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Full Length Research Paper

Effect of post-anthesis water deficit on yield and some physiological parameters on two wheat cultivars

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This work investigates the effects of water deficit on physiologic parameters related to yield in two wheat cultivars (*Triticum aestivum* L.), Marvdasht and Zagros (sensitive and tolerant to terminal season drought, respectively) grown in pots under well watered and water-stressed starting from anthesis until maturity. All physiological parameters were affected by drought stress. Results showed 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 display a strict correlation with the drought sensitivity of the genotypes and showed an early reduction in Marvdasht. Water stress resulted in a marked increase in leaf proline content of the drought-tolerant that led to alleviate the deleterious effect of water stress whereas, a slightly increment at the end of grain development observed in drought sensitive cv. The effect of drought on grain yield was primarily due to the significant reduction in grain weight, particularly in drought-sensitive. The results indicate that grain filling processes under water restriction are limited by low substrate availability and reduced synthesis capacity of the sink. These results raise the possibility that water stress-induced elevated levels of proline in Zagros contribute to reduce harmful of stress during grain filling.

Key words: Chlorophyll, flag leaves, grain yield, proline, soluble proteins, wheat (*Triticum aestivum* L.).

INTRODUCTION

In semi arid areas of the world with a Mediterranean climate, wheat crop often enters the reproductive phase when rainfall decreases and soil evaporation increases, leading to adverse water deficit conditions (Blum, 1998; Ehdaie and Waines, 1989). Environment inadequate conditions due to water (Ortiz et al., 2007) can cause reductions in morphological and agronomical parameters, as well as disorders at physiological, biochemical and molecular levels (Bartels and Sunkar, 2005). Drought stress decreases the rate of photosynthesis (e.g., Kawamitsu et al., 2000). The effects of drought on leaf photosynthesis are well documented (for example, Kaiser, 1987; Chaves, 1991). It is generally accepted that genotypes that are able to sustain photosynthesis in the flag leaf for a longer time tend to yield more. Sharkey and Seemann (1989) concluded that reductions in whole leaf photosynthesis caused by mild drought stress are primarily the results of stomatal closure and that there is no indication of damage to chloroplast reactions. At more severe drought stress, photosynthesis continues to decrease, while the ratio of intercellular/ambient CO_2 concentration increases significantly to values similar to those obtained in well watered plants (Rekika et al., 1998). Thus, the decrease in photosynthesis could result from non-stomatal factors affecting photosynthetic capacity, for example, reduced activity of some Calvin cycle enzymes, inhibition of photosynthetic electron transport, and impaired photophosphorylation capacity (Sharkey and Seemann, 1989; Kicheva et al., 1994). There exist genotypic variations in the effect of drought stress on stomatal conductance (g_s) and net photosynthetic rate (P_N) (Johnson et al., 1987; Matin et al., 1989). Severe drought stress also inhibits the photosynthesis of plants by causing changes in chlorophyll content, by affecting cholorophyll components and by damaging the photosynthetic apparatus (IturbeOrmaetxe et al., 1998). 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). Ommen et al. (1999)

reported that leaf chlorophyll content decreases as a result of drought stress. The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species (Smirnoff, 1995). Plants can partly protect themselves against mild drought stress by accumulating osmolytes. Proline accumulation in leaves of drought-stressed plants and its role as an osmolyte or osmoprotectant has been the theme of a long-standing debate (Seki et al., 2007; Szabados and Savoure, 2009). Proline does not interfere with normal biochemical reactions but allows the plants to survive under stress (Stewart, 1981). The accumulation of proline in plant tissues is also a clear marker for environmental stress, particularly in plants under drought stress (Routley, 1966). Proline accumulation may also be part of the stress signal influencing adaptive responses (Maggio et al., 2002). In addition to the physiological and biochemical responses of plants to water stress, the information on the molecular mechanisms of drought stress adaptation could be useful for the genetic improvement of drought-resistant crops/genotypes. Generally, drought induces metabolic changes related to protein turnover (alterations in protein synthesis, maintaining the level of some proteins or protein degradation) (Bray, 1997). Changes in protein patterns induced due to drought play a pivotal role in the adaptive response of plants to the stress (Riccardi et al., 1998). In line with these findings, drought stress initiated at different growth stages may induce quantitative and qualitative changes in wheat leaf proteins. A better understanding of the mechanisms that enable wheat plants to adapt to drought stress and maintain growth, development, and productivity during stress periods would help in breeding for drought resistance (Seropian and Planchon, 1984). The objective of this study was to investigate the effect of drought stress on yield and some physiological parameters in two wheat (*Triticum aestivum* L.) genotypes differing in degree of drought resistance.

