Full Length Research Paper

Relationship between abscisic acid (ABA) concentration and some physiological traits in two wheat cultivars differing in post-anthesis drought-resistance

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This work investigated the effects of endogenous abscisic acid (ABA) and 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 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 CO2 assimilation rate (PN) in flag leaves during water deficit displayed a strict correlation with the drought sensitivity of the genotypes and showed an early reduction in Marvdasht. Water stress resulted in a marked increase just in the ABA content of the drought-sensitive that led to reduced transport of sucrose into the grains and lowered the starch synthesis ability of grains, whereas, sucrose uptake and conversion by grains was stimulated by low ABA concentrations in Zagros. 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 raised the possibility that water stress-induced elevated levels of endogenous ABA contribute to reduced grain growth.

Key words: Abscisic acid, chlorophyll, flag leaves, grain yield, soluble proteins, soluble sugar, starch, wheat (Triticum aestivum L.).

INTRODUCTION

Reports on drought-induced reduction in seed yield of the crop are highly variable due to differences in the timing and intensity of the stress imposed and the genotypes used (Ramirez-Vallejo and Kelly, 1998). Drought stress decreases photosynthetic rate, thereby decreasing the amount of assimilates available for export to the sink organs (Kim et al., 2000). Plants grown under drought condition have a lower stomatal conductance in order to conserve water. Consequently, CO2 fixation is reduced and photosynthetic rate decreases, resulting in less assimilate production for growth and yield of plants. Severe drought stress also inhibits the photosynthesis of plants by causing changes in chlorophyll content, by affecting chlorophyll components and by damaging the photosynthetic apparatus (IturbeOrmaetxe et al., 1998).

Drought can also affect carbohydrate metabolism in plant reproductive organs (Liu et al., 2004). For example, Setter et al. (2001) found higher or at least similar levels of sucrose in maize ovaries between drought-stressed and well watered controls. These results imply that in addition to assimilate availability per se, the capacity for utilizing them in the reproductive structures may also be affected under drought stress. Thus, drought-induced

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Abbreviations: ABA, Abscisic acid; DAA, days after-anthesis; HI, harvest index; WW, well-watered; gs, stomatal conductance.
changes in carbohydrate status and metabolism in crop reproductive structures are crucial for successful fruit set. In addition to photosynthetic supply, decrease in water potential and higher abscisic acid (ABA) accumulation in the reproductive structure of plants subjected to drought may also contribute to the loss of fruit or seed set (Liu et al., 2004).

ABA also promoted dry matter accumulation in several organs and its level was strongly correlated with the growth rates of both fruits and seeds (Wang et al., 1998). Isogenic lines of wheat containing high levels of endogenous ABA appear to be better at osmoregulation and exhibit better growth and higher yields under water stress (Quarrie et al., 1999). This suggests that an appropriate level of ABA will be necessary for plants to grow successfully under stress conditions (Spollen et al., 2000).

Although, it has been widely speculated that ABA may be causally related to growth inhibition (Dodd and Davies, 2005).

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. Among the stress induced proteins identified, are those implicated in the biosynthesis of osmolytes (Ishitan et al., 1995), in the uptake and compartmentation of ions (Lisse et al., 1996), in hydroxyl-radical scavenging (Ingram and Bartels, 1996) and protection of cellular structure (Neslihan-Ozturk et al., 2002). Proteins that show significant down regulation under drought stress were observed for photosynthesis-related function (Neslihan-Ozturk et al., 2002). 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.

The objective of this study was to investigate the differential effect of drought stress on seed ABA content and some physiological parameters and yield 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 (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 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 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 x 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 (Pn), 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 LI-6400-02 light source. The fully expanded flag leaves on the stated dates were homogenized in ice cold 100% (v/v %) acetonitrile (1.5 ml for 250-250 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 %) acetonitrile (1.5 ml for 250 mg sample) for 24 h. After centrifugation (5,000 g, 15 min, 4°C), the supernatants were collected. The chlorophyll composition was measured with a double-beam spectrophotometer using the method of Lichtenthaler and Wellburn (1993). 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

For seed sugar, starch analyses, protein and ABA content of the seed samples which obtained at 7, 15 and 31 days after the commencement of drought stress were dried at 80°C for 48 h.

