

## ALLEVIATION OF DROUGHT-INDUCED ADVERSE EFFECTS IN SPRING WHEAT (*TRITICUM AESTIVUM* L.) USING PROLINE AS A PRE-SOWING SEED TREATMENT

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### Abstract

An experiment was conducted to assess the effect of exogenous application of proline as a pre-sowing seed treatment on morpho-physiological and yield attributes of 5 wheat cultivars viz., SARC-I, Inqlab-91, MH-97, Bhakkar and S-24 under well watered or water deficit conditions. Plants of the 5 wheat cultivars raised from proline (control, 20 mM and 40 mM) treated seeds were subjected to water stress i.e. well watered and 60% field capacity for 63 days. Water stress reduced shoot and root fresh and dry weights, shoot length, total leaf area per plant, grain yield and gas exchange characteristics and increased shoot P contents. However, the effect of pre-sowing proline on shoot  $K^+$  and  $Ca^{2+}$  and root N,  $Ca^{2+}$  and  $K^+$  was not-significant. Exogenous application of proline as a pre-sowing seed treatment improved shoot and root fresh and dry weights, shoot length and grain yield under both non-stress and stress conditions and total leaf area per plant only under stress conditions. Proline level, 20 mM was effective for Inqlab-91 and MH-97, while for others, 40 mM proline was more effective in promoting plant growth and other related attributes under water deficit conditions. Performance of Bhakkar and MH-97 was poor as compared to the other cultivars under drought stress conditions.

### Introduction

Of various abiotic factors, water scarcity adversely affects the crop productivity (Jones & Corlett, 1992). Generally, drought stress reduces growth (Levitt, 1980) and yield of various crops (Dhillon *et al.*, 1995) by decreasing chlorophyll pigments and photosynthetic rate (Asada, 1999), and stomatal conductance as well as transpiration rates (Lawlor, 1995). Drought stress reduces the nutrient uptake in plants (Baligar *et al.*, 2001).

However, it is now well evident that drought stressed plants exhibit various physiological, biochemical and molecular changes to thrive under water limited conditions (Arora *et al.*, 2002). Under various environmental stresses, high accumulation of proline is a characteristic feature of most plants (Rhodes *et al.*, 1999; Ozturk & Demir, 2002; Hsu *et al.*, 2003; Kavi-Kishore *et al.*, 2005). Its accumulation is generally correlated with stress tolerance because tolerant species accumulate more proline as compared to sensitive ones. For example, salt-tolerant alfalfa (Fougere *et al.*, 1991; Petrusa & Winicov, 1997) and drought tolerant wheat (Nayyar & Walia, 2003) accumulated higher amount of proline than the sensitive cultivars.

Exogenous application of proline is known to induce abiotic stress tolerance in plants (Claussen, 2005; Ali *et al.*, 2007; Ashraf & Foolad, 2007), because proline may protect protein structure and membranes from damage, and reduce enzyme denaturation (Iyer & Caplan, 1998; Rajendrakumar *et al.*, 1994; Saradhi *et al.*, 1995; Smirnov & Cumbe, 1989). It may also act as a regulatory or signaling molecule to activate a variety of responses (Maggio *et al.*, 2002). Its storage is also beneficial for plants as a source of nitrogen (Hare *et al.*, 1998). Ali *et al.*, (2007) found that exogenous application of proline enhances gas

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exchange attributes like net CO<sub>2</sub> assimilation rate, transpiration rate and stomatal conductance. However, effect of proline is concentration dependent (Ashraf & Foolad, 2007). Exogenous application of proline in low concentration decreased the potassium efflux from the barley root under salt stress (Cuin & Shabala, 2005). In grasses, high nitrogen uptake due to high proline accumulation is also reported (Tanguiling *et al.*, 1987).

Different osmotica can be applied exogenously to plants in three different ways i.e., through the rooting medium, as a foliar spray or pre-sowing seed treatment. Reports on the effects of foliar application of proline in alleviating the adverse effects of abiotic stresses can be deciphered from the literature (Claussen, 2005; Ali *et al.*, 2007; Ashraf & Foolad, 2007), but there is little information available on the effect of proline application as a pre-sowing seed treatment on mitigating the inhibitory effects of abiotic stresses on plants. Thus, the main objective of the present study was to investigate the effect of pre-sowing seed treatment with proline on morpho-physiological and yield attributes of wheat. Secondly, to explore which level of proline would be more effective when wheat is grown under drought stress.