MATERIALS AND METHODS

Experimental procedure and design

Based on preliminary experiments (Saeidi et al., 2006), two contrasting winter wheat cultivars (*T. aestivum* L.) Marvdasht and Zagros (drought susceptible and tolerant during grain filling, respectively were used in pot culture experiments during the growing season from 2009 to 2010 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 P_2O_5 of 30.9 µg g^{-1}, available K_2O 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 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 treatment. Each of the treatment had four replications with three sub-samples, in a complete randomized block design.

Physiological measurements

The net photosynthetic rate (P_N), stomatal conductance (g_s) 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.

Chemical analysis

Protein content determination

Leaf samples were ground in liquid nitrogen and the powder was dissolved in 1 ml of 50 mM HEPES-NaOH buffer pH 7.6 containing 3 mM DTT. After centrifugation for 10 min at 13000 g, the protein concentration was measured using the method of Sedmak and Grossberg (1977), using BSA as standard protein. This allowed all enzymatic activities to be expressed relative to the soluble protein concentration.

Proline content

Assessments of proline content were performed during the experimental period, at 7, 15 and 31 days after the imposed water stress at anthesis. Proline was extracted from a sample of 0.5 g fresh leaf material samples in 3% (w/v) aqueous sulphosalycylic acid and estimated using the ninhydrin reagent according to the method of Bates et al. (1973). The absorbance of fraction with toluene aspired from liquid phase was read at a wave length of 520 nm. Proline concentration was determined using a calibration curve and expressed as µ mol proline g^{-1} FW.

RESULTS

Chlorophyll content

In the well water and drought stress plants, relevant differences were recorded in the leaves (Chl) throughout the experiment (Figure 1A and C). Chl a and b contents decrease steadily in response to water deficit treatment and a significant changes were found in the Chl a and b contents at 31 DAA between treatments (Figure 1B and D). Irrespective to water regime the lower Chl levels were measured in flag leaves of the drought-sensitive

