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Full Length Research Paper

Water stress causes differential effects on germination indices, total soluble sugar and proline content in wheat (*Triticum aestivum* L.) genotypes

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Different cultivars differ inherently in their response to drought and those cultivars best adapted to growth in arid and semiarid conditions form the most uniform and vigorous stands when grown under water deficits. The seeds of five wheat cultivars (GA-2002, Chakwal-97, Uqab-2000, Chakwal-50 and Wafaq-2001) were subjected to five different levels of osmotic stress; 0 bars (distilled water, control), -2, -4, -6 and -8 bars to assess the effect of osmotic stress on germination percentage, mean germination time, coleoptile length, proline and sugar amounts. The investigations were performed as factorial experiments under complete randomized design (CRD). Germination percentage, mean germination time and coleoptile length were shown to decrease with increasing osmotic stress, whereas a progressive increase in proline and sugar content were observed with increasing osmotic stress. The response of five cultivars examined under various levels of osmotic stress differed dramatically. Chakwal-50 and GA-2002 were amongst best performers, showing high germination rate, longest coleoptile length, highest proline values and sugar contents when compared with other cultivars under stress conditions. These were proven to be the most tolerant cultivars. Performance of Wafaq-2001 and Uqab-2000 were poor when compared to the other cultivars under limited water stress conditions.

Key words: Wheat, *Triticum aestivum*, water stress, osmotic stress, proline, sugar, seedling, germination.

INTRODUCTION

Drought is a worldwide problem, seriously constraining global crop production. Recent global climate change has made this situation even more serious (Pan et al., 2002). Current estimates indicate that 25% of the world's agricultural land is now affected by high levels of water stress (Jajarmi, 2009). Drought is connected with almost all aspects of biology (Bayoumi et al., 2008), and is one of the major causes of crop loss worldwide, which commonly reduces average yield for many crop plants by more than 50% (Wang et al., 2003).

Wheat (*Triticum aestivum* L.) is one of the world's most widely adapted crop supplying one-third of the world population with more than half of their calories and nearly half of their protein. In Pakistan, wheat is a staple food and occupies a central position in setting farming and agriculture policies. It contributes 14.4% value added to agriculture and 3.1% to GDP (Government of Pakistan, 2009 -10). Wheat is mainly grown on rainfed lands without supplementary irrigation. About 37% of land area in developing countries consists of semiarid environments in which available moisture constitutes a primary constraint to wheat production. Drought negatively affects seedling emergence and establish-ment, root to shoot ratio, solute accumulation (Blum, 1996), photosynthesis (Brar et al., 1990) and ultimately the yield of the crop. Drought stress affects yield by depressing both sink and source, depending on the timing and the severity of stress with respect to plant phenology (Blum, 1996). Plants may be affected by drought at any time in life, but certain stages such as germination and seedling growth are critical (Pessarakli, 1999).

Seed germination and seedling vigor are prerequisites for successful stand establishment and under rainfed

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conditions of arid and semiarid regions; low moisture is the main limiting factor for germination. The rate and degree of seedling establishment are extremely important factors in determining yield (Brigg and Aylenfisu, 1979; Rauf et al., 2007). As such, drought stress at the seed germination and seedling stage is a major determinant of wheat production in many parts of the world. In particular, seed vigor index and shoot length are among the most sensitive to drought stress, followed by root length and coleoptile length (Dhandas et al., 2004). And it has been demonstrated that under drought conditions there is a significant positive relationship between coleoptile length and drought resistance index in wheat (Song-ping et al., 2007).

Osmotic adjustment is a well-known mechanism by which plants tolerate drought. Compatible solutes are produced at higher levels when plants experience osmotic stress as a means to facilitate osmotic adjustment (Hasegawa et al., 2000; Zhu, 2000; Shao et al., 2005). These compounds accumulate in high amounts mainly in cytoplasm of stressed cells and behave as osmoprotectants of membrane and protein integrity (Yancey, 1994). High accumulation of proline (Rhodes et al., 1999; Ozturk and Demir, 2002; Hsu et al., 2003; Kavi-Kishore et al., 2005) and sugars (Mohammadkhani and Heidari, 2008) under stress is a characteristic feature of most plants. Selection for drought tolerance at the early seedling stage is frequently accomplished using simulated drought induced by chemicals. Polyethylene glycol (PEG-6000) is the most widely used chemical for this purpose, generally used to modify the osmotic potential of nutrient solution and induce plant water deficit in a relatively controlled manner (Carpita et al., 1979). Polyethylene glycol molecules are inert, non-ionic, virtually impermeable to cell membranes and can induce uniform water stress without causing direct physiological damage (Carpita et al., 1979; Lu and Neumann, 1998; Kulkarni and Deshpande, 2007).

