td>5.2±0.33</td>
<td>5.4±1.77</td>
<td>6.1±1.70</td>
<td>6.9±1.04</td>
<td>5.1±1.19</td>
<td>3.6±92</td>
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<tr>
<td>% Reduction</td>
<td>WW</td>
<td>24</td>
<td>39</td>
<td>45</td>
<td>50</td>
<td>56</td>
<td>42</td>
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<td>54</td>
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<tr>
<td>gs (mmol m$^{-2}$s$^{-1}$)</td>
<td>WW</td>
<td>150.0±17.0</td>
<td>150±5.0</td>
<td>180.0±36.0</td>
<td>186.0±29.6</td>
<td>186.0±29.6</td>
<td>226.6±18.0</td>
<td>216.1±8.0</td>
<td>153.1±31.1</td>
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<tr>
<td></td>
<td>WD</td>
<td>80.0±36</td>
<td>66.0±14.0</td>
<td>63.0±3.0</td>
<td>86.6±42.5</td>
<td>80.0±26.1</td>
<td>103.3±31.1</td>
<td>93.3±43.7</td>
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<td>56</td>
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<td>54</td>
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<td>54</td>
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<td>65</td>
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<td>MC (mmol m$^{-2}$s$^{-1}$)</td>
<td>WW</td>
<td>31.0±1.0</td>
<td>37.0±4.0</td>
<td>41.0±11.0</td>
<td>46.6±8.3</td>
<td>43.6±1.0</td>
<td>49.4±9.1</td>
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<tr>
<td></td>
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<td>30±3.0</td>
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<td>25.0±2.9</td>
<td>23.9±7.0</td>
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<td>22.4±7.0</td>
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<td>% Red</td>
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<td>32</td>
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<td>45</td>
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<td>60</td>
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<tr>
<td>WL (g g$^{-1}$dw)</td>
<td>WW</td>
<td>0.22±0.02</td>
<td>0.29±0.01</td>
<td>0.26±0.06</td>
<td>0.26±0.03</td>
<td>0.31±0.04</td>
<td>0.23±0.001</td>
<td>0.36±0.02</td>
<td>0.56±0.09</td>
</tr>
</tbody>
</table>
**Gaseous Exchanges**

Leaf gas exchange parameters indicated that under soil moisture stress, $P_n$, $g_s$, $E$, $C_o$, and $P_n/C_i$ declined in all genotypes tested. The mean decreases were 45%, 59%, 39%, 10%, and 37%, respectively (Table 2). By the imposition of water stress, the reduction of $P_n$ ranged from 24% for triticale No. 1 to 54% for Bahman. However, on the average, these reductions were 39, 48, and 54% in triticale, wheat, and barley, respectively. In contrast to the triticale No. 1, which had a low $P_n$ under normal condition (7.85±0.87 µmol CO$_2$ m$^{-2}$ s$^{-1}$), the Pishgam had higher $P_n$ in both stress (11.8±1.66 µmol CO$_2$ m$^{-2}$ s$^{-1}$) and non-stress (6.86±1.04 µmol CO$_2$ m$^{-2}$ s$^{-1}$) conditions, suggesting high ability of photosynthetic machinery in Pishgam under different soil moisture regimes. This might have resulted in more stability of this cultivar in the case of $P_n$.

A significant and positive relationship was found between $P_n$ and BYAA (Figure 2-A).

A gradual reduction in $g_s$ (47-71%) and $P_n/C_i$ (20-58%) was observed (Table 2) under water deficit. Average reduction of $g_s$ in barley (65%) was more than wheat (61%) and triticale (55%). The lowest reduction of $g_s$ was observed in triticale No. 1 (47%), and the highest was found in Alvand (71%), with similar pattern for $P_n/C_i$, suggesting an increased susceptibility of stomatal conductivity to water stress, resulting in an increase in the apparent mesophyll resistance, which can be differentiated among different types of cereals. However, under soil water deficit, Pishgam had the highest $g_s$ (103.3±31.1 mmol m$^{-2}$ s$^{-1}$) and Bahman had the lowest (53.3±12.3 mmol

![A](https://example.com/image1.png)

$y = 1.5979x + 0.0098$

$R^2 = 0.6699$

![B](https://example.com/image2.png)

$y = 15.677x - 7.318$

$R^2 = 0.6244$

![C](https://example.com/image3.png)