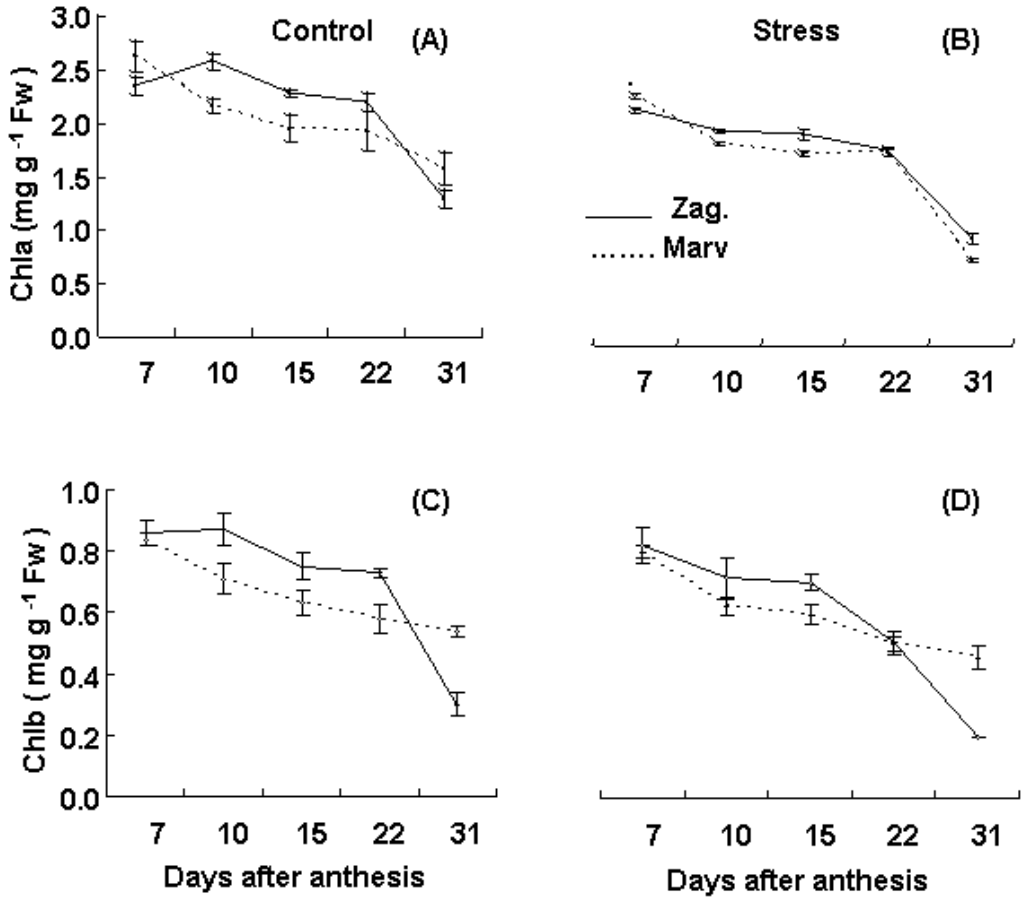

Figure 1. Changes in chlorophyll a and b content in control, (A) and (C) and water stress treatments, (B) and (D) in flag leaves during grain filling in two wheat cultivars (drought Sensitive cv. Marvdasht and drought Tolerant cv. Zagros). Vertical bars represent ± SE of the mean (n=4) Data are means ± SE of three independent samples.

Marvdasht during 7 to 22 DAA. Drought stress imposed at anthesis contrast to control treatment led to the senescence process started earlier in plants of both cultivars (Figure 1B and D).

Photosynthetic performance

The P_N of both cultivars under well-watered condition was significantly higher than under water stress and the difference became more pronounced with time (Figure 2C and D). The P_N 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 P_N nearly 9 contrast to 14 μmol m^{-2} s^{-1} CO_2 at the end of experiment. At the beginning of water stress imposing, the P_N reduced by 67 and 50% in Marvdasht and Zagros compared with those of control treatments, respectively. These reduction remain constant in drought-tolerant while dropped to 75% at the end of experiment in drought-sensitive cultivar (Figure 2D). Similar to P_N, values of *g*s in 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 sampling and the differences kept remain with development. The water stress resulted in evident reduction in *g*s at early stage (7 DAA). A substantial reduction in *g*s of both cultivars during 7 DAA was followed by a further slight reduction till the end of experiment.

Leaf protein and proline contents

The amounts of soluble proteins reduced with time in both treatments (Figure 3A and B), although, considerable differences were detected between treatments, as substantial reduction occurred in both cultivars under water stress compared with the control treatment. Irrespective of treatment, Zagros revealed higher soluble proteins content than Marvdasht

