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Comparison of some metabolites alteration in two wheat cultivars subjected to terminal drought stress during grain filling

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The aim of this study is to study 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 to maturity. 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. Hence, 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. The higher assimilate in Zagros grains under drought could also be related to drought resistance. These results raise the possibility that water stress-induced elevated levels of proline in Zagros contribute to reduced harmful stress during grain filling.

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

INTRODUCTION

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. The plant response to drought consists of numerous processes that must function in coordination to alleviate both cellular hyperosmolarity and ion disequilibrium. To cope with drought stress, plants respond with physiological and biochemical changes. These changes aim at the retention of water in spite of the high external osmoticum and the maintenance of photosynthetic activity, while stomatal opening is reduced to counter water loss. Accumulation of low molecular compounds, according to the metabolic responses has drawn much attention. Adaptation to all these stresses is associated with metabolic adjustments that lead to the accumulation of several organic solutes like sugars, polyols, betaines and proline (Ashraf, 2004; Irigoyen et al., 1992; Kohl et al., 1991). In addition to synthesis of these osmolytic compounds, specific proteins and translatable mRNA are induced and increased by drought stress (Reviron et al., 1992). Metabolic benefits of osmolyte accumulation may augment the classically accepted roles of these compounds. In reassessing the functional significance of

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compatible solute accumulation, it is suggested that proline and glycine betaine synthesis may buffer cellular redox potential (Hare et al., 1998). In the present study, we have analyzed the biochemical responses involved in two contrasting wheat genotypes to cope with drought stress. Such study will provide valuable information that can be used for 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 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.

Estimation of metabolites

For seed sugar analyses, samples were obtained from the harvests made at 7, 15 and 31 days after the commencement of drought stress. The seeds were dried at 80°C for 48 h.

Sugars

Three-hundred milligram ground plant material was weighed into a 50 ml volumetric flask and 30 ml of double-demineralised water was added. The material was then extracted by incubating in a shaking water bath at 60°C for 30 min. The flask was quickly cooled on ice, and filled up to the mark with double-demineralised water followed by filtration with (blue-band) filter paper (Faltenfilter 5951/2, Scheicher and Schüll Co., Dassel, Germany). Sugars were determined by using enzymatic test kits and absorbances of the solutions were read at 340 nm.

Soluble protein

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 express relative to the soluble protein concentration.

Proline

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 µmol proline g^{-1} FW.

RESULTS

Leaf sucrose, protein and proline contents

Drought stress altered the sucrose concentration in the grains of the two wheat genotypes, as available sugar concentration was suppressed due to drought for Marvdasht but not for Zagros during 15 days after anthesis (DAA) (Figure 1B). The reduction in sucrose content of stressed grains became much pronounced (47% of control) at 15 DAA in drought-sensitive compared with those of the control whereas, a further slight elevation (3%) occurred during similar stage sampling in drought-tolerant compared with their respective controls (Figure 1B).

The amounts of soluble proteins reduced with time in both treatments (Figure 1C, D), 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 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 1D). The leaf proline contents increased by water stress imposed in both cultivars, however, considerable differences were detected between them, as substantial increment occured 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 in respective to those control treatment in tolerant cultivar whereas, this difference was not evident until 31 DAA in Marvdasht (Figure 1F). 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 1F). In comparison, under well water treatment, no significant difference was observed between cultivars at all stages sampling (Figure 1E).

