



Full Length Article

The Rates of Photosynthesis, Chlorophyll Content, Dark Respiration, Proline and Abscisic Acid (ABA) in Wheat (*Triticum durum*) under Water Deficit Conditions

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ABSTRACT

A controlled environment pot experiment was conducted to evaluate the effects of water stress on wheat crop. A number of parameters were estimated; two physiological functions; photosynthesis and dark respiration, two osmolytes (amino acid & free proline & Abscisic acid ABA) and the chlorophyll contents. Four cultivars of wheat used in this study were Al-gaimi, Sindy-1, Sindy-2 and Hab-ahmar. The plants were subjected to three water regimes (1) well-watered plants at 80% of the field capacity of soil (FC) and two levels of water stress; (2) moderate stress at 50% FC and (3) severe stress at 30% FC. The water stress reduced the photosynthesis rates in Hab-ahmar and Sindy-2, but not in Al-gaimi and Sindy-1. The dark respiration was increased as a result of water stress in all cultivars, except Hab-ahmar. Chlorophyll content was reduced in all cultivars, except Al-gaimi. Both free proline and ABA showed generally an increase under water stress conditions. The differences between the wheat varieties in tolerance to water stress are discussed. © 2011 Friends Science Publishers

Key Words: Wheat; Water stress; Photosynthesis; Dark respiration; ABA; free Proline

INTRODUCTION

Drought is among the environmental constraints that affect crop growth and crop production worldwide (Ashraf & Foolad, 2007; Farooq *et al.*, 2009). It has been estimated that up to 45% of the world agricultural lands are subjected to drought (Bot *et al.*, 2000). Crop tolerance to abiotic stresses, such as drought is complex at the whole plant and cellular levels (Foolad *et al.*, 2003; Ashraf & Harris, 2004). Water deficit leads to the perturbation of all or some of physiological and biochemical processes and consequently reduces plant growth and yield (Ingram & Bartels, 1996; Boutraa, 2010). Many authors reported that water deficit reduces the rate of photosynthesis in plants (Sharkey, 1990; Cornic, 2000; Lawlor, 2002). The mechanism(s) behind such reduction are not well understood. However, stomatal closure is considered as the major cause for decline in photosynthesis when plants are under water deficit (Cornic, 2000; De Souza *et al.*, 2005). Other mechanisms are also reported such as the inhibition of the activity of the enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Parry *et al.*, 2002) and impairment of ATP synthesis (Tezara *et al.*, 1999). Similarly, dark respiration was found to decline in crop plants (Lawlor & Cornic, 2002; Mohammadkhani & Heidari, 2007) and a desert plant under moderate water stress (Akhkha, 2009). However, an

increase (Akhkha, 2009) and no change (Ribas-Carbo *et al.*, 2005) in dark respiration were also observed. In addition, drought conditions were found to decrease chlorophyll content (Beltrano & Ronco, 2008; Nikolaeva *et al.*, 2010).

Among the common response in plants to abiotic stresses is the production of different types of organic solutes (Serraj & Sinclair, 2002), which include small molecules such as proline (Shao *et al.*, 2006; Tan *et al.*, 2006; Szabados & Savaure, 2010). Those so-called osmotic regulators or compatible osmolytes protect plants from stresses by cellular adjustment through the protection of membranes integrity and enzymes stability (Ashraf & Foolad, 2007; Farooq *et al.*, 2009). A number of studies using transgenic plants demonstrated that proline has a complex effect on stress responses, suggesting that proline is important in stress tolerance (Hong *et al.*, 2000; Mattioli *et al.*, 2008). For example, in wheat, it has been found that proline concentration was higher in stress-tolerant cultivars than in sensitive-tolerant cultivars (Nayyar & Walia, 2003). Proline can enhance stress tolerance by different means; it has been reported that proline has the ability to function as molecular chaperone protecting protein integrity and preventing protein aggregation and stabilisation (Rajendrakumar *et al.*, 1994) and protects the nitrate reductase under osmotic stress conditions (Sharma & Dubey, 2005). Proline plays a role in stabilising membranes

and proteins (Ashraf & Foolad, 2007). It can also act as antioxidant (Sharma & Dietz, 2006) and regulates the cytosolic acidity (Sivakumar *et al.*, 2000).