Sugars

300 mg 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.

Starch

Starch determination was performed following enzymatic assay procedure using the starch determination kit from Boehringer (Mannheim, Germany). Homogenised ground seed samples of 300 mg were weighed into Erlemeyer flasks, and 20 ml of dimethylsulfoxide and 5 ml HCl (8 mol l⁻¹) were added. The sealed flask was then incubated for 30 min at 60°C in a shaking water bath. The sample solutions were cooled quickly to room temperature and approximately 50 ml water was added. The pH was adjusted to 4.5 with sodium hydroxide (5 M) under vigorous shaking. The solution was then transferred to a 100 ml volumetric flask, rinsed with water, filled up to the mark with water and filtered.
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.

using Falten filter 5951/2 (Scheicher and Schüll Co., Dassel, Germany).

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 Sedmak and Grossberg (1977), using BSA as standard protein. This allowed all enzymatic activities to be expressed relative to the soluble protein concentration.

ABA

Metabolite extraction from freeze-dried grains (second and third kernel from each spikelet) or flag leaf of two wheat genotypes was performed. Extracts were passed through a Sep Pak C18-cartridge. Methanol was removed under reduced pressure and the aqueous residue was partitioned three times against ethyl acetate at pH 3.0. The ethyl acetate of the combined organic fractions was removed under reduced pressure. The newly obtained residue was taken up in TBS-buffer (Tris buffered saline; 150 mmol L⁻¹ NaCl 1 mmol L⁻¹ MgCl₂ and 50 mmol L⁻¹ Tris at pH 7.8) and subjected to an immunological ABA assay (ELISA) as described earlier (Mertens et al., 1985).

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 1C). Chl a and b contents decreased steadily in response to water deficit treatment and a significant change were found in the Chl a and b contents at 31 DAA between treatments (Figure 1B and
Irrespective to water regime, the lower Chl levels were measured in flag leaves of the drought-sensitive 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 1D).

Photosynthetic performance

The net CO₂ assimilation rate \( (P_N) \) of both cultivars under well-watered (WW) condition was significantly higher than under water stress and the difference between cultivars became more pronounced under stress condition (Figure 2C and 2D). 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 CO₂ at the end of the experiment. At the beginning of water stress, imposing the \( P_N \) reduced by 67 and 50% in Marvdasht and Zagros compared with those of the control treatments, respectively. These reduction remain constant in drought-tolerant while dropped to 75% at the end of the experiment in drought-sensitive cultivar (Figure 2D). Similar to \( P_N \), values of \( g_s \) in WW treatment were significantly higher than under water stress (Figure 2A and 2B). Stomatal conductance \( (g_s) \) 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 to end of experiment.