Germination and seedling stage is considered to be the most critical growth stage, especially under water stress conditions for the successful stand establishment of crop plants. The present study was conducted to evaluate five wheat cultivars for drought resistance at germination and seedling stage. PEG-6000 was used as an osmoticum to induce stress conditions. The objective of this study was to evaluate wheat varieties for drought resistance at germination and seedling stage.

MATERIALS AND METHODS

The experiments were conducted in Crop Physiology Laboratory, Department of Agronomy, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan. Five wheat (*Triticum aestivum* L.) varieties (GA-2002, Chakwal-97, Uqab-2000, Chakwal-50 and Wafaq-2001) were subjected to five stress levels at 0 bars (distilled water, control), -2, -4, -6 and -8 bars to test germination and seedling growth. The seeds of the wheat genotypes were obtained from National Agriculture Research Council, Islamabad. Osmotic stress (-2, -4, -6 and -8 bars) was created using different concentrations of polyethylene glycol (PEG) 6000 at 20°C according to the method as used by Michel and Kaufmann (1973). Experiments were laid out in a two factorial design using complete randomize design (CRD) with four replications.

Determination of mean germination time (MGT)

Forty healthy and uniform seeds of each genotype were selected and then sterilized with 1.0% sodium hypochlorite solution for 3 min (Mcgee, 1988). Seeds were then put in sterilized 9 cm Petri dishes containing germination paper moistened with 8 ml of the four solutions of PEG-6000. The Petri dishes were kept in an incubator at 20 ± 0.5°C (Rehman et. al., 1996; Ghodsi, 2004) and the number of seeds germinated in each Petri dish recorded daily for 8 days. For germination purposes, only those seeds that presented approximately 2 mm of root length were considered germinated (Sapra et al., 1999; Afzal et al., 2004). The numbers of seeds germinated were counted daily and the germination percentage and mean germination time were estimated. The mean germination time (MGT) was determined by using formula as described by Sadeghi et al. (2011).

$$MGT = \sum Dn / \sum n,$$

Where, Dn is the number of seeds which germinated on day D and n is the number of days from beginning of germination test to day D.

Determination of CRI

At the end of the eighth day, five seedlings were randomly selected (from each treatment) and the coleoptile length measured and mean length determined. Coefficient of relative inhibition (CRI) was calculated by the formula given by Mercado (1973), which is a measure of growth inhibition based on the stress treatments suppression of overall plant biomass accumulation;

$$CRI = \frac{\text{Biomass of unstressed plants} - \text{Biomass of stressed plants}}{\text{Biomass of unstressed plants}}$$

A second experiment was laid out in two factorial design using completely randomize design (CRD) with three replications. Seeds were sown in Petri dishes and after seven days the seedlings were transplanted into hydroponics having modified MS medium solution (Murashige and Skoog, 1962). After 3 to 4 days, the modified MS medium was supplemented by PEG 6000 to induce osmotic stress of -2, -4, -6 and -8 bars. MS medium without PEG served as control. After 2 days, there was a visible effect of the treatments on growth and plants were harvested. Proline and sugar contents of the stressed and control seedling were then measured.

Determination of proline content

Proline (mg/g fresh weight) amounts were determined following the method of Bates et al. (1973). 0.1 g of fresh sample of leaves was added in 5 ml of 3% sulfosalicylic acid in test tubes, ground and then allowed to settle. Then 2 ml from supernatant was mixed with 2 ml each of glacial acetic acid and ninhydrin reagent and was boiled for 1 h in water bath at 100°C. After 1 h, the reaction was stopped in ice and finally 4 ml of toluene was added, vortexed and the absorbance of the supernatant was read at 520 nm on the UV Spectrophotometer (Biochem, 2100). Toluene was used as blank

Table 1. Effect of polyethylene glycol (PEG)-induced osmotic stress on germination indices: germination percentage, mean germination time and coleoptile length of five wheat cultivars