Abscisic acid (ABA) is a plant hormone that is also accumulated in plants under stresses conditions. It enables the plant to overcome abiotic stresses, such as drought, cold, salt and wounding (Morgan, 1984; Finkelstein *et al.*, 2002). For example, it has been reported that drought tolerance was improved in transgenic plants that overexpressed ABA biosynthetic genes (Iuchi *et al.*, 2001). Furthermore, ABA is also involved in osmotic adjustment in some plant species (Trewavas & Jones, 1991).

The objective of this work was to evaluate the changes in the photosynthesis rates, chlorophyll content, dark respiration and two biochemical markers accumulated in plants under stress conditions that believed to contribute in stress tolerance; the amino acid, Proline and the plant hormone, Abscisic acid (ABA), in four Saudi wheat cultivars under water deficit conditions.

MATERIALS AND METHODS

Plants material: Grains of four cultivars of wheat (*Triticum durum*) were used in this study. The wheat cultivars were; Sindy-1, Sindy-2, Al-gaimi and Hab-Ahmar. Grains were sown in 12 cm plastic pots containing sand: compost mix (1:3 v:v). The experiments were conducted in growth room at the Department of Biology, Faculty of Sciences, University of Taibah, Al-Madinah Al-Munawarh, KSA. Plants were subjected to three soil moisture regimes of the soil field capacity (FC). Each treatment consisted of three pots in a replicate, each pot contains three plants.

The soil moisture for all pots was kept at 80% FC until 21 days after sowing (DAS). After 21 days, the water stress treatments were initiated. Pots were subjected to one of the three water stress treatments; a well watered control, 80% FC and two water stress treatments; moderate water stress, at 50% FC and severe water stress treatment, at 30% FC. In the stressed treatments, moisture levels were allowed to fall from the initial 80% FC to 50% FC and 30% FC, respectively. All pots were weighed on daily basis. The loss in pots weight represents transpiration and evaporation. Cumulative water loss was added to each pot to compensate for transpiration and evaporation. Accumulated water loss was calculated as the differences in pots weights between successive weights.

Gas exchange measurements: Gas exchange rates were measured on intact leaves using a LICOR Infra Red Gas Analyser IRGA, LI-6400 XT (LICOR Inc., Lincoln, NE, USA), fitted with a leaf chamber mounted with a source of light. Fully expanded youngest fourth leaves were used for photosynthesis measurements. The inside temperature of the leaf cuvette was set at $30 \pm 2^\circ\text{C}$. The light responses curves were carried out at ambient CO_2 concentrations (300-350 $\mu\text{mol/mol}$). Measurements were made on five weeks old plants after two weeks of water stress treatments. The red-

blue LED light source attached to the sensor head was applied to the leaves. Leaves were illuminated with a series of light intensities ranging gradually from 0 to 1500 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$ over 7 steps (0, 50, 100, 500, 750, 1000 & 1500 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$).

Chlorophyll estimation: Chlorophyll content in the 4th leaf of five weeks old wheat plants were determined as Chlorophyll Index using a non-destructive method that uses an optical instrument called Chlorophyll Meter (Apogee Instruments Inc., 721W 1800N, Logan, UT 84321, USA).

Proline estimation: Free proline concentrations in plant tissues were determined by the colorimetric method adopted from Bates *et al.* (1973).

ABA estimation: ABA was extracted from plant tissue samples (50 μg dry weight) with 80% aqueous (vol/vol) and then 100% methanol (2 μg of tissue dry weight per μL of extract) for 24 and 6 h, respectively at 4°C in darkness in 6×10 mm borosilicate glass tubes. The extracts were combined and dried using a Rota-Vapor. The resulting residue was dissolved in a small volume of methanol. The methanolic extracts were diluted with Tris-buffered saline [TBS; 50 mM Tris (pH 7.8)/1 mM MgCl_2 /10 mM NaCl] to no more than 10% methanol dilution. The Phytodetek Kit (Agdia, 30380 County Road 6, Elkhart, IN 46514) was used to determine ABA concentrations in different leaf samples from the four wheat cultivars and different water regimes treatments.

Statistical analyses: Three replicates of each treatment were used for all wheat cultivars. The means and standard errors (shown in graphs) were calculated using Excel (Microsoft Office 2007). Analysis of variance was performed using Minitab's ANOVA and General Linear Model GLM (version 15).