## Materials and Methods

A pot experiment was conducted to assess the effect of pre-sowing seed treatment on morpho-physiological and biochemical attributes of wheat (*Triticum aestivum* L.) under well watered or drought stress conditions. Wheat cultivars used were SARC-I, Inqlab-91, MH-97, Bhakkar and S-24. Seed of cvs. Inqlab-91, MH-97 and Bhakkar was obtained from the Ayub Agricultural Research Institute, Faisalabad, while, that of SARC-I from the Department of Plant Breeding and Genetics and of S-24 from the Department of Botany, University of Agriculture, Faisalabad. The experiment was carried out in the Botanical Garden of the Department of Botany, University of Agriculture, Faisalabad during the year 2007-08. There were two drought levels i.e., well-watered and 60% field capacity, three proline (M. wt. = 115.3) of Sigma-Aldrich) levels (0, 20 and 40 mM) for pre-sowing seed treatment. The grains were surface sterilized with 5% sodium hypochlorite solution for 10 minutes and then soaked in the earlier mentioned proline solutions for 16 h. Plastic pots of uniform size (20 cm diameter and 24 cm depth) containing 9 kg dry sandy loam soil were used. The saturation percentage of the soil used was 45 and pH 7.69. Twelve seeds were sown in each pot. The plants were thinned to maintain 6 plants per pot and allowed to establish for 42 days before the start of water deficit conditions i.e., well watered and 60% field capacity. Plants samples were harvested, after 63 days of drought treatments. Plants were uprooted carefully and washed with distilled water. After recording fresh weights of all plant samples they were dried in an oven at 65°C to constant dry weight. Shoot length and total leaf area per plant were also measured.

**Gas exchange characteristics:** A portable infrared gas analyzer (model LCA-4; Analytical Development Company, Hoddesdon, England) was used to measure various gas exchange characteristics such as net CO<sub>2</sub> assimilation rate (*A*), transpiration rate (*E*), stomatal conductance (*g<sub>s</sub>*) and sub-stomatal CO<sub>2</sub> concentration (*C<sub>i</sub>*). A fully expanded second leaf of each plant was used for all these measurements. Measurements were performed from 10.00 to 13.00 h with the following specifications/adjustments of the leaf chamber: molar flow of air per unit leaf area 403.3 mmol m<sup>-2</sup> s<sup>-1</sup>, atmospheric pressure 99.9 kPa, water vapor pressure into chamber ranged from 6.0 to 8.9 mbar, PAR at leaf surface was maximum upto 1711 μmol m<sup>-2</sup> s<sup>-1</sup>, temperature of leaf ranged from 28.4 to

32.4°C, ambient temperature ranged from 22.4 to 27.9°C, and ambient CO<sub>2</sub> concentration was 352 µmol mol<sup>-1</sup>.

**Determination of mineral elements:** The dried ground material (0.1 g) of shoots or roots were digested following Allen *et al.*, (1986). Potassium and calcium contents in the roots and shoots were determined with a flame photometer (Jenway PFP 7). Nitrogen was estimated by micro-Kjeldhal's method (Bremner, 1965), while phosphorus was determined spectrophotometrically (Jackson, 1962).

**Yield attributes:** Grain yield per plant and 100-grain weight were recorded at maturity.

**Statistical analysis:** Analysis of variance of the data for each attribute was computed using the MSTAT-C Computer Program (MSTAT Development Team, 1989). Mean values of each attribute were compared using the least significance difference test (LSD) at 5% levels of probability following Snedecor & Cochran (1980).

## Results

Imposition of water stress (60% of field capacity) reduced shoot fresh and dry weights of all wheat cultivars (Table 1; Fig. 1). However, exogenous application of proline as a pre-sowing treatment improved the shoot fresh and dry weights of all cultivars except Bhakkar and MH-97 under both non-stress and drought stress conditions. Pre-sowing seed treatment with 40 mM proline was more effective than 20 mM proline in enhancing shoot fresh and dry weights of all wheat cultivars except cv. Bhakkar and MH-97 under stress conditions.

Root fresh and dry masses of five wheat cultivars decreased significantly due to the imposition of water stress (Table 1; Fig. 1). However, water stress-induced reduction in root fresh and dry biomass was more in cv. MH-97 and Bhakkar than that in to the other cultivars. Exogenous application of proline as a pre-sowing treatment increased the root biomass of non-stressed or water stressed plants of all cultivars except those of S-24 under non-stress and those of SARC-I under drought stress conditions. Furthermore, 20 mM proline was more effective in mitigating the adverse effects of water stress particularly on cvs. Inqlab-91 and MH-97.

Imposition of drought stress reduced shoot length of all wheat cultivars (Table 1; Fig. 1). However, exogenous application of proline increased the shoot length of only stressed plants of all cultivars, whereas the shoot length of non-stressed plants remained unchanged due to pre-sowing seed treatment with proline.

