Appraisal of some physiological traits in two wheat cultivars subjected to terminal drought stress during grain filling

Saeed Saeedipour

Department of Agronomy, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran. E-mail: saeeds79@gmail.com. Tel: +98-916-317-1978.

Accepted 23 July, 2012

In a greenhouse experiment, two winter wheat (*Triticum aestivum* L.) varieties differing in post anthesis drought resistance, tolerant (cv. Zagros) and sensitive (cv. Marvdasht), were subjected to either well-watered (WW) or water-stressed (WS) from anthesis to maturity. All physiological parameters were affected by drought stress. Results show that water deficits enhanced the senescence by accelerating loss of leaf chlorophyll and soluble proteins and the loss was more in Marvdasht than Zagros. The net CO$_2$ assimilation rate ($P_N$) in flag leaves during water deficit displayed a strict correlation with the drought sensitivity of the genotypes and showed an early reduction in Marvdasht. The effect of drought on grain yield was primarily due to the significant reduction in grain weight, particularly in drought-sensitive Marvdasht. The results indicate that the main physiological factor associated with yield stability of Zagros under drought stress may be attributed to the capacity for chloroplast activity in the flag leaf, which apparently allows sustained $P_N$ of flag leaf during grain filling under drought stress.

**Key words:** Assimilate, chlorophyll, flag leaves, grain yield, wheat (*Triticum aestivum* L.).

**INTRODUCTION**

Grain growth in wheat depends on carbon from three sources: Current assimilation, remobilization of pre-anthesis assimilates stored in the stem and other plant parts, and re-translocation of assimilates stored temporarily in the stem after anthesis (Kobata et al., 1992). Post-anthesis drought reduces carbon assimilation and hence the availability of current assimilates for grain filling, but probably does not affect the translocation of carbon to the grain (Wardlaw, 1967; Johnson and Moss, 1976). Moisture stress generally prevails during grain filling in wheat due to shortage of irrigation, low winter rainfall and high evaporation demand. Under this terminal drought condition, leaf senescence is accelerated and photosynthetic activity declines.

Wheat genotypes vary in the timing of senescence initiation and also in the subsequent rate of leaf senescence. The quest of the causes of differences in leaf photosynthetic rate among interspecies and/or intraspecies of crops may be one of the important strategies of crop engineering (Jiang et al., 2002). So delaying leaf senescence has become an agronomically desirable trait (Quirino et al., 2000; Subhan and Murthy, 2001). Flag leaf photosynthesis in wheat contributes about 30 to 50% of the assimilates for grain filling (Sylvester-Bradley et al., 1990) and initiation of grain filling coincides with the onset of senescence, therefore, photosynthesis of the flag leaf is the most important basis of the formation of grain yield, and the onset and rate of senescence are important factors for determining grain yield. The primary signs of leaf senescence are the breakdown of chlorophyll (Chl) and the decline of photosynthetic activity (Yang et al., 2001; Gregersen and Holm, 2007). It is generally accepted that genotypes that are able to sustain photosynthesis in the flag leaf for a longer time tend to yield more. Under drought, there is a rapid decline in photosynthesis after anthesis; due to a decrease in leaf stomatal conductance and net CO$_2$ assimilation, limiting the contribution of current
assimilates to the grain. Most of the drought-mediated reduction in CO₂ assimilation was attributed to stomatal closure; a part of it was attributed to the direct effect of water stress on the inhibition of CO₂ fixation (Sharkey and Seemann, 1989).

The relative magnitude of stomatal and non-stomatal factors in limiting photosynthesis depends on the severity of stress (Kicheva et al., 1994). Historically, research on biochemical changes that occur during leaf senescence has focused on loss of photosynthetic pigments, degradation of protein, and re-absorption of mineral nutrients. In the present study, we analyzed some physiological responses involved in two contrasting wheat genotypes to cope with drought stress. Such study will provide valuable information that can be used for the genetic basis of improvement of wheat to enhance yield and quality under stress conditions.