RESULTS AND DISCUSSION

Physiological Parameters

Net photosynthesis rates under drought: In the current study, the results (Figs. 1a, b & c) confirmed that water stress has affected the photosynthesis rates of wheat cultivars, but not at the same level. For example, the photosynthesis rates were not affected in both Sindy-1 and Al-gaimi cultivars under the moderate water stress. In contrast, the photosynthesis activity increased under the influence of the severe water stress, but this rise was not statistically significant ($p > 0.05$). The photosynthesis rate has fallen significantly under the moderate water stress for the Hab-Ahmar and Sindy-2 cultivars. With the increase of intensity of water deficit, the effect on photosynthesis was less and the photosynthesis rate was close to that for the control plants under 80% water regime. This indicates that the moderate water stress at 50% field capacity did not affect the drought-tolerant wheat cultivars, while the impact was clear on the photosynthesis efficiency in most drought-sensitive cultivars. It has been reported by many researchers that impact of water deficit at different growth stages on

growth is due to reduction in photosynthesis (Cornic, 1994; Lawlor, 1995).

In a study conducted on the high drought-tolerant desert shrub, *Calotropis procera*, it has been reported that the moderate drought, at 50% field capacity, resulted in an increase in the photosynthesis rate, while the severe stress, at 30% field capacity, did not affect the photosynthesis efficiency, suggesting that drought-tolerant plant species can maintain the integrity of the photosynthesis apparatus to perform their functions normally under stress conditions (Akhkha, 2009). The processes by which drought-tolerant plant species protect the photosynthesis machinery from any damage, as a result of water stress, are not yet clear. Nonetheless, Massacci *et al.* (2008) suggested that the photosynthetic electron transport system is stimulated in the drought-tolerant plants as a result of the high efficiency of the open photosystem II centers. The mechanisms by which the water stress affects the photosynthesis process is not clearly understood yet, but the stomatal closing is one of the most important reasons for the low rates of photosynthesis in plants suffering from water stress (Cornic, 2000; De Souza *et al.*, 2005).

Other mechanisms are also proposed, such as the inhibition of Rubisco activity (Parry *et al.*, 2002). Siddique *et al.* (1990) reached the same result by using four varieties of wheat, suggesting the intervention of other mechanisms other than the stomatal closing that cause decrease in the photosynthesis rates. But Cornic (2000) concluded that the low rate of photosynthesis, as a result of water stress, was partially due to the stomatal closing, as the reduction of stomatal conductance is the most effective way to reduce water loss, but acts as a barrier against the CO₂ diffusion to the photosynthetic cells, which results in the reduction of CO₂ concentration inside the leaves and therefore reduction photosynthesis rate.

Stomatal conductance: To understand the role of the stomatal closing impact on the rate of photosynthesis in the current study, we investigated the impact of water stress on stomatal conductance. The results (Fig. 2) showed that the moderate water stress led to a decline in the rate of stomatal conductance for all cultivars, with Hab-Ahmar and Sindy-2 being more affected. The effect of the severe water stress on stomatal conductance did not differ from that at 80% treatment in Hab-Ahmar and Sindy-1 cultivars, while the Sindy-2 cultivar showed a reduction in stomatal conductance similar to that under the moderate water stress. In contrast, stomatal conductance in Al-gaimi significantly ($p < 0.01$) increased, which might explain slight increase in the photosynthesis rates for this cultivar. The apparent decrease in the photosynthesis rates in Sindy-2 cultivar can be explained by the clear decline in the stomatal conductance under the moderate water stress, indicating that stomatal closing is the main reason for the low photosynthesis rate, but under the severe water stress, the decline in stomatal conductance did not result in a clear reduction in photosynthesis (Sharkey, 1990; Cornic, 2000).