Total leaf area per plant of wheat cultivars (Table 1; Fig. 1) decreased significantly under water deficit conditions. However, exogenous application of proline as a seed treatment improved the leaf area of all cultivars except Bhakkar under both well watered and water stress conditions. The proline level 40 mM, was more effective for SARC-I and S-24, while 20 mM proline for Inqlab-91 and MH-97 under both normal and water deficit conditions. Overall, S-24 followed by SARC-I was better as compared to others in leaf area.

Water stress caused a significant reduction in photosynthetic rate of all wheat cultivars (Table 1; Fig. 2). Cultivars differed significantly in photosynthetic rate under non-stress or water stress conditions. Exogenous application of proline as a seed treatment did not significantly affect net CO<sub>2</sub> assimilation rate. Of all cultivars, Bhakkar was the lowest of all cvs. in photosynthetic rate under both stress and non-stress conditions.

**Table 1.** Mean squares from analyses of variance of data for different growth, gas exchange characteristics and shoot and root mineral nutrients of wheat plants (raised from proline pre-treated grains) subjected to control or drought stress conditions for 63 days.

Source of variation	df	Shoot f. wt.	Shoot d. wt.	Root f. wt.	Root d. wt.
Cultivars (Cvs)	4	194.33***	1.261***	1.145***	0.346***
Drought (D)	1	661.4***	20.135***	22.07***	2.944***
Proline (Pro)	2	56.03***	1.694***	0.188**	0.0344ns
Cvs x D	4	9.618ns	1.166***	0.292***	0.0461**
Cvs x Pro	8	8.103ns	0.154ns	0.377***	0.037**
D x Pro	2	5.706ns	0.048ns	0.038ns	0.0094ns
Cvs x D x Pro	8	12.93**	0.359*	0.377***	0.054***
<b>Error</b>	<b>60</b>	<b>4.056</b>	<b>0.144</b>	<b>0.246</b>	<b>0.012</b>
		Shoot length	Total leaf area	Grain weight	100-grain weight
Cultivars (Cvs)	4	180.67***	119378.3***	2.725***	3.575***
Drought (D)	1	2888.3***	460909.4***	43.89***	17.96***
Proline (Pro)	2	139.85**	48761.31**	0.763***	1.306**
Cvs x D	4	167.68***	112653.2***	1.646***	1.983***
Cvs x Pro	8	24.596ns	41060.8***	0.053ns	0.114ns
D x Pro	2	95.68*	3959.8ns	0.109ns	1.065**
Cvs x D x Pro	8	92.197***	25526.4**	0.142ns	0.202ns
<b>Error</b>	<b>60</b>	<b>20.804</b>	<b>8668.58</b>	<b>0.085</b>	<b>0.199</b>
		A	E	g <sub>s</sub>	A/E
Cultivars (Cvs)	4	331.1***	0.758ns	85459.1***	44.43***
Drought (D)	1	3088.4***	45.44***	312936.1***	54.43***
Proline (Pro)	2	3.976ns	0.291ns	2852.01ns	0.617ns
Cvs x D	4	87.51***	2.708***	22375.5***	6.732**
Cvs x Pro	8	7.301ns	1.254***	14399.4***	8.832***
D x Pro	2	4.939ns	0.807ns	4398.1ns	10.199***
Cvs x D x Pro	8	12.55**	0.984**	12276.4***	4.634**
<b>Error</b>	<b>60</b>	<b>4.316</b>	<b>0.324</b>	<b>2982.01</b>	<b>1.281</b>
		Shoot N	Shoot K <sup>+</sup>	Shoot Ca <sup>2+</sup>	Shoot P
Cultivars (Cvs)	4	54.71***	216.78***	1290.2**	5.265***
Drought (D)	1	167.6***	80.27ns	350.06ns	3.387**
Proline (Pro)	2	74.31***	43.01ns	8.258ns	0.262ns
Cvs x D	4	36.24***	31.416ns	586.2ns	2.076**
Cvs x Pro	8	32.56***	88.96*	843.8*	1.595**
D x Pro	2	1.916ns	31.477ns	330.1ns	0.926ns
Cvs x D x Pro	8	24.50**	52.022ns	230.96ns	1.836***
<b>Error</b>	<b>60</b>	<b>6.665</b>	<b>37.327</b>	<b>345.6</b>	<b>0.469</b>
		Root N	Root K <sup>+</sup>	Root Ca <sup>2+</sup>	Root P
Cultivars (Cvs)	4	26.62ns	99.18***	307.2ns	0.806*
Drought (D)	1	0.469ns	6.346ns	107.8ns	2.949**
Proline (Pro)	2	7.185ns	8.101ns	0.369ns	0.145ns
Cvs x D	4	7.856ns	32.08*	316.64ns	1.279**
Cvs x Pro	8	24.83*	14.624ns	251.96ns	2.723***
D x Pro	2	10.54ns	1.559ns	67.04ns	2.893***
Cvs x D x Pro	8	38.39**	22.38*	339.72*	0.678*
<b>Error</b>	<b>60</b>	<b>11.33</b>	<b>10.458</b>	<b>131.73</b>	<b>0.290</b>