Treatment	Germination (%) ±SE	Mean germination time ± SE	Coleoptile length (cm) ± SE
Control	100.00 ± 0[a]	17.10 ± 0.02[a]	4.29 ± 0.02[a]
-2 Bars	95.63 ± 0.11 [b]	13.31 ± 0.02[b]	3.89 ± 0.02[b]
-4 Bars	82.00 ± 0.38[c]	10.38 ± 0.05[c]	2.99 ± 0.03[c]
-6 Bars	67.63 ± 0.52[d]	7.31 ± 0.05[d]	2.03 ± 0.03[d]
-8 Bars	59.25 ± 0.46[e]	5.69 ± 0.04[e]	1.51 ± 0.02[e]

Significant at P<0.05.

sample.

$$\text{Proline} = \frac{\text{Absorbance of sample} \times \text{K value} \times \text{dilution factor}}{\text{Weight of sample} \times 100}$$

Determination of sugar content

Total soluble sugar (mg/g fresh weight) was determined based on the method given by Dubois (1951). Fresh leaves (0.1 g) were added with 5 ml of 80% ethanol to test tubes, placed in water bath and heated for 1 h at 80°C. Then 1 ml of the sample extract was taken in another set of test tubes and mixed with 1 ml each of 18% phenol and distilled water, and then allowed to stand at room temperature for 1 h. Finally, 5 ml of sulphuric acid was added and the whole mixture was vortexed. Absorbance was read at 490 nm wavelength on the UV spectrophotometer (Biochem, 2100). 80% Ethanol was used as blank of sample.

$$\text{Total soluble sugar} = \frac{\text{Absorbance of sample} \times \text{K value} \times \text{dilution factor}}{\text{Weight of sample} \times 100}$$

Statistical analysis

The data collected was analyzed statistically using analysis of variance techniques to identify significant differences among wheat varieties. Least significant difference test was applied at five percent level of probability to compare the treatment means as explained by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Data pertaining to effect of PEG induced stress on germination percentage, mean germination time and coleoptile length is shown in Table 1. Significant differences were observed among all these parameters. An inverse relationship was observed between osmotic stress level and the following: germination percentage, MGT and coleoptile length. Germination percentage decreased from 100 in control to 59.25% in -8 bars osmotic stress, while MGT decreased from 17.10 in control to 5.69 under -8 bars osmotic stress. Increase in stress level caused a linear decrease in germination percentage and MGT in all wheat cultivars. Germination is a critical stage of plant life. Seed germination and vigor are prerequisite for the successful establishment of plants. Water stress at this stage can result in delayed and reduced germination or may prevent germination completely (Hegarty, 1977). It has been suggested (Hunter and Erickson, 1952) that once a seed attains a critical level of hydration it will precede without cessation toward full germination. However, physiological changes do occur at hydration levels below this critical level that can cause an inhibition of germination. Therefore, water stress is reported to delay and reduced germination or may prevent germination completely (Hegarty, 1977). For example, reduction in germination percentage can result from PEG treatments that decrease the water potential gradient between seeds and their surrounding media (Dodd and Donovan, 1999). Water stress significantly reduces the MGT, thus reducing the resistance of young plants to withstand other unfavorable field conditions. Our findings which revealed that moderate stress intensities only delay germination, while high stress intensities have an impact on the final germination percentages are consistent with that of Almansouri et al. (2001). Resistance to water stress during the germination stage can make a plant stable for later growth.

PEG-induced osmotic stress also had an adverse effect on coleoptile length. Coleoptile length decreased significantly from 4.29 in control to 1.51 cm in -8 bars osmotic stress. In cereals, coleoptile is a specialized tissue that provides a protective shield to the primary leaf until it reaches to the soil surface. In the dark underground environment, coleoptile elongation must equal or exceeds that of leaf it encloses as they grow upwards together (Salisbury and Ross, 1992). Water availability affects coleoptile growth and thus affects the seedling emergence and stand establishment. Wang et al. (1999) found a significant relation between coleoptile length (CL) and drought resistance index (DRI) in wheat under drought, and suggested that the CL can be used to evaluate drought tolerance (DT) in wheat and to screen drought-tolerant genotypes.