Leaf protein contents

The amounts of soluble proteins decreased within the period of after anthesis in both treatments (Figure 3A and
3B), although, considerable differences were detected between treatments, as substantial reduction occurred in both cultivars under water stress compared with the WW 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 3B). Opposite to other physiological parameter mentioned, the leaf ABA contents increased by water stress imposed in both cultivars, however, the increment was more pronounced in drought-tolerant than sensitive one during 15 DAA (Figure 3D). In comparison, ABA levels under stress regime was significantly more than respective to controls at all stages except for day 31, and the differences reached to maximum value by day 15 and then underwent a rapid reduction during 15 to 31 DAA. In addition, an obvious differences in absolute ABA concentration was observed between cultivars under WW treatment and the ABA level was markedly more during 20 DAA in drought-tolerant than drought-sensitive cultivar (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 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, harvest index (HI) decreased under water stress condition, although, the reduction was more in drought-sensitive (37%) than to drought-tolerant genotypes.
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 DAA (Figure 4B). 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 4B). Likewise, a rapid loss of sucrose began in stressed grains than in controls from day 15 onwards in Zagros and this event was simultaneous with mark starch accumulation during the same period (Figure 4B and 4D). A nearly linear pattern of starch accumulation was observed by stressed grains during all stage sampling in both cultivars (Figure 4D). However, a period of 16 days water stress (15 to 31 DAA) caused a sharp enhanced in starch concentration per grain especially in drought-tolerant. A comparison between grain age under stress-watered conditions indicates a greater capacity (50 and 27%) of grains for starch synthesis at day 31 than at day 15 in Zagros and Marvdasht, respectively. A period of 8 days (7 to 15 DAA) water stress had no effect on the starch synthesis ability of the sink (Figure 4D). On a grain starch accumulation, differences between treatments were still not significant at day 15. However, at day 31 differences between treatments were apparent on this basis. The percentage conversion, as a proportion of total sucrose uptake, was considerably higher in stressed grains at day 31 in comparison with grains from control plants, implying that the conversion of sucrose taken up by the endosperm cells had been effectively raised by water stress.

Under limited conditions, ABA content of both cultivars significantly enhanced in all stage sampling compared to their respective controls (Figures 4E and 4F). A pattern of grain ABA levels similar to that of control treatment was observed under stress conditions in both cultivars. The grain ABA content achieved a maximum value by day 15, and then undertakes a rapid reduction during the later stage of grain growth (15 to 31 DAA) (Figure 4F). Regardless of treatments; grain ABA concentration was more in drought-sensitive than drought-tolerant. In comparison, under water deficit, the grain ABA content in Marvdasht reached 1.8 fold of Zagros at the end of the experiment (Figure 4F). On the basis of our results, total sucrose conversion was stimulated by the lower ABA concentration in drought-tolerant in water stress treatment, but inhibited by higher ABA level in drought-sensitive; the higher ABA concentration in Marvdasht cv. caused the greater inhibition of sucrose conversion in grains (Figure 4B and 4F). Increase in Marvdasht grain ABA content caused a significant reduction in sucrose availability and starch accumulation when compared with their counterpart Zagros ABA concentration.

DISCUSSION

Varieties significantly differed in photosynthetic activities, 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 gs decreases (example, Tenhunen et al., 1987; Nilsen and Orcutt, 1996). Chaves and Oliveira (2004) concluded that gs only affect 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 CO₂ deficit in the chloroplasts is the main cause of decreased photosynthesis under mild and moderate stresses (Flexas and Medrano, 2002). The study observation also showed that soluble proteins of the flag leaves declined with age in both cultivars under WW 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 3). 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).

Irrespective to treatments, drought-tolerant showed a higher chlorophyll content during 7 to 22 DAA, and the differences between cultivars can only be expressed under well water treatment and not evident under stress condition for Chl a. Similar changing pattern was observed for Chl b, although, the differences between cultivars was distinct under the water deficit. 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 addition, one must consider that the protective role of ABA over pigments may be related to stimulation of the nonphotochemical quenching imposed to increase the level of xanthophylls. Ivanov (1995) found that barley seedlings treated with ABA had markedly increased pigments levels, which play