\*, \*\*, \*\*\* = Significant at 0.05, 0.01 and 0.001 levels, respectively.  
ns = Non-significant

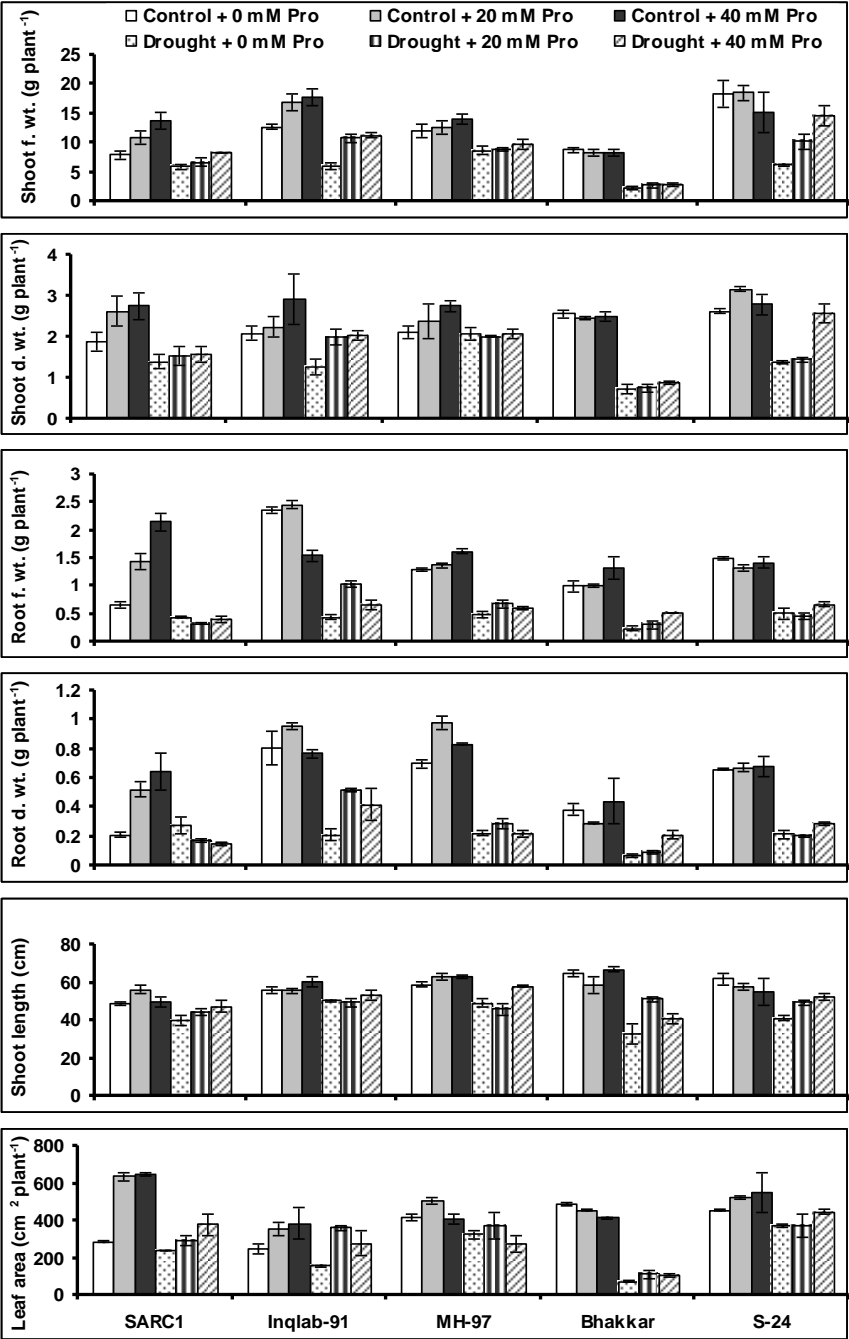


Fig. 1. Growth attributes of wheat plants (raised from proline pre-treated grains) subjected to control or drought stress conditions for 63 days.