$y = 4.9979x - 1.6091$

$R^2 = 0.914$

![D](https://example.com/image4.png)

$y = 1.5925x - 55.217$

$R^2 = 0.5438$

Figure 2. Correlations of photosynthetic rate, $P_n$ and biomass yield at anthesis, BYAA (A), $P_n$ and stomatal conductance, $g_s$ (B), $P_n$ and apparent carboxylation efficiency, $P_n/C_i$ (C), % reduction of $P_n/C_i$ and % reduction of $g_s$ (D) among four genotype of triticale, three genotypes of bread wheat and one genotype of barley under water deficit ($\Psi_{soil} = -12$ bar).
The studied species exhibited significant reduction in transpiration rate, $E$, by soil water deficit conditions. A relatively high level of transpiration rate under stress condition ($4.29\pm0.71 \text{ mmol m}^{-2} \text{s}^{-1}$) concomitant with low reduction (27%) was found for Pishgam. A similar trend was observed for triticale No. 1 (Table 2). The distinct decrease of $E$ (49 and 43%) was observed in Alvand and Bahman, respectively. On the average, triticale and wheat transpired more than barley under both stress (15 and 25%) and non-stress (7 and 15%) conditions, respectively.

The examined genotypes had relatively significant differences in $C_i$ in their response to soil water deficit. The $C_i$ reduction by water stress ranged from 2% for Bahman to 23% for triticale No. 1 (Table 2). However, the reduction in barley was the least (2%), followed by wheat (12%) and triticale (15%). Under stress condition, the least $C_i$ was observed for triticale No.1 (192.7±19.3 ppm) and the highest was found in Bahman (242.1±9.4 ppm).

### Fluorescence Parameters

Table 3. Mean fluorescence parameters under well watered (WW, $\Psi_{wil} = -3$ bars) and water deficit (WD, $\Psi_{wil} = -12$ bars) conditions. The data are shown as Mean±SE. Negative values represent the percent increase by water deficit.

<table>
<thead>
<tr>
<th>trait</th>
<th>treatment</th>
<th>No. 1</th>
<th>No. 2</th>
<th>No. 3</th>
<th>No. 4</th>
<th>Alvand</th>
<th>Pishgam</th>
<th>Zarrin</th>
<th>Bahman</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{v}/F_{m}$</td>
<td>WW</td>
<td>0.75±0.02</td>
<td>0.73±0.01</td>
<td>0.76±0.01</td>
<td>0.74±0.01</td>
<td>0.77±0.01</td>
<td>0.79±0.01</td>
<td>0.79±0.01</td>
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anthesis and the $Fv/Fm$ under the stress treatment (Figure 3-D).

The results showed a smaller increase for the $DIo$ in triticale (13%) than wheat (27%) and barley (78%) (Table 3). A distinct decrease of the $Fv/Fo$ was noted for barley (40%) and wheat (26%) in contrast to the lowest value (11%) in triticale (Table 3). Under stress treatment, the highest values of $Fv/Fo$ were observed in both triticale No. 1 and Pishgam (2.93±0.38 and 2.52±0.47, respectively) and the lowest values were found in Alvand and Bahman (2.15±0.47 and 2.20±0.42, respectively).

Overall, the water stress caused less damage to PS II in triticale than wheat or barley (Table 3), as indicated by the smaller reduction of the PI in the former (21%)
against larger reduction for wheat (38%) and barley (79%). The results revealed that the PI was higher under normal conditions than under stress, as evidenced by the significant correlation between the RWC and PI (Figure 3-F).

DISCUSSION

The results of this study showed that water deficit affected the growth of barley more than that of bread wheat and triticale. In triticale, genotype No. 1 had the lowest biomass yield reduction, whereas No. 2 showed a large decrease when the moisture was limited. In the case of wheat, Pishgam performed better than the other two varieties, Alvand and Zarrin, when confronted with water stress. These results suggest a high variation within triticale and wheat genotypes under water-limiting conditions, an observation that is in agreement with the findings of Grzesiak et al. (2003) and Izanloo et al. (2008). The better performance of wheat genotypes compared with barley under water deficit indicated that barley would not necessarily perform better than wheat under limited environments, as reported by Mariano Cossani et al. (2009).