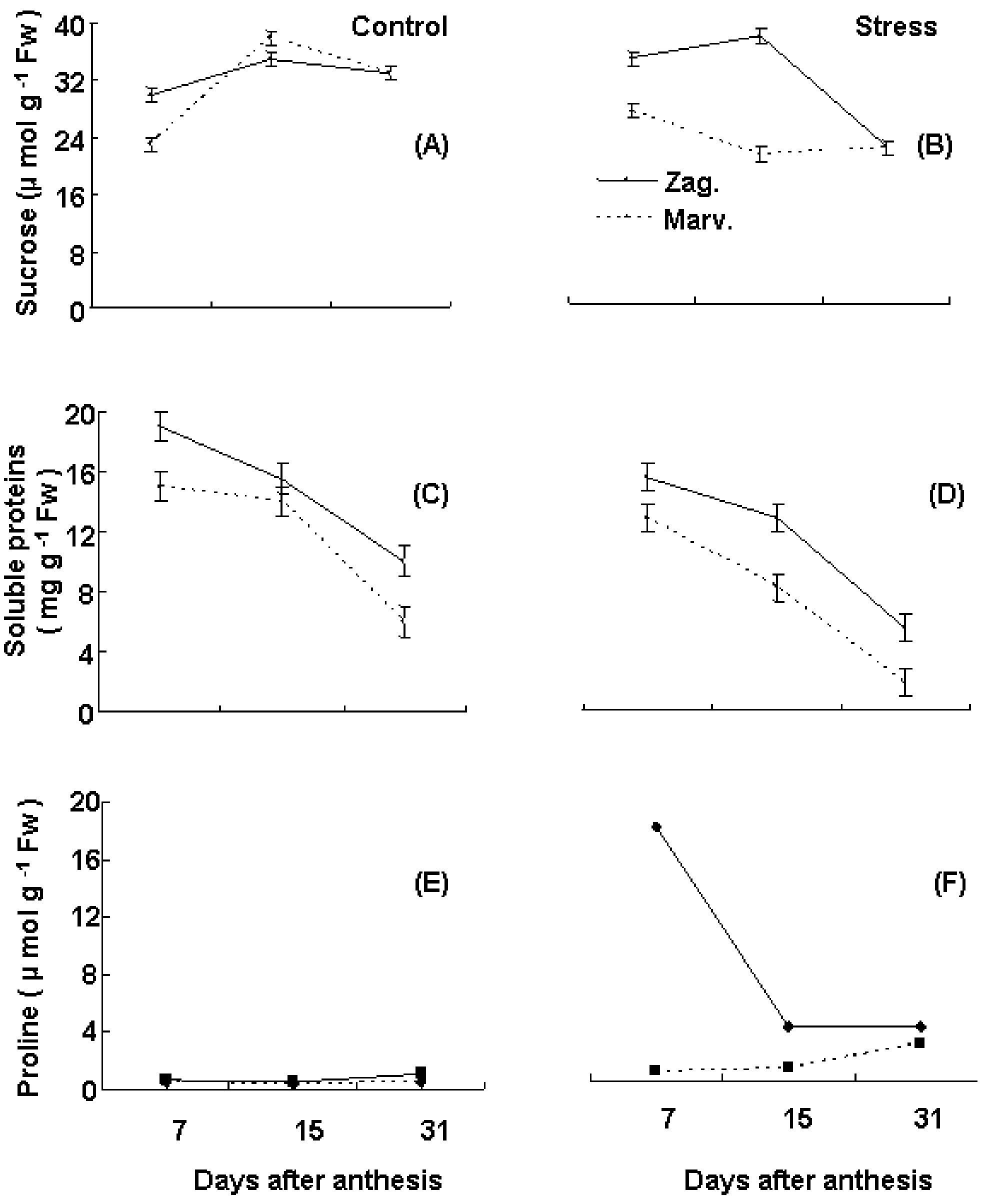

Figure 1. Changes in sucrose of grains and soluble ptoteins and proline concentration of flag leaves in control: (A, C and E) and water stress treatments: (B, D and F) during grain filling in two wheat cultivars (drought sensitive cv. Marvdasht and drought Tolerant cv. Zagros). Vertical bars represent ± 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.

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 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 (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

The study observation showed that, soluble proteins of the flag leaves declined with age in both cultivars under control treatment, but water stress enhanced such a

Table 1. Effect of different water treatment, well watered (control), withholding water (stress) from anthesis 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 ear	Grain yield per ear (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.

decline with a more extent in Marvdasht than Zagros, although, Marvdasht showed earlier reduction under stress treatment than Zagros cv (Figure 1D). 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 proline 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 increasing 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). Varietal differences were found in terms of the level of sucrose available for metabolism in the grains under drought stress conditions (Figure 1B). In Marvdasht, drought initiated at early stage of grain filling (15 DAA) caused a marked reduction in seed sucrose concentration relative to the non-stressed plants. On the contrary, seed sucrose concentrations for Zagros increased by about 3% as a consequence of the drought stress imposed during similar period. Sucrose metabolism is pivotal in seed development and is particularly susceptible to drought stress (Pinheiro et al., 2005).

The decrease in seed sucrose concentration due to drought at all durations of stress in Marvdasht (Figure 1B), reflected the lower availability of the assimilate at source level. A direct relationship between sucrose availability and export rate at source level and the establishment of new sink organs has been shown for several crops (Setter et al., 2001; Liu et al., 2004). In line with these reports, we suppose that the higher decrease in sink size (number of endosperm cell) of the drought susceptible genotype due to drought stress is partly attributed to reduced availability of the assimilation at source level (Ho, 1988). Although a genotypic difference was evident for the length of the stress period at which the effects began to manifest.

Conclusion

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 grain dry mass accumulation followed by numbers of grains per spike were the most affected yield components under drought stress. Compared with Marvdasht, Zagros had larger grain weight and higher harvest index under drought stress. Different grain sucrose concentration found between the contrasting watering regimes for both genotypes imply that sucrose availability, as well as the capacity for utilizing the assimilate affected under drought stress.

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