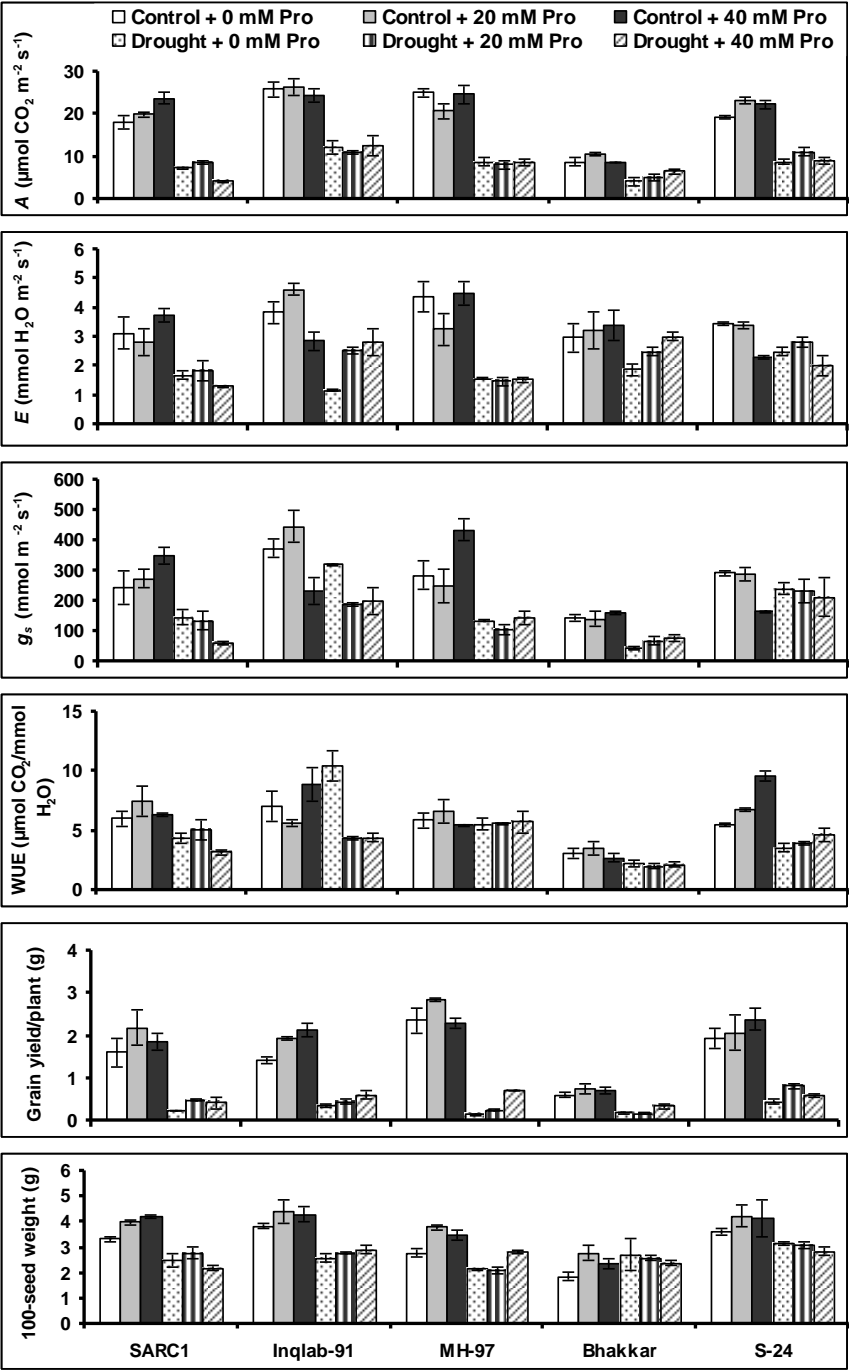


Fig. 2. Gas exchange characteristics of wheat plants (raised from proline pre-treated grains) subjected to control or drought stress conditions for 63 days.

Transpiration rate of 5 wheat cultivars was markedly suppressed due to water deficit conditions. Cultivars did not differ significantly in this attribute, while, the effect of exogenous application of proline was variable under stress or non-stress conditions. Under water deficit conditions, the effect of proline was positive in promoting Inqlab-91 and Bhakkar, while in the remaining cvs. the effect of proline was non-significant (Table 1; Fig. 2).

A marked reduction in stomatal conductance in all wheat cultivars was observed due to water stress (Table 1; Fig. 2). All cultivars differed significantly in this gas exchange attribute. Although the exogenous application of proline as a seed treatment significantly improved stomatal conductance, the effect was variable in different cultivars. The proline level 40 mM was effective in promoting  $g_s$  in SARC-I and MH-97, while 20 mM proline for Inqlab-91 under well watered conditions.