Fig. 1a: Effect of 80% FC water regime on photosynthesis rates

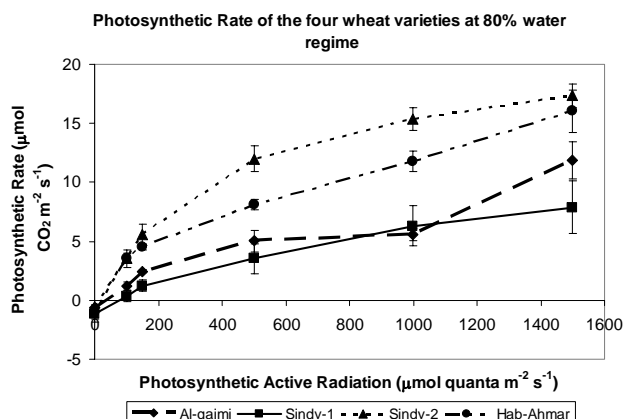


Fig. 1b: Effect of moderate water stress (50% FC) on photosynthesis rates

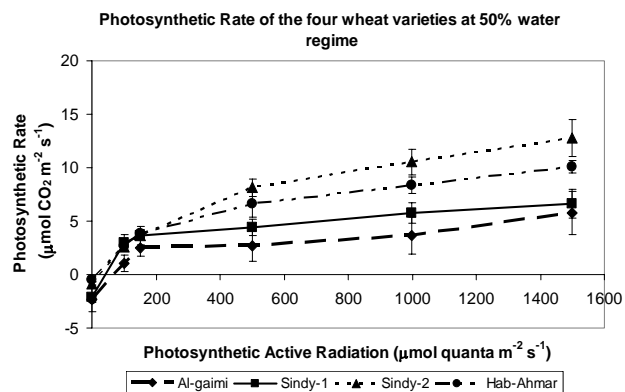
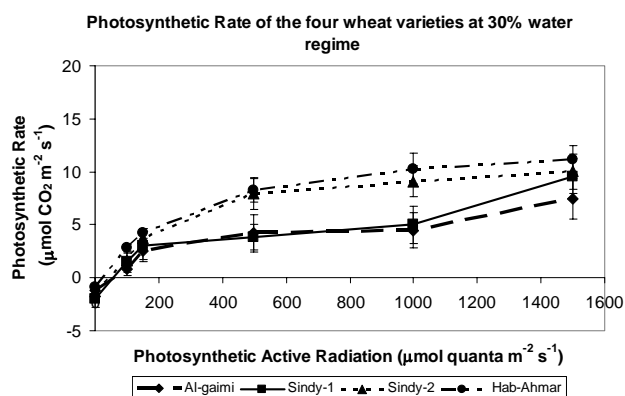


Fig. 1c: Effect of severe water stress (30% FC) on photosynthesis rates



Lawlor (2002) related the decline in photosynthesis activity under water stress to the metabolic limitations, where Tezara *et al.* (1999) proposed that the decline in ATP synthesis was the main reason for the low photosynthesis rates under water stress conditions. However, Cornic and Fresneau (2002) strongly supported the stomatal closing to be the main reason in reducing the photosynthesis rates as a

result of water stress. This is because the maximum value of the photosynthesis can be recovered by supplying sufficient amount of CO₂ to the leaves. Thus, the causes of low of photosynthesis under water deficit depend not only on the stress and plant species or cultivars, but also on the complex interaction between the age of the plant and leaves, the light intensity and other factors (Flexas *et al.*, 2004). The rise in the stomatal conductance in Al-gaimi cultivar might be due to the processes that control the mechanisms of stomatal closing and opening, or to the damage in plant leaves tissues, that result in the loss of water.

Dark respiration: Most physiological functions have been investigated to understand the impact of water deficit, but the dark respiration has not been thoroughly examined, despite its importance in plant functions. The current study examined the impact of different treatments of water stress on dark respiration in the four wheat cultivars to evaluate the effect of water stress on this physiological function. The results showed a general increase in the dark respiration rates, which vary between the cultivars, except the Hab-Ahmar cultivar that showed a clear decline in the dark respiration rates ($p < 0.05$), especially under the medium water stress. The maximum rate of dark respiration was found in two cultivars Al-gaimi and Sindy-1 under the moderate and severe water stress conditions, respectively (Fig. 3). The decline in Hab-Ahmar is in accordance with the findings of Mitchell *et al.* (1991) and Vassileva *et al.* (2009) who reported greater decline in dark respiration in the drought-sensitive wheat plants. Similarly, it has been reported by Mohammadkhani and Heidari (2007) that water deficit caused a decrease in respiration rate in maize as well. It has been also reported that water stress reduced stomatal density and stomatal frequency of wheat (*Triticum aestivum*) genotypes (Vassileva *et al.*, 2010). The decrease in the respiration rate may be due to the direct damage of the mitochondria or the decline in the organic compounds as a result of lower rates in photosynthesis (Mohammadkhani & Heidari, 2007). Similar results have been already reported by Akhkha (2009) in our lab using the drought-tolerant desert plants *Calotropis procera*, where it was noted that the respiration rate declined under moderate water stress conditions, while increased under severe water stress conditions. In contrast, other researchs showed no effect of water stress on the rate of dark respiration (Lawlor, 1976; Loboda, 1993). This increase in the rate of dark respiration was observed in the wheat cultivar Al-gaimi, Sindy-1 and Sindy-2. This is likely that water deficit caused a significant shift of electrons from cytochrome to the alternative pathway to maintain high respiration rates (Ribas-Carbo *et al.*, 2005), which was not tested in the current search.