The wheat cultivars under study in this report differed significantly in response to osmotic stress conditions. Chalwal-50 followed by GA-2002 gave a better germination, MGT and coleoptile length (Table 2). Water stress generally reduces seed germination, coleoptile length and delays germination, and ultimately the crop yield is

Table 2. Performance of five wheat cultivars germinated under PEG-induced osmotic stress on three germination indices: germination percentage, mean germination time and coleoptile length

Cultivar	Germination (%) ±SE	Mean Germination Time ±SE	Coleoptile length (cm) ±SE
GA-2002	84.38 ± 0.72^{b}	11.12 ± 0.20^{b}	3.41 ± 0.05^{b}
Chakwal-97	82.25 ± 0.75^{b}	10.87 ± 0.20^{b}	2.91 ± 0.05^{c}
Uqab-2000	73.00 ± 1.11^{d}	9.88 ± 0.24^{d}	2.33 ± 0.06^{e}
Chakwal-50	87.13 ± 0.62^{a}	11.55 ± 0.20^{a}	3.59 ± 0.05^{a}
Wafaq-2001	77.75 ± 0.92^{c}	10.36 ± 0.22^{c}	2.46 ± 0.06^{d}

Significant at P<0.05.

reduced. Germination percentage and speed are important for seedling establishment, which in turn determines both yield and time of maturity (Rauf et al., 2007). Coleoptile length is related with drought resistance in wheat (Song-ping et al., 2007). Chakwal-50 and GA-2002 based on germination response and coleoptile length under stress conditions in these experiments may be considered better drought tolerant cultivars. Uqab-2000 exhibited the lowest values for germination (73%), MGT (9.88) and coleoptile length (2.33 cm), thereby manifesting the least resistance against this water deficiency stress. Differential cultivar response to these osmotic stress treatments suggests a great deal of genetic variation among cultivars that could be utilized to develop new wheat cultivars adapted to arid and semiarid regions. Alaei et al. (2010), Jajarmi (2009) and Bayoumi et al. (2008) all reported variable response of wheat cultivars for germination percentage, MGT and coleoptile length to various osmotic stress levels. Results presented here are consistent with previous findings that certain germination criteria such as germination percentage, MGT, coleoptile length, root growth and shoot growth can all be used for selecting drought-resistant cultivars.

Coefficient of relative inhibition (CRI) is a measure of growth inhibition; the greater the coefficient, the more the inhibition of plant growth. The differences among the water stress treatment for CRI were significant (Figure 5). An increase in osmotic stress caused a significant increase in CRI from 0.17 at -2 bars to 0.62 under -8 bars osmotic stress. Increase in CRI indicates a progressive inhibition of plant growth in conformity to the observation of Meiri and Poljakoff-Mayber (1970) who found that the reduction in the plant growth was dependent on the ultimate level of stress condition. These results are also in line with the findings of Mercado (1973) who reported positive relationship between CRI and growth reduction under salinity. Differences among cultivars were also highly significant for CRI (Figure 6). Chakwal-50 and GA-2002 showed minimum values of CRI (0.32 and 0.34, respectively), hence showed maximum resistance against water stress as compared to other cultivars. Uqab-2000 exhibited the maximum (0.48) values of CRI and showed minimum resistance against water stress. Therefore, Chakwal-50 may be considered more stress resistant variety followed by GA-2002 and Uqab-2000 being the most susceptible.

The difference among the water stress treatments for proline and sugar were highly significant at 5% level of probability (Figures 1 and 2) and there was a progressive increase in proline and sugar contents with increased osmotic stress. Proline content increased from 0.33 in control to 2.65 mg/g in -8 bars osmotic stress, while sugar content increased to 3.78 under -8 bars osmotic stress from 1.49 mg/g in control. Proline plays an important role in minimizing the damage caused by dehydration (Nayer and Heidari, 2008). Recent studies have demonstrated that the manipulation of genes involved in the biosynthesis of low molecular weight metabolites such as proline, can improve plant tolerance to water deficiency. High proline synthesis in stressed plants under field conditions could favor a better recovery of the plants (Tatar and Gevrek, 2008). Plant cells achieve their osmotic adjustment by the accumulation of different kinds of compatible solutes such as proline, betaine and polyols to protect membranes and proteins (Delauney and Verma, 1993).