Although water use efficiency decreased significantly in all cultivars due to water deficit conditions, the effect of exogenous proline as a seed treatment remained non-significant on this attribute (Table 1; Fig. 3).

Imposition of water stress reduced grain yield per plant and 100-grain weight in all wheat cultivars (Table 1; Fig. 2). However, pre-sowing seed treatment with proline improved the grain yield of all cultivars. Proline applied as 20 mM was very effective in enhancing yield of non-stressed plants SARC-I and MH-97, while in others 40 mM proline was better than the other levels under both stress and non-stress conditions except in Bhakkar where its effect was not prominent under both stress treatments. The effect of pre-sowing proline treatment was not prominent in terms of 100-seed weight.

Water deficit conditions caused a slight increase in shoot N of SARC-I and MH-97, while in other cultivars the effect was not so prominent (Table 1; Fig. 3). Exogenous application of proline as a seed treatment significantly improved shoot N in cv Bhakkar under both stress and non-stress conditions and that of SARC-I under stress conditions.

Although cultivars differed significantly in shoot  $K^+$  and  $Ca^{2+}$ , the effect of water stress or exogenous proline as a seed treatment was non-significant on these attributes (Table 1; Fig. 3). Water deficit conditions caused a significant increase in P content in all cultivars except cv. Bhakkar in which a substantial decrease in shoot P was observed due to drought stress. Effect of pre-sowing seed treatment of proline on different cultivars was variable (Table 1; Fig. 3).

Imposition of drought stress did not affect the root N and  $Ca^{2+}$ . Furthermore, cultivar difference was also not significant. Pre-sowing seed treatment with proline did not alter the levels of these two nutrients in either cultivar under either water stress treatment (Table 1; Fig. 4). Drought stress had no significant effect on root  $K^+$  of the wheat cultivars. Also the effect of pre-sowing treatment with proline remained non-effective in altering the levels of root  $K^+$  (Table 1; Fig. 4). Although drought stress had a significant effect on root P content of the wheat cultivars, exogenously applied proline as pre-sowing seed treatment did not show any prominent effect on root P (Table 1; Fig. 4).

## Discussion

Water deficit conditions caused a substantial reduction in growth of all 5 wheat cultivars. However, exogenous application of proline as a pre-sowing seed treatment with varying levels of proline ameliorated the adverse effects of water deficit conditions on the growth of all 5 wheat cultivars. These findings of the present study are similar to some earlier studies in which foliar applied proline alleviated the adverse effects of water stress on the growth and/or yield of rice plants (Kavi-Kishore *et al.*, 1995) and *Allenrolfea occidentalis* (Chrominski *et al.*, 1989).