**MATERIALS AND METHODS**

**Experimental procedure and design**

Based on preliminary experiments (Saeidi et al., 2006), two contrasting winter wheat cultivars (Triticum aestivum L.) Marvdasht and Zagros (drought susceptible and tolerant during grain filling, respectively) were used in pot culture experiments during the growing season from 2010 to 2011 in the greenhouse of Agricultural Biotechnology Research Institute of Iran (48°20 N; 31°41 E; 20 m above sea level). Pots with a diameter of 23 cm and height of 25 cm were each filled with 8 kg pot⁻¹ sieved yellow drab soil mixed with 20 g pot⁻¹ manure fertilizer and 3.3 g pot⁻¹ compound fertilizer (N:P:K = 9:8:8). The soil contained organic matter of 1.48%, total N of 0.12%, available N of 82.3 µg g⁻¹, available P₂O₅ of 30.9 µg g⁻¹, available K₂O of 126.7 µg g⁻¹. Drought stress was imposed by withholding the amount of water applied in order to keep the soil moisture level at about 50% of the field capacity (FC). For non-stressed (control) treatments, the soil moisture was maintained at field capacity until the plants were harvested. Fifteen seeds per pot were initially sown and later thinned to five at the third-leaf stage. The pots were weighed daily and watered to restore the appropriate moisture by adding a calculated amount of water. The experiment was 2 × 2 (two cultivars and two water regimes) factorial design with four treatments. Each of the treatment had four replications with three sub-samples, in a complete randomized block design.

**Physiological measurements**

The net photosynthetic rate (Pₙ) and stomatal conductance (gs) were measured with a portable photosynthesis system Li-6400 (LI-COR, Lincoln, USA) on the flag leaves on 7, 10, 15, 22 and 31 days after anthesis. Photosynthetically active radiation (PAR) of 300 µmol m⁻² s⁻¹ was provided at each measurement by the 6400-02 light source. The fully expanded flag leaves on the stated dates were homogenized in ice cold 100% (v/v%) acetone (1.5 ml for 250 mg sample) and extracted for 24 h. Samples were centrifuged at 5,000 g for 15 min at 4°C. The pellet was extracted again with 80% (v/v %) acetone (1.5 ml for 250 mg sample) for 24 h. After centrifugation (5,000 g, 15 min, 4°C), the supernatants were collected. The pigment composition was measured with a double-beam spectrophotometer using the method of Lichtenthaler and Wellburn (1983). This method involves measurement of the light absorbed in the plant extract at 646.8 and 663.2 nm. Six leaves were used for each treatment.

**RESULTS**

**Chlorophyll content**

In the well watered and drought stressed plants, relevant differences were recorded in the leaves (Chl) throughout the experiment (Figure 1). Chl a and b contents decreased steadily in response to water deficit treatment and a significant changes were found in the Chl a and b contents at 31 days after anthesis (DAA) between treatments (Figure 1B and D). Regardless of water regime, lower Chl levels were measured in flag leaves of the drought-sensitive Marvdasht during 7 to 22 DAA. Drought stress imposed at anthesis contrasted to the control treatment led to the senescence process starting earlier in plants of both cultivars (Figure 1B and D).

**Photosynthetic performance**

The Pₙ of both cultivars under well-watered conditions was significantly higher than under water stress and the difference became more pronounced with time (Figure 2C and D). The Pₙ of flag leaf in both cultivars under WW treatment exhibited a more moderate decline with a similar changing pattern in both cultivars, however, Marvdasht had lower values in Pₚ nearly 9 contrasted to 14 µmol m⁻² s⁻¹ CO₂ at the end of the experiment. At the beginning of imposing water stress, the Pₙ was reduced by 67 and 50% in Marvdasht and Zagros compared with those of control treatments, respectively. These reductions remained constant in drought-tolerant Zagros while dropping to 75% at the end of the experiment in drought-sensitive Marvdasht (Figure 2D). Similar to Pₙ, values of gs in the well-watered treatment were significantly higher than under water stress (Figure 2A and B). Stomatal conductance under water withholding was significantly lower than the respective controls at all stages of sampling and the differences remained during development. The water stress resulted in an evident reduction in gs at early stage (7 DAA). A substantial reduction in gs of both cultivars at 7 DAA was followed by a further slight reduction at the end of the experiment.

**Seed yield and yield components**

In both genotypes, drought stress imposed at anthesis resulted in significant seed yield reduction (Table 1). Drought stress that lasted for 31 days resulted in 45.6 and 8.2% seed yield reductions in Marvdasht and Zagros, respectively. The effect of drought on seed yield was primarily due to the significant reduction in grain weight per plant (seeds per ear and 1000 grain weight) (Table...
Figure 1. Changes in chlorophyll a and b content in control (A) and (C) and water stress treatments, (B) and (D) in flag leaves during grain filling in two wheat cultivars (drought sensitive cv. Marvdasht and drought tolerant cv. Zagros). Vertical bars represent ± SE of the mean (n=4). Data are means ± SE of three independent samples. SE bars are not shown where they are smaller than symbols.