Our data showed considerable variation (24-56%) among our tested genotypes in the reduction of the net photosynthetic rate, $P_n$, under water stress, irrespective of the plant species. There are genotypic variations in the effect of drought stress on $P_n$ (Johnson et al., 1987; Matin et al., 1989). At the whole-plant level, the effect of water stress has been perceived as reduction in photosynthesis (Blum, 1996; Mwanamwenge et al., 1999; Inoue et al., 2004; Ghaderi et al., 2011). Even though triticale had lower $P_n$ values under WW conditions in comparison with wheat (Singh and Singh, 2001) and barley, the reduction by water deficit in this species was lower (39% against 48 and 54%, respectively) indicating the better acclimation of this new crop in relation to photosynthesis inhibition caused by water stress.

Monitoring gas exchange in plants is a common approach, with stomatal conductance (gs) reported as one of the most sensitive indicators of stress under drought for C3 species (Medrano et al., 2002). However, it has been reported that decreasing leaf water content initially induces stomatal closure (Pasban Eslam, 2011), imposing a decrease in the supply of CO$_2$ to the mesophyll cells and consequently, results in a decrease in the rate of leaf photosynthesis (Lawlor and Cornic, 2002). A genetic variation in this case has been reported for triticale (Grzesiak et al., 2003; Hura et al., 2007) and wheat (Loggini et al., 1999).

The $C_i$ reduction by water stress was 9.8% in all tested species, but was not statistically significant. Generally, lower $C_i$ should be accompanied by lower stomatal conductance (gs). However, in barley, despite more decline in the $g_s$, the $C_i$ was unaffected by water deficit conditions (Table 2), suggesting an inability of photosynthesis machinery to utilize internal CO$_2$ (Luo, 1991; Ahmadi and Siosemardeh, 2005) and non-stomatal factors predominating over stomatal factors in the limitation of CO$_2$ assimilation activity (Rivelli et al., 2002). The significant relationship ($P \leq 0.01$) between the reduction of the $g_s$ and $P_{n}/C_i$ (Figure 2-D) in the drought stress treatment revealed that, under unfavorable conditions accompanied by stomatal limitations, some physical (solubility of CO$_2$, surface area of the apoplastic, and symplastic routes of CO$_2$) and metabolic components (aquaporins and carbonic anhydrase) (Pinheiro and Chaves, 2010), in particular, the activity, quantity, and regeneration of Rubisco, would be impaired. This could induce a non-stomatal limitation, such as mesophyll resistance. Such a limitation has also been reported in other studies (Flexas et al., 2008, Chaves et al., 2009).

Both stomatal and non-stomatal limitations were more pronounced in barley than the other two crops because of high...
reduction of $g_s$ (65%), $P_{n}/C_i$ (60%) and $P_n$ (54%) in this crop. There were two different scenarios between the genotypes with low sensitivity to water deficit, e.g. triticale No. 1 and Pishgam. Low reduction of $P_{n}/C_i$ (3%) in triticale No. 1 against more reduction in Pishgam (38%) accompanied by higher reduction of $P_n$ (42%) revealed the fact that Pishgam was more sensitive to non-stomatal limitation of photosynthesis in comparison with triticale No. 1. However, higher correlation between the $P_n$ and $P_{n}/C_i$ (Figure 2-C) rather than the $g_s$ (Figure 2-B) under this condition suggests that the $g_s$ might play an important role in the high $P_n$ under well watered or mild drought stress, but, under severe drought stress, the high $P_n$ is related more to the maintenance of a higher capacity for mesophyll photosynthesis (Farquhar and Sharkey, 1982; Johnson et al., 1987; Reikka et al., 1998; Shangguan et al., 1999).

During water deficit conditions, the activity of the photosynthetic apparatus was lower, especially for some varieties of wheat (Alvand and Zarrin) and barley (Bahman) than that for the control, resulting in lower values of such parameters as $F_v/F_m$ and $F_i/F_o$ (Table 3). As described by Baker and Rosenquist (2004) and Hura et al. (2007), under stress treatment, the stomatal limitation on photosynthesis was accompanied with decrease in the rate of consumption of ATP and NADPH for CO2 assimilation that could result in decrease in the rate of linear electron transport and, consequently, in $F_v/F_m$. However, the distinct decrease in gas exchanges parameters, for instance $g_s$, that occurred in some genotypes (e.g. No. 1, No. 3 and Pishgam) when only relatively small drop of $F_v/F_m$ were found may suggest that damage of PSII in this genotypes was less marked (Wu and Bao, 2011). On the other hand, in these genotypes, despite considerable stomatal limitation, the $F_v/F_m$ was not significantly changed, likely due to the buffering effects of photorespiration as an electron sink, which maintained the rate of electron flow similar to that of non-stress conditions (Flexas et al., 2002; Ort and Baker, 2002). The low or lack of sensitivity of the $F_v/F_m$ in different types of cereals has also been observed in other studies (Kocheva et al., 2004; Hura et al., 2007).