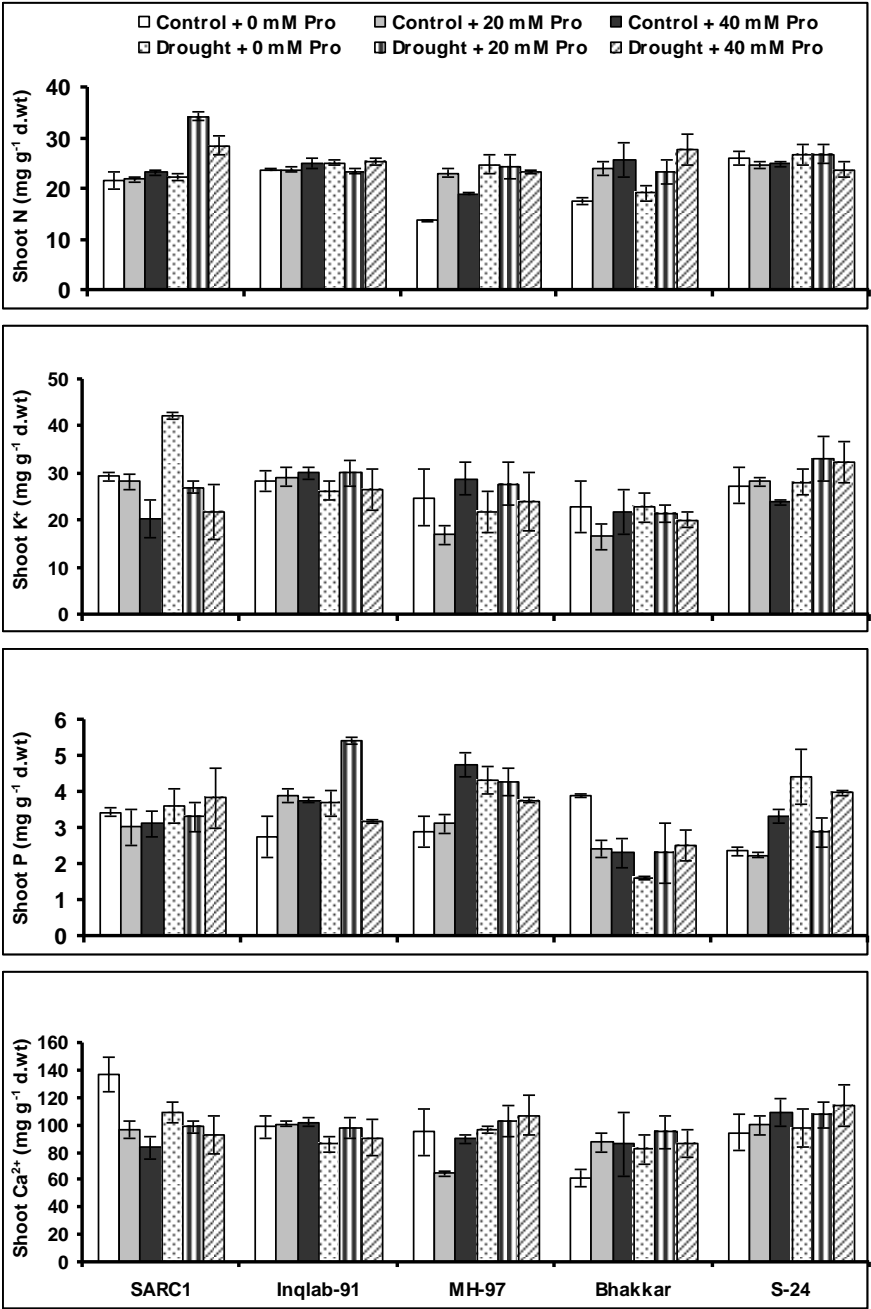


Fig. 3. Shoot N, K, P and Ca of wheat plants (raised from proline pre-treated grains) subjected to control or drought stress conditions for 63 days.