It has also been shown that proline also plays a key role in stabilizating cellular proteins and membranes in presence of high concentrations of osmoticum (Yancey, 1994; Errabii et al., 2006). These results are in accordance with the findings of Mohammadkhani and Heidari (2008), Tatar and Gevrek (2008) and Kameli and Losel (1996) who reported that wheat sugar and proline content increased under drought stress conditions. Higher proline content in wheat plants after water stress has also been reported by Vendruscolo et al. (2007) and Patel and Vora (1985). Increasing amounts of proline under several stress conditions including water deficit stress, was also observed in wheat (Charest and Phan, 1990; Nayyar, 2003; Poustini et al., 2007; Tian and Lei, 2007). Sugars also play a role in osmotic adjustment, with increasing soluble sugars under water stress reported by Johari et al. (2010). Higher amount of soluble sugars and a lower amount of starch were found under water stress conditions in maize plants (Mohammadkhani and Heidari, 2008). Kerepesi and Galiba (2000) also found that tolerant genotypes accumulated more total water-soluble carbohydrate (WSC), glucose, fructose and sucrose than did sensitive ones when subjected to PEG induced drought stress. Moreover, the wheat cultivars

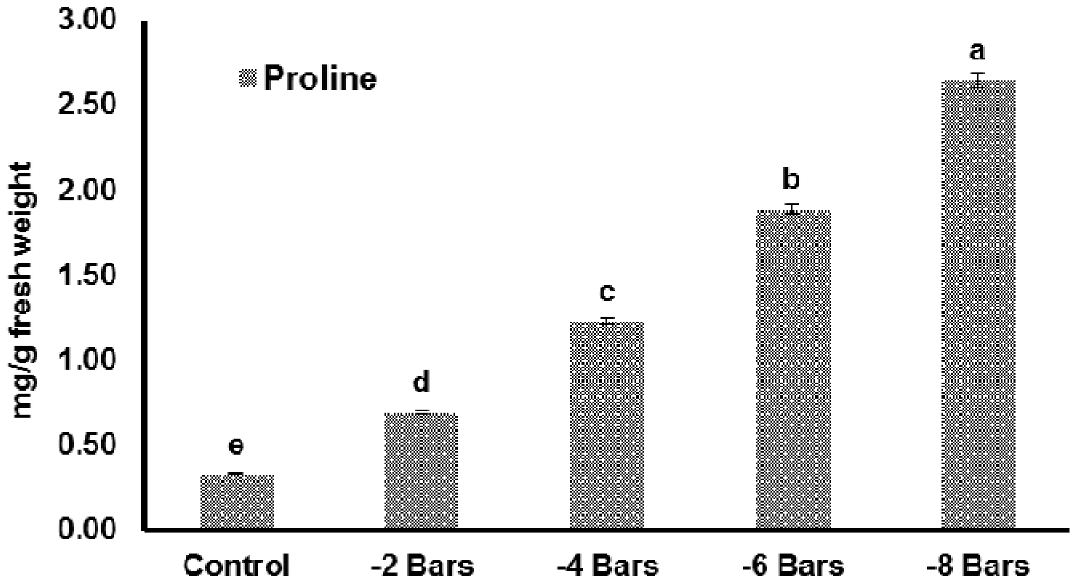

Figure 1. Proline content at different osmotic stress levels of 14-day old wheat seedling of five wheat cultivars (all means [± S.E.] are significantly different at P<0.05).