Chlorophyll content: The results showed that chlorophyll content was not affected in Al-gaimi cultivar, but slightly decreased in the Hab-Ahmar under the moderate water stress conditions. However, chlorophyll content decreased further with the increase of water deficit (Fig. 4). A significant decrease was found in the two cultivars, Sindy-1

Fig. 2: Effect of different water deficit regimes on stomatal conductance of four wheat cultivars

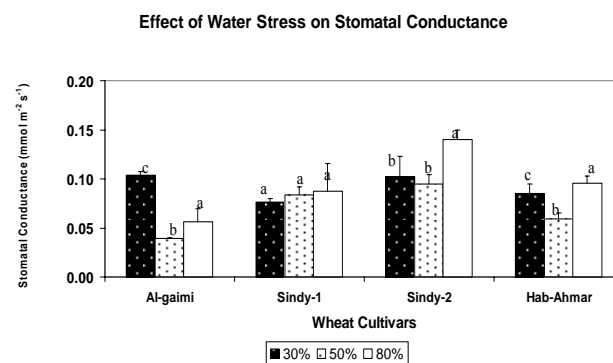


Fig. 3: Effect of different water deficit regimes on dark respiration of four wheat cultivars

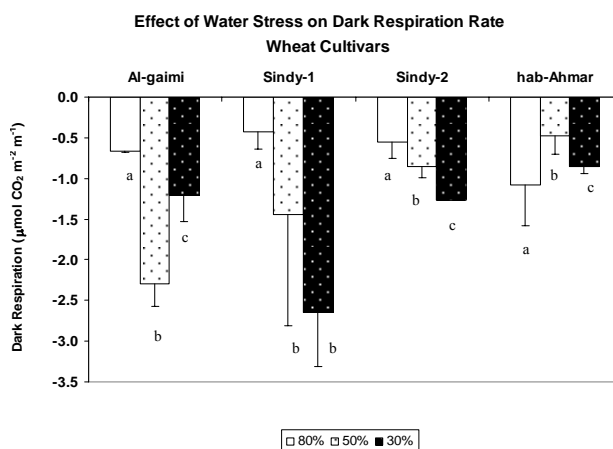
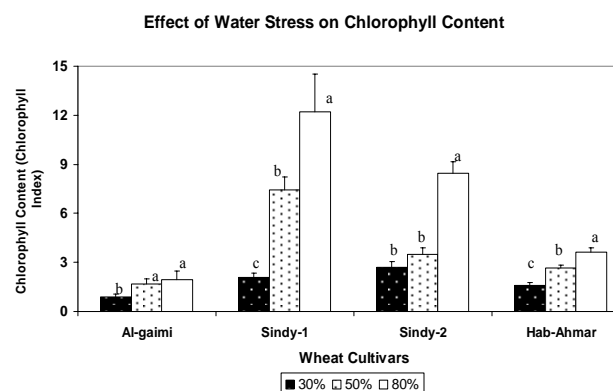


Fig. 4: Effect of different water deficit regimes on chlorophyll content of four wheat cultivars



and Sindy-2 to the maximum level under both moderate and severe water stress levels. Nikolaeva *et al.* (2010), noted a decline in chlorophyll content from 13% to 15% in water stressed wheat compared with the well-watered plants in three varieties of wheat. Beltrano and Ronco (2008) also noted that the content of chlorophyll in leaves of wheat decreased under the influence of moderate and severe water

stress. This is supported by the findings of Jones (1985) and Sikuku *et al.* (2010) noting the inhibition of chlorophyll synthesis and inability of sensitive wheat to withstand water deficit. Contrastingly, Shangguan *et al.* (2000) did not observe any changes in the chlorophyll content in wheat cultivars under different treatments of water deficit.