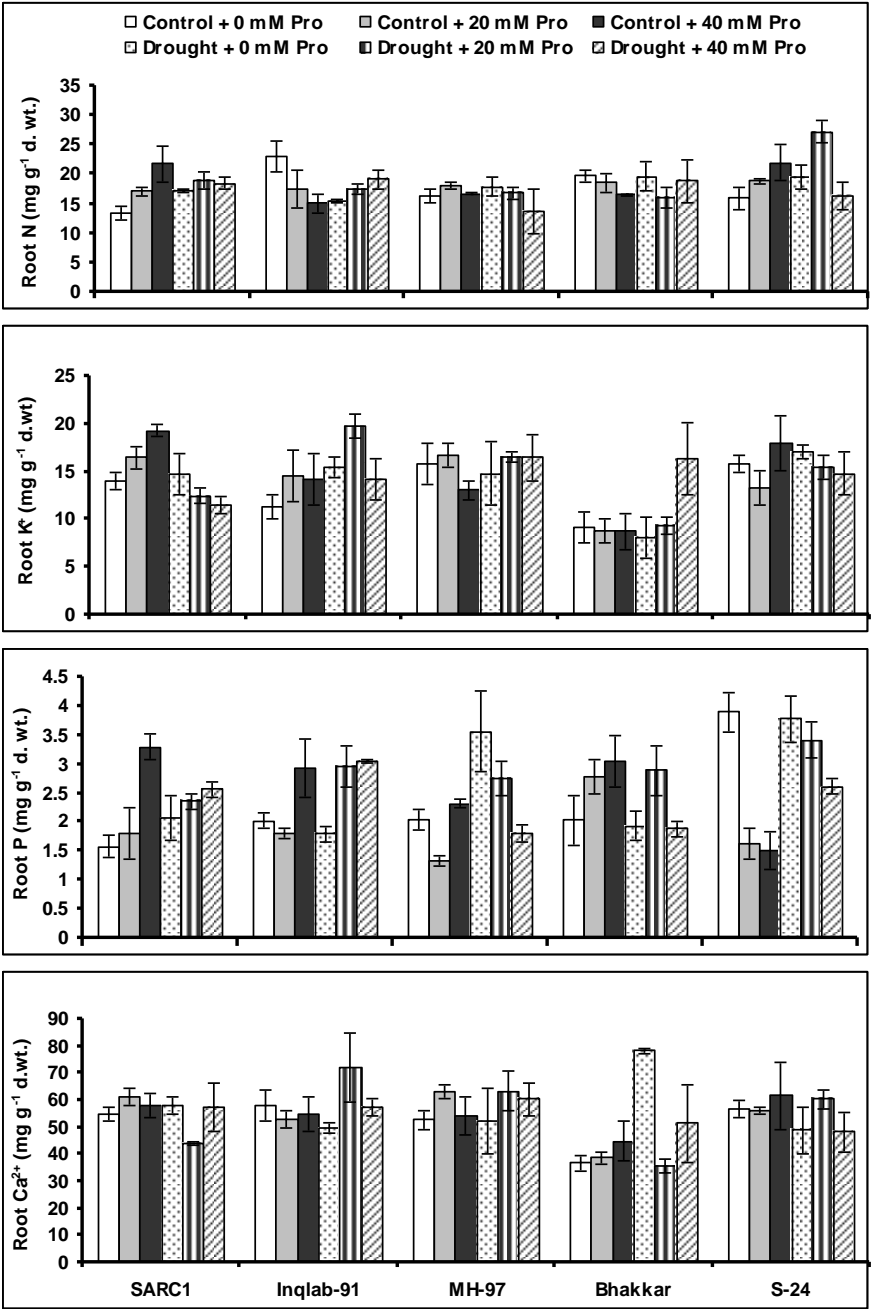


Fig. 4. Shoot and root Ca, P and N of wheat plants (raised from proline pre-treated grains) subjected to control or drought stress conditions for days.

It is now well established that accumulation of proline in plants provides energy for their growth and stress tolerance. Proline also plays an important role in protection of membrane organelles, proteins and enzymes (Iyer & Caplan, 1998; Ashraf & Foolad, 2007; Hoque *et al.*, 2007). Of different levels of proline used for pre-sowing treatment of wheat seed, 40 mM proline was found to be more effective in promoting growth of wheat plants under stress or non-stress conditions. Earlier, in rice, foliar applied 30 mM proline proved to be beneficial when applied at the seedling stage (Roy *et al.*, 1993), while for mung bean (*Vigna radiata*), the effective levels of proline were 20-30 mM applied in cell cultures, and 10 mM proline applied to tobacco suspension cells under stress conditions (Okuma *et al.*, 2000). Increase in growth was not prominent in Bhakkar and MH-97 due to exogenous application of proline, while in others, application of proline significantly increased the growth. These findings confirm the argument of Garg (2003) that genotypes of the same species may vary in their response to exogenous application of proline.

Under water deficit conditions, photosynthetic rate of all wheat cultivars was reduced significantly. This reduction in net CO<sub>2</sub> assimilation rate may have been due to low transpiration rate and stomatal conductance as observed here under water deficit conditions. It is now well evident that reduction in photosynthetic rate occurs due to stomatal closure under water deficit conditions which may limit CO<sub>2</sub> diffusion into the leaves (Flexas *et al.*, 2004; Athar & Ashraf, 2005). However, pre-sowing seed treatment with proline did not affect the net CO<sub>2</sub> assimilation rate in all five wheat cultivars. Thus, increase in growth attributes of wheat was not associated with gas exchange characteristics, however, it might have been due to physiological/metabolic processes other than photosynthetic rate.

In response to drought, plants accumulate various organic and inorganic solutes in the cytosol to maintain osmotic adjustment (Rhodes & Samaras, 1994). Water stress can reduce the accumulation of mineral nutrients i.e., N, P, K<sup>+</sup> and Ca<sup>2+</sup> (Ali *et al.*, 2007). This reduction in mineral contents might occur due to drought-induced reduction in transpiration rate and stomatal conductance (Pessarakli, 1999). Exogenously applied proline as a pre-sowing seed treatment did not affect the shoot and root K<sup>+</sup>, Ca<sup>2+</sup> and P and root N, while the effect of proline on shoot N contents was inconsistent. In cv. Bhakkar and SARC-I shoot N contents were increased due to pre-sowing proline treatment while in others, the effect was not much prominent. However, it needs to be elucidated how exogenous proline alters the uptake and accumulation of different nutrients in wheat plants under water deficit conditions.

Overall, drought stress adversely affected the plant biomass, gas exchange characteristics and grain yield of all wheat cultivars under investigation. Exogenous application of proline as a pre-sowing seed treatment mitigated the adverse effects of drought on growth and yield. Increase in growth with proline was not found to be associated with net CO<sub>2</sub> assimilation rate. Of various proline levels used for pre-sowing seed treatment, 40 mM was more effective in enhancing growth of wheat cultivars.

## Acknowledgements

The authors gratefully acknowledge the funding from the Pakistan Science Foundation (PSF) (Grant No. PSF/Res/P-AU/Agr (318). The results presented in this paper are a part of M. Phil. studies of Mr. Muhammad Kamran.

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(Received for publication 12 December 2008)