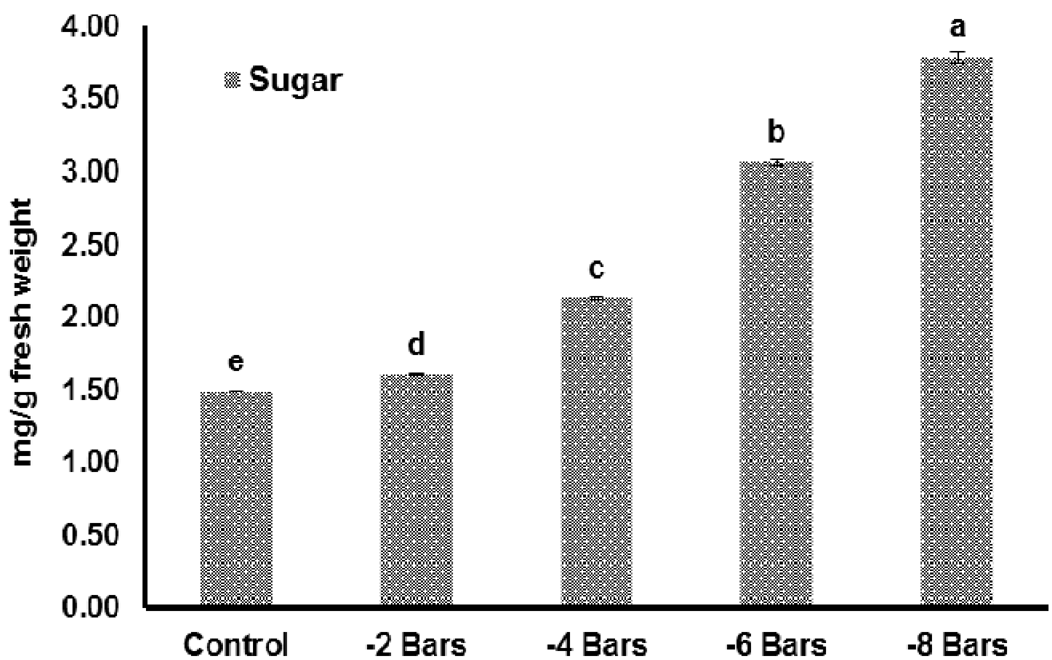

Figure 2. Soluble sugar content at different osmotic stress levels of 14-day old wheat seedling of five wheat cultivars (all means [± S.E.] are significantly different at P<0.05).

examined here differed significantly in their seed germiation response to osmotic stress. Chalwal-50 produced higher proline (1.80 mg/g) and sugar (2.64 mg/g) contents (Figures 3 and 4) with lowest (0.32) CRI, hence it was more stress tolerant than the other cultivars tested. Uqab-2000 however, produced the lowest values for proline (0.89 mg/g) and sugar (2.14 mg/g) contents and was the least resistance to water stress.

Our results therefore indicated that accumulation of high proline content could be a very good criterion for selecting tolerant genotypes (Vendruscolo et al., 2007). These results are similar to the findings of Keyvan (2010), Mohammadkhani and Heidari (2008) and Hong-Bo et al. (2006) in wheat. The wheat variety Chalwal-50,

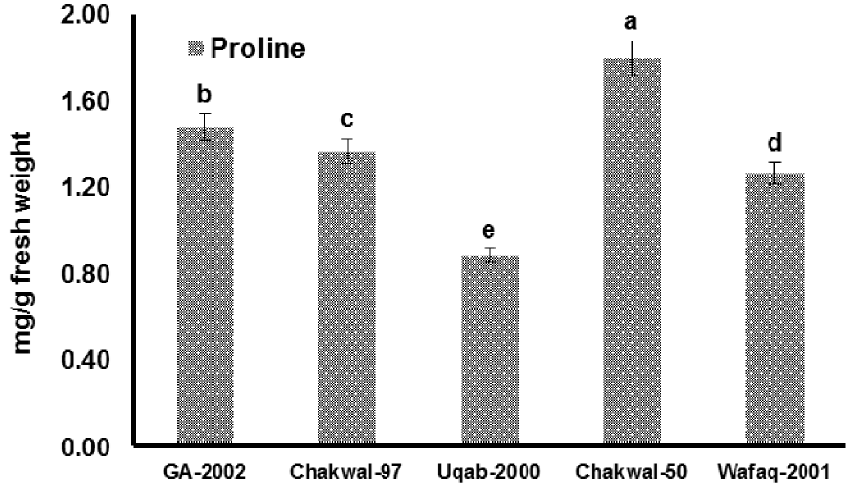

Figure 3. Proline content of 14-day old seedlings of five wheat cultivars (all means [± S.E.] are significantly different at P<0.05).

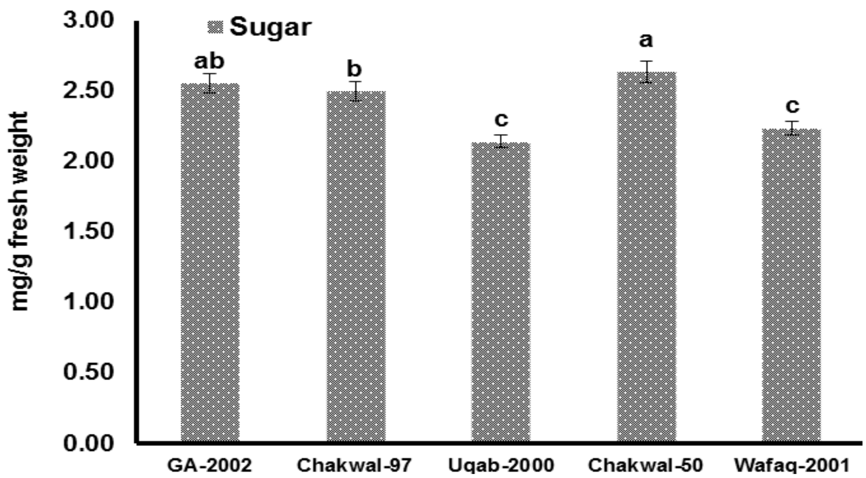

Figure 4. Total soluble sugar content of 14-day old seedlings of five wheat cultivars (all means [± S.E.] are significantly different at P<0.05).