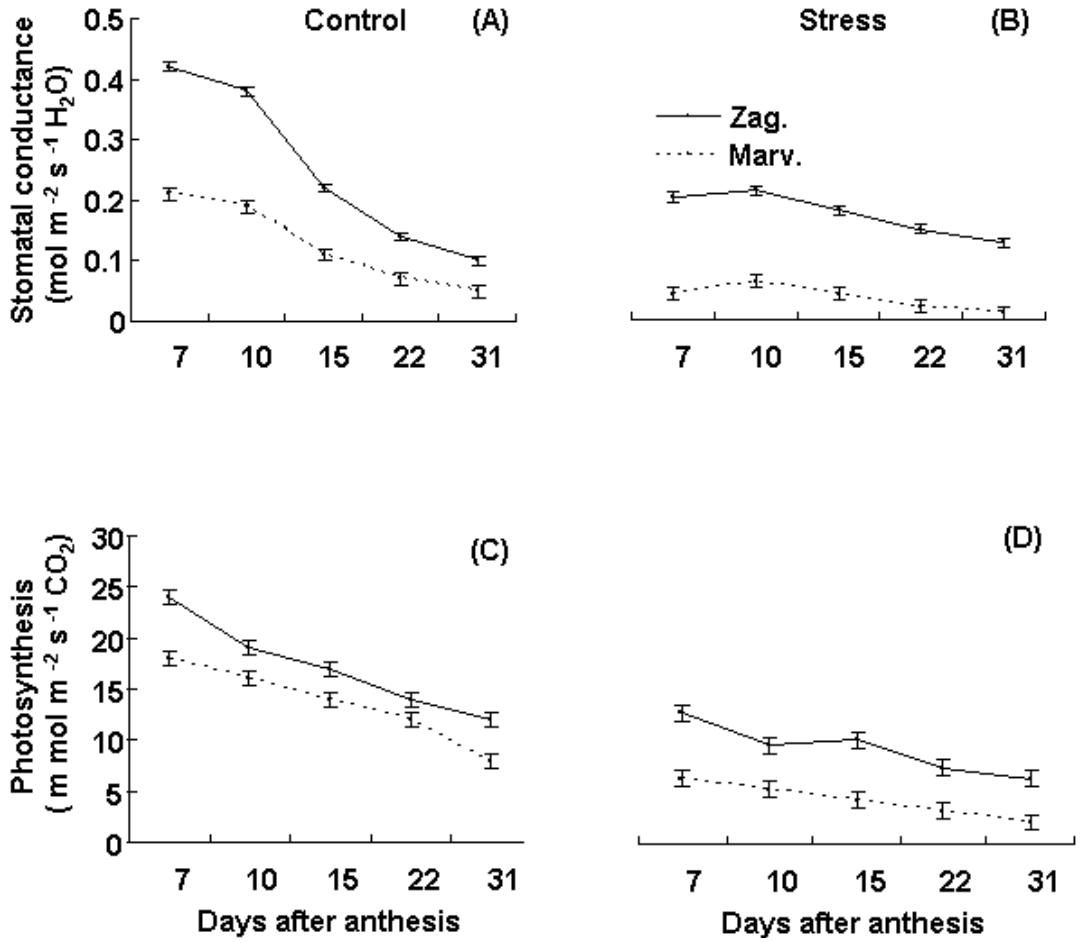

Figure 2. Changes in stomatal conductance (g_s) and net photosynthetic rate in control, (A) and (C) and water stress treatments, (B) and (D) in flag leaves during grain filling in two wheat cultivars (drought Sensitive cv. Marvdasht and drought Tolerant cv. Zagros). Vertical bars represent ± SE of the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

throughout all stages sampling. Reduction in soluble proteins under water stress was more remarkable than well watered treatment from day 10 onwards in Marvdasht, since this difference was not evident until 31 DAA in Zagros (Figure 3B). The leaf proline contents increased by water stress imposed in both cultivars, although, considerable differences were detected between them, as substantial increment occurred in Zagros cultivar throughout all stages sampling under water stress compared with Marvdasht. The water stress, at early stage (by day 7) elevated leaf proline level 28 fold respective to those control treatment in tolerant cultivar whereas, this difference was not evident until 31 DAA in Marvdasht (Figure 3D). Under stress condition, Zagros leaf proline concentration reduced sharply between 7 to 15 DAA and then underwent slightly reduction during later stage (31 DAA) of grain growth (Figure 3D). In comparison, under well water treatment, not significant differences observed between cultivars at all stages sampling (Figure 3C).