Figure 2. Changes in stomatal conductance (g_s) and net photosynthetic rate in control, (A) and (C) and water stress treatments, (B) and (D) in flag leaves during grain filling in two wheat cultivars (drought sensitive cv. Marvdasht and drought tolerant cv. Zagros). Vertical bars represent ± SE of the mean (n=4). Data are means ± SE of three independent samples.


<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Water-deficit treatment</th>
<th>Number of grains per ear</th>
<th>Grain yield per ear (g)</th>
<th>1000 grain dry mass (g)</th>
<th>Aerial biomass (g plant⁻¹)</th>
<th>Harvest Index (HI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marvdasht</td>
<td>WW</td>
<td>58.21ᵃ</td>
<td>1.58ᵃ</td>
<td>36.66ᵃ</td>
<td>3.72ᵃ</td>
<td>65.1ᵃ</td>
</tr>
<tr>
<td></td>
<td>WS</td>
<td>52.16ᵇ</td>
<td>0.767ᵈ</td>
<td>17.04ᶜ</td>
<td>2.39ᵇ</td>
<td>40.1ᶜ</td>
</tr>
<tr>
<td>Zagros</td>
<td>WW</td>
<td>46.17ᶜ</td>
<td>1.233ᵇ</td>
<td>31.24ᵇ</td>
<td>2.3ᵇ</td>
<td>62.6</td>
</tr>
<tr>
<td></td>
<td>WS</td>
<td>46.4ᵃᵇᶜ</td>
<td>1.115ᶜ</td>
<td>27.5ᵇ</td>
<td>2.42ᵇ</td>
<td>54.6ᵇ</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td>4.5</td>
<td>0.477</td>
<td>4.428</td>
<td>0.371</td>
<td>4.91</td>
</tr>
</tbody>
</table>

ᵃᵇᶜᵈ, Indicate statistical significance at $p < 0.05$ within the same cultivar.

It is noteworthy that water stress led to 10.4% reduction in numbers of grains per ear in Marvdasht, but had no effect on numbers of grains per ear for Zagros (Table 1). A similar changing pattern was found for aerial biomass in both cultivars. Generally, HI decreased under water stress conditions, although the reduction was more in the drought-sensitive (37%) than in the drought-tolerant (12%) cultivar.

**DISCUSSION**

Varieties differed significantly in photosynthetic activity, and these differences became more obvious under water stress. Photosynthesis decreases when $g_s$ decrease (Tenhunen et al., 1987; Nilsen and Orcutt, 1996). Chaves and Oliviera (2004) concluded that $g_s$ only affects photosynthesis at severe drought stress. The decrease in photosynthesis in drought-stressed plants can be attributed both to stomatal (stomatal closure) and non-stomatal (impairments of metabolic processes) factors. Under stress conditions, Zagros showed higher photosynthesis and grain yield. At present most researchers agree that stomatal closure and the resulting CO₂ deficit in the chloroplasts is the main cause of decreased photosynthesis under mild and moderate stresses (Flexas and Medrano, 2002). Regardless of treatments, drought-tolerant Zagros showed higher chlorophyll content during 7 to 22 DAA. The difference between cultivars for Chl a (Figure 1B) was only expressed under the well watered treatment. A similar changing pattern was observed for Chl b, although the differences between cultivars were distinct under the water deficit (Figure 1D). Decreased or unchanged chlorophyll level during drought stress has been reported in other species, depending on the duration and severity of drought (Kpyoarissis et al., 1995). A decrease of total chlorophyll with drought stress implies a lowered capacity for light harvesting. Since the production of reactive oxygen species is mainly driven by excess energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments (Herbinger et al., 2002). In line with these reports, we propose that the higher decrease in sink size (number of endosperm cells) of the drought susceptible genotype due to drought stress is partly attributable to a reduced availability of assimilates at the source level (Ho, 1988). However, a genotypic difference was evident for the length of the stress period at which the effects began to manifest.

**Conclusion**

All physiological parameter responses of the drought-tolerant (Zagros) and the drought-sensitive (Marvdasht) wheat cultivar to limited water supply showed similar patterns: Decreased chlorophyll a, b, net photosynthesis, stomatal conductance and yield. Drought stress significantly but differentially affected the growth and yield of the two contrasting genotypes. The grain dry mass accumulation followed by numbers of grains per spike was the most affected yield components under drought stress. Compared with Marvdasht, Zagros had larger grain weight and higher harvest index under drought stress.

**ACKNOWLEDGEMENT**

The corresponding author gratefully acknowledges the funding from the Islamic Azad University, Shoushtar branch through Grant.

**REFERENCES**