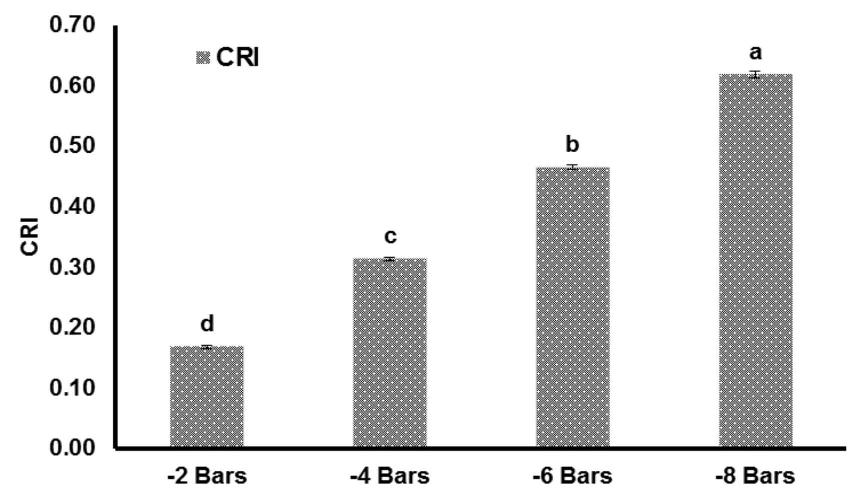

Figure 5. Coefficient of relative inhibition at different osmotic stress levels of five wheat (all means [± S.E.] are significantly different at P<0.05).

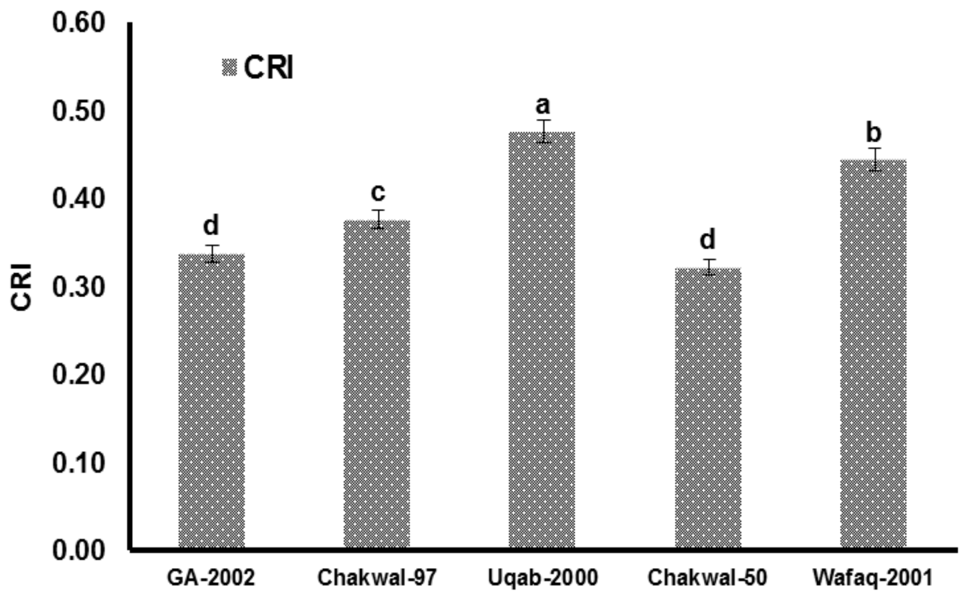

Figure 6. Coefficient of relative inhibition of eight-day-old seedlings of five wheat (*Triticum aestivum*) cultivars (all means [± S.E.] are significantly different at P<0.05).

Chalwal-97 and GA-2002 may be excellent cultivars to grow in regions where water deficiency stress may be common during the germination and early seedling growth stages. These also may serve as excellent parents to initiate a breeding program using recurrent selection to develop even better water stress tolerant lines.

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