Seed yield and yield components

In both genotypes, drought stress imposed at anthesis stage resulted in significant seed yield reduction (Table 1). Drought stress that lasted for 31days 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 (Table 1). It is noteworthy that, water stress led to 10.4% numbers of grains reductions in Marvdasht, whereas had no effect on Zagros grain number (Table 1). A similar changing pattern was found for aerial biomass in both cultivars. Generally, HI decreased under water stress condition, although the reduction was more in drought-sensitive (37%) than to drought-tolerant (12%).

DISCUSSION

Varieties significantly differed in photosynthetic activities,

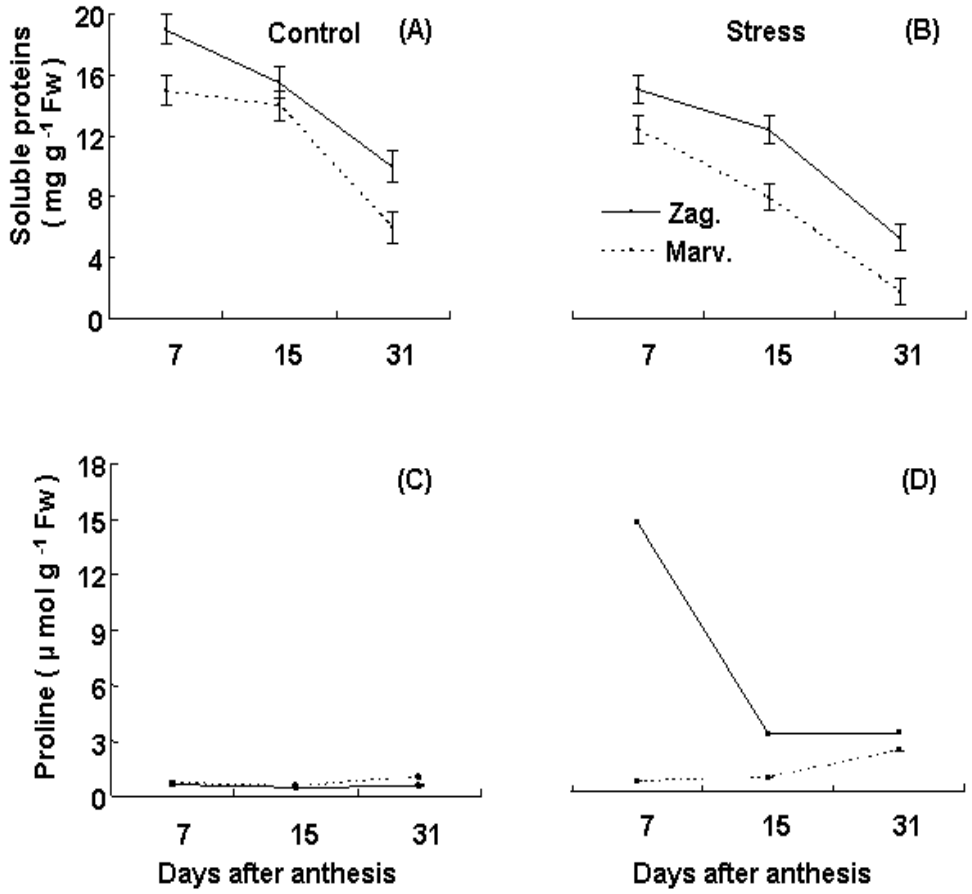

Figure 3. Changes in soluble ptoteins and proline content in control, (A) and (C) and water stress treatments, (B) and (D) in flag leaves during grain filling in two wheat cultivars (drought Sensitive cv. Marvdasht and drought Tolerant cv. Zagros). Vertical bars represent ± SE of the mean (n=4) Data are means ± SE of three independent samples. SE bars are not shown where they are smaller than symbols.

Table 1. Effect of different water treatment, well watered (control), withholding water (stress) from anthesis till to maturity on the final number of kernel per spike, kernel weight per spike, the thousand-kernel weight, aerial biomass of plant and harvest index in two wheat cultivars.