Under stress conditions, a significant correlation was found between the $F_v/F_m$ and biomass yield at anthesis (Figure 3-D). Unlike stress free conditions (data not shown), the significant positive correlation (Figure 2-B) between the $F_v/F_m$ and $P_{n}/C_i$ and the strong relationship (Figure 3-A) between the $F_v/F_m$ and $P_n$ under water stress indicated that chlorophyll fluorescence under water limiting condition acts as a non-stomatal factor in limiting photosynthesis apparatus (Hura et al., 2007).

Moreover, photo inhibition is considered to be more accurately identified by an increase in the $D_{lo}$ (Force et al., 2003). Under water stress, energy dissipation rises with an increasing degree of PSII injuries resulting in an increase in the $D_{lo}$ (Rapacz and Wozniczka, 2009). High increase in the $D_{lo}$ in barley compared to low decrease in triticale means that PS II was less damaged in the later species.

The parameter of $F_v/F_o$ decreased in comparison to the well-irrigated plants, which has been associated with the donor part of PS II (Hura et al., 2007), especially in the water-splitting system. As mentioned by Maxwell and Johnson (2000), this parameter is more sensitive to change. It was confirmed by more variation of $F_v/F_o$ than $F_v/F_m$ as shown in Table 3.

The performance index, $PI$ is one of the chlorophyll fluorescence parameters that can provide useful and quantitative information about the state of plants and their vitality (Oukarroum et al., 2007). As mentioned, the $PI$ decreased after water stress. This parameter is sensitive to change in either the antenna properties, trapping efficiency, or electron transport beyond $Q_A$ (Oukarroum et al., 2007). The decrease in the $PI$ observed in the water deficit treatment may be related to damage in both PSII reaction center and antenna, as well as electron transfer disturbance (Rapac and Wozniczka, 2009). The slight reduction of
the PI in triticale No. 1 and Pishgam may explain the slight decrease in the photosynthetic machinery that, consequently, reduced the sensitivity to drought in these genotypes. In fact, the performance index is closely related to the final outcome of plant’s activity, such as growth or survival under stress conditions (Yusuf et al., 2010). The variability of the PI in response to water deficit on the basis of the reduction rate ranged from 5 to 79%, which was more than that of \( F_v/ F_m \), suggesting a higher sensitivity of the former to drought compared to the latter. This was in agreement with the results of Oukarroum et al. (2007) who reported limited differences in the \( F_v/ F_m \) compared to the PI and a higher effectiveness of the latter parameter in the evaluation of the susceptibility of genotypes to environment with considerable water limitation. The significant correlation between the PI and the \( P_n/C_i \) under water deficit condition in conjunction with the lack of relationship between the PI and \( g_s \) (Figure 3-E) also confirmed our abovementioned inference that non-stomatal limitations have considerable impact on the photosynthetic activity under limiting water conditions. Finally, the significant relationship between \( P_n \) and the PI (Figure 3-C) also emphasized the reliability of the PI for screening genotypes with high photosynthetic activity in reproductive stage under moisture-stress conditions.

In conclusion, stomatal and non-stomatal inhibition to \( P_n \) under stress condition may vary with genotypes as well as plant species. High leaf \( P_n \), RWC, \( g_s \), \( P_n/C_i \), \( F_v/F_m \) and PI appear to be involved in triticale No. 1 and Pishgam regardless of their species. However, these traits are associated with better performance of these genotypes under water deficit condition. On average, triticale had a lower reduction in photosynthetic traits under water deficit in comparison with wheat and barley. The better performance of Pishgam wheat variety in reducing stomatal and non-stomatal limitations confirms higher adaptability of Pishgam for water limiting conditions in Iran.

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REFERENCES