Figure 4. Changes in sucrose, starch and ABA content in control, (A), (C) and (E) and water stress treatments, (B), and (D) and (F) in grains 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.
an important role in maintaining the integrity of the photosynthetic membranes under situations of oxidative stress (Havaux, 1998; Munne-Bosch and Alegre, 2002). In our experiments, the higher Zagros leaf ABA level promoted a greater concentration of chlorophylls and therefore yellowness was prevented. Consequently, ABA may promote (although indirectly) greater stability of the photosynthetic apparatus, allowing more photosynthesis and thus higher accumulation of dry matter in the harvested products (Thomas and Howarth, 2000). Varietal differences were found in terms of the level of sucrose available for metabolism in the grains under drought stress conditions (Figure 4B). In Marvdasht, drought initiated at early stage of grain filling (15 DAA) caused a marked reduction in seed sucrose concentration relative to the well-watered 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 4B), 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-sensitive genotype due to drought stress is partly attributed to reduced availability of the assimilate 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, seed starch concentrations only for drought-sensitive wheat genotype was decreased under drought stress (Figure 4D). Drought induced decrease in seed starch accumulation was more consistent across the stress period considered for Marvdasht than for Zagros. In Marvdasht, drought stress at day 15 resulted in 39% less seed starch concentrations than the corresponding well-watered plants (Figure 4C and 4D). On the other hand, drought stress that lasted up to 15 days did not affect seed starch accumulation of the drought-resistant genotype, Zagros. When the stress period was prolonged to 31 days, seed starch concentration of the genotype increased by ca. 28 and 100% relative to the WW treatment in drought-sensitive and drought-tolerant respectively (Figure 4D). In wheat endosperm, Jenner et al. (1991) found a relationship between the seed carbohydrates and the rate of storage starch accumulation was a function of the concentration of sucrose. Based on these relationships, it appears that shortage of assimilate (sucrose) could be one of the prime factors responsible for the reduced starch accumulation in the seeds of the drought-sensitive wheat genotype. On the basis of our previous study, the failure to set reproductive sinks under drought stress in Marvdasht addition to the decrease in sucrose concentration, attributed from the lower invertase activities in the reproductive structures. In the above context, we suppose that besides to sucrose availability, the capacity for utilizing the assimilate may have been differently affected in the two wheat genotypes under drought stress. The variation in sink may, therefore, partly explain the observed genotypic difference in the establishment and growth of reproductive structures under drought conditions. These results imply that apart from assimilate availability; drought stress may induce other factors that contribute to decreased seed starch synthesis. Limitations of sink activities due to the inhibition of the activities of key enzymes of sucrose metabolism, invertases and sucrase synthase (Weber et al., 2005), and starch synthesis (ADP-glucose pyrophosphorylase and starch synthase) (Ho, 1988) have been cited as principal factors responsible for reduced starch synthesis under drought situations.

As depicted in Figure 4, endogenous grain ABA levels increase with water stress in both genotypes. Relative to the corresponding WW treatments, drought stress increased sink grain ABA concentration of Marvdasht by ca. two-fold at early (7 DAA) and at late stage (31 DAA) of grain development compared with not evident differences for the drought-resistant genotype, Zagros (Figure 4F). We suppose that the differences in ABA accumulation due to drought relative to WW conditions (higher for Marvdasht compared with Zagros) may partly explain the differences found in grain abortion between the two wheat genotypes. Studies have shown that ABA accumulation in reproductive organs during early developmental inhibits cell division and subsequent abortion or failure to set seed (Wang et al., 1999).

Overall, the results obtained from our study furnish valuable information regarding the relationship between ABA content and yield probably has an optimum ABA content which is likely to differ for each environment and crop. Below their optima, increases in ABA content might be reflected in higher yields, but too much ABA perhaps adversely affects grain filling and reduces yield (Quarrie, 1991). The positive relationship between grain growth in vivo and grain ABA content is likely to be via the effect of ABA on stomatal aperture, enhancing water use efficiency (WUE) and leading to a better yield under field conditions. The negative response to ABA may reflect adverse effects on source activity (example, reducing current photosynthesis and accelerating senescence) and a decreased sink ability to attract and utilize available carbohydrates. Increased ABA levels under water stress conditions are likely to be above this optimum and thus negatively affect physiological processes including grain fill.

Conclusion

Drought stress significantly affected the growth and yield
of the two contrasting genotypes. 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. The trend of seed starch accumulation of the genotypes due to the stress imposed maybe influenced by different grain ABA content. The decrease in seed starch accumulation of Marvdasht corresponded with marked increased in grain ABA level whereas the increased starch found for Zagros could be due to the stimulatory effect of appropriate sink ABA level activity factors accompany with sucrose availability.

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