Cultivars	Water-deficit treatment	No.of grains per year	Grain yield per year (g)	1000 grain dry mass (g)	Aerial biomass (g plant^{-1})	Harvest index (HI)
Marvdasht	WW	60.41^{a}	1.78^{a}	38.96^{a}	3.82^{a}	67.3^{a}
	WS	54.16^{b}	0.967^{d}	19.24^{c}	2.59^{b}	42.3^{c}
Zagros	WW	48.37^{c}	1.433^{b}	33.44^{b}	2.5^{b}	64.8
	WS	48.67^{bc}	1.315^{c}	29.71^{b}	2.62^{b}	568^{b}
LSD(0.05)		5.5	0.577	4.528	0.371	5.91

Letters indicate statistical significance at $p_{0.05}$ within the same cultivar.

and these differences could not only be expressed under the control condition but also became more obviously under water stress. In many experiments it has been shown that photosynthesis decreases when g_s decreases (e.g., 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 condition, Zagros showed higher photosynthesis and grain yield. At present most researchers agree that the stomatal closure and the resulting CO_2 deficit in the chloroplasts is the main cause of decreased photosynthesis under mild and moderate stresses (Flexas and Medrano, 2002). Irrespective to treatments, drought-tolerant showed a higher chlorophyll content during 7 to 22 DAA, and the differences between cultivars only be expressed under well water treatment and not evident under stress condition for Chl a (Figure 2B). Similar changing pattern was observed for Chl b, although the differences between cultivars was distinct under the water deficit (Figure 2D). 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). The study observation also showed that, soluble proteins of the flag leaves declined with age in both cultivars under control treatment, but water stress enhanced such a decline with a more extent in Marvdasht than Zagros, although, Marvdasht showed earlier reduction under stress treatment than Zagros cv (Figure 3B). The changes in leaf protein corroborate with previous reports on the responses of plants to drought stress (Riccardi et al., 1998; Salekdeh et al., 2002). Among amino acids, the accumulation of proline is frequently reported in many plants or tissues in response to a variety of abiotic stresses (McCue and Hanson, 1990). In maize primary root, for example, the proline level increases as much as a hundred fold under a low water potential (Voetberg and Sharp, 1991). The increase in proline content drought-tolerant cultivar due to drought stress was more severe (28.4 fold) compare to control treatment at early stage (during 7 DAA) of grain development and then declined with time (31 DAA) but remain at the higher level (6 fold) in respective to control treatment. Accumulation of proline in plants under stress is a result of the reciprocal regulation of two pathways: increased expression of proline synthetic enzymes and repressed activity of proline degradation (Delauney and Verma, 1993; Peng et al., 1996). During the experiment, we found that Zagros had increase of proline content higher than Marvdasht. It is possible that these differences are due to up-regulation of proline degrading enzymes such as praline dehydrogenase (PDH) in drought stressed Zagros. These results prove that proline accumulation by Zagros flag leaf is due to up-regulation of proline biosynthesis pathway rather than inhibition of catabolic process, this increases roles as an osmotic compatible and adjust osmotic potential which resulted in drought stress avoidance in Zagros. Accumulation of proline has been advocated as a parameter of selection for stress tolerance (Yancy et al., 1982).

Conclusion

All physiological parameters responses of drought-tolerant (Zagros) and drought-sensitive (Marvdasht) wheat cultivar to limited water supply showed similar patterns: decreased chlorophyll a, b, net photosynthesis, stomatal conductance, soluble proteins and yield were associated with increased proline. Differences between cultivars were mainly found in water relation parameters, which indicates adaptations in physiology (stomata) or osmotic adjustments. Proline (Pro) accumulation is a common physiological response in many plants in response to drought stress. Photosynthesis is limited by drought stress due to stomatal and nonstomatal (impairments of metabolic processes) factors. The drought stress imposed in this study affected the yield; however, yield was the most affected, limiting significantly the grain weight.

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