Biochemical Parameters

Free proline content: The results of this study showed that water deficit led to generally high free proline levels in Hab-Ahmar cultivar, Sindy-1 and Sindy-2 with the latter being not significant ($p > 0.05$). In contrast, water stress caused a decrease in the proline content in Al-gaimi cultivar (Fig. 5). This increase in free proline content due to water deficit has been reported by many authors (Delauney & Verma, 1993; Johari-Pireivatlou *et al.*, 2010). Bajji *et al.* (2000) and Shao *et al.* (2005) reported that free proline accumulation is very important in enhancing osmotic stress tolerance in plants. However, Tatar and Gevrek (2008) suggested that proline is mainly involved in protection against oxidative stress that osmotic adjustment during the onset of water stress. It has been also proven that proline has an essential role in stabilising proteins and cellular membranes in plant cells in the presence of high levels of osmolytes (Errabii *et al.*, 2006; Farooq *et al.*, 2009). In addition, Vendruscolo *et al.* (2007) suggested that proline plays an important role in water stress tolerance mechanism(s) in plants due to its ability in opposing oxidative stress; and considered this as the most important strategy in plants to overcome water deficit effects.

Abscisic acid (ABA) content: Many studies confirmed a high amount of the plant stress hormone ABA as a result of increased water stress, where it plays an important role in the organisation of plant responses to water stress (Davies & Zhang, 1991). Under intense water stress, the concentration of ABA in plants increases, which triggers a number of processes starting from decrease in turgor pressure, decline in cellular expansion then stomatal closure to reduce water loss in leaves (Thompson *et al.*, 1997). The results in the present study did not differ from what has been reported before that water stress causes ABA accumulation in stressed plants (Ünyayar *et al.*, 2004). All cultivars in this study showed an increase in ABA but under moderate water stress and Al-gaimi cv. was the least affected (Fig. 6). However, when water stress becomes severe, water stressed Al-gaimi plants were not significantly different ($p > 0.05$) from the control plants, while ABA content decreased in Sindy-1 and Sindy-2 to levels lower than those of the control plants and especially in the latter. In contrast, in Hab-Ahmar ABA content was lesser under moderate water stress but was still above the control plants. This is assigned to the fact that increased sensitivity of the plants to ABA results in an increase in the ability to withstand stress as it was confirmed using Arabidopsis plants (Cutler *et al.*, 1996). This may explain the slight change in the amount of ABA in Al-gaimi cv., as a small increase in the amount of

Fig. 5: Effect of different water deficit regimes on free proline content of four wheat cultivars

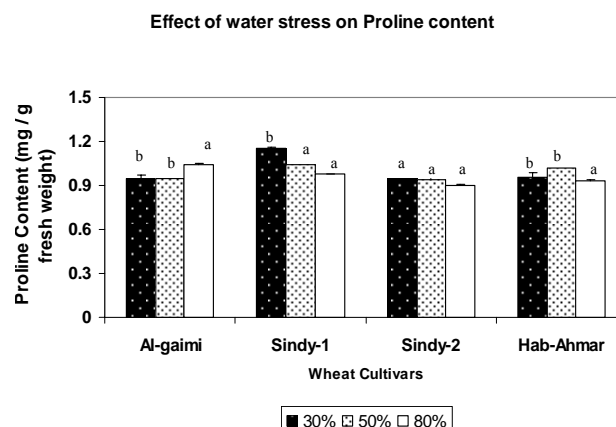
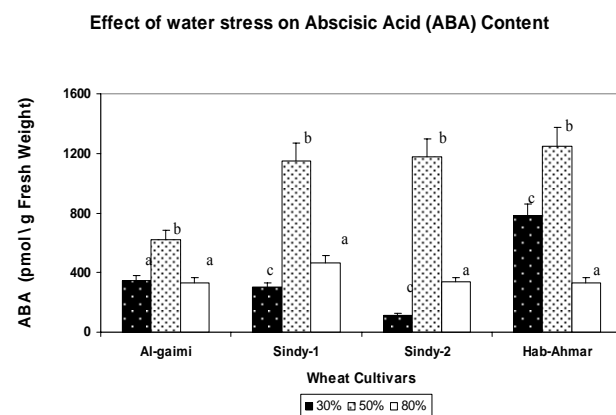


Fig. 6: Effect of different water deficit regimes on abscisic acid content of four wheat cultivars



ABA was enough to make this cultivar more tolerant to water stress than the other cultivars, while the other cultivars needed large amounts of ABA to cope with the water stress.

CONCLUSION

Despite the complexity of the interactions between the effects of water stress on a number of physiological and biochemical parameters in the four Saudi wheat cultivars, we can conclude from these results that Al-gaimi was the most drought-tolerant wheat cultivar, followed by Sindy-1, while the most drought-sensitive cultivar was Hab-Ahmar followed by Sindy-2.

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