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DOES EXOGENOUS APPLICATION OF GLYCINEBETAINE AS A PRE-SOWING SEED TREATMENT IMPROVE GROWTH AND REGULATE SOME KEY PHYSIOLOGICAL ATTRIBUTES IN WHEAT PLANTS GROWN UNDER WATER DEFICIT CONDITIONS?

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Abstract

The ameliorative effect of pre-sowing seed treatment with glycinebetaine (GB) on growth attributes, gas exchange characteristics, and root and shoot mineral nutrients of wheat was observed under water deficit conditions. Five wheat cultivars viz., SARC-I, Inqlab-91, MH-97, Bhakkar and S-24 were grown under well-watered and 60% field capacity. Three levels of GB (0, 50 and 100 m*M*) were applied as pre-sowing seed treatment. Drought stress caused reduction in shoot fresh and dry biomass, shoot length, leaf area per plant, grain yield, net CO_2 assimilation and transpiration rates, and stomatal conditions. However, exogenous application of GB as a pre-sowing seed treatment increased shoot fresh biomass and leaf area per plant while its effect was non-significant on net CO_2 assimilation rate, stomatal conductance and water use efficiency, and shoot and root N, K^+, Ca^{2+} and P. Overall, GB applied @ 50 m*M* showed the maximum effect in ameliorating growth of wheat plants under drought stress. Cultivars SARC-I, Inqlab-91 and S-24 were better as compared to others in their response to drought or GB application.

Introduction

Water stress reduces plant growth and yield of crops and this is a major threat particularly in the arid and semi-arid regions of the world (Souza *et al*., 2004). Like other physiological/biochemical processes affected by drought, the process of net CO_2 assimilation rate is also adversely affected by drought (Dubey, 1997). It has been observed that decrease in photosynthetic rate under drought stress conditions is mainly due to stomatal and/or non-stomatal factors (Seemann & Sharkey, 1986; Meyer & de Kouchkovsky, 1993). Application of compatible solutes under drought stress is an important approach to mitigate the adverse effects of water deficit conditions. Of various compatible solutes, application of glycinebetaine (GB) is an effective approach to mitigate the adverse effects of drought stress on plants because GB is involved in stabilizing the quaternary structures of enzymes and complex proteins, lipids of photosynthetic apparatus as well as in maintaining the highly ordered state of membranes under desiccation stress (Papageogiou & Murata, 1995; Xing & Rajashekar, 1999).

A variety of chemicals, both inorganic and organic, are used as priming agents so as to ensure maximum germination and crop stand establishment in soils exposed to environmental factors (Rudrapal & Nakamura, 1998; Lee *et al*., 2002). Seed priming is an effective means to reduce the time between seed sowing and seedling emergence and the synchronization of emergence (Azam *et al*., 2005). Harris *et al*., (2002) reported that priming in maize (*Zea mays* L.) led to better crop establishment and growth, earlier flowering and greater yields. However, the physiological processes leading to these enhancements are not well understood. In particular, the effects of seed priming in later stages of crop growth were more pronounced than on crop emergence (Azam *et al*., 2005). It is not explicitly clear whether priming directly affects crop growth, or whether its effect is indirect through more rapid establishment.

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Application of GB under drought stress conditions improves the growth and yield of plants e.g., *Nicotiana tabacum* (Agboma *et al*., 1997b), *Zea mays* (Agboma *et al*., 1997a), *Glycine max* (Agboma *et al*., 1997c). However, reports with reference to wheat are contradictory. According to Borojevic *et al*., (1980) application of GB improved the growth of wheat while non-significant effect was observed by Agboma *et al*., (1997a).

Very little is known about the effects of exogenously applied glycinebetaine as a pre-sowing seed treatment on later growth stages of crop plants. So the objectives of the present study were to assess the effectiveness of exogenously applied GB as a pre-sowing seed treatment on wheat plants in terms of improvement in biomass production, seed yield and physiological and biochemical processes such as photosynthetic rate and nutrient uptake parameters in water-stressed wheat plants.

Materials and Methods

The influence of GB as pre-sowing seed treatment on growth and physiological and biochemical parameters of wheat (*Triticum aestivum* L.) under non-stress or drought stress conditions was assessed in a pot experiment. Five wheat cultivars (SARC-I, Inqlab-91, MH-87, Bhakkar and S-24) were used for this study. Seed of the cultivars was obtained from various sources i.e., Inqlab-91, MH-97 and Bhakkar from the Ayub Agricultural Research Institute, Faisalabad, SARC-I from the Department of Plant Breeding and Genetics and S-24 from the Department of Botany, University of Agriculture, Faisalabad. The experiment was conducted in the Old Botanical Garden of the Department of Botany, University of Agriculture, Faisalabad during the period from 2007-08. There were two water regimes i.e., well-watered and 60% field capacity and three glycinebetaine (M. wt. = 117.1 of MP Biomedicals) levels (0, 50 and 100 m*M*) for pre-sowing seed treatment. Before soaking seeds in the above mentioned levels, they were surface sterilized with 5% Sodium hypochlorite solution for 10 minutes. Seeds were sown in plastic pots of uniform size (20 cm diameter and 24 cm depth) containing 9 kg dry sandy loam soil. The pH of the soil was 7.69, while saturation percentage 45. In each pot, 12 seeds were sown. After germination, the plants were thinned to maintain 6 plants per pot. The plants were allowed to establish for 42 days before the onset of various water regimes i.e. well watered and 60 % field capacity. After 63 days of drought treatment, plant samples were harvested. Two plants from each pot were uprooted carefully and washed with distilled water. After recording plant fresh biomass, they were dried in an oven at 65°C to constant dry weight. Other growth attributes like shoot length and total leaf area per plant were also measured.

Gas exchange characteristics: An IRGA (infrared gas analyzer, model LCA-4; Analytical Development Company, Hoddesdon, England) was used to measure various gas exchange characteristics like photosynthetic rate (*A*), transpiration rate (*E*) and stomatal conductance (g_s). All the measurements were performed on a fully expanded second leaf of each plant. 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^{-2} s^{-1}, 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^{-2} s^{-1}, temperature of leaf ranged from 28.4 to 32.4^{o}C, ambient temperature ranged from 22.4 to 27.9 ^{o}C, and ambient CO_2 concentration 352 µmol mol^{-1}.

Determination of mineral elements: The dried ground material (0.1 g) of leaves or roots was digested following Allen *et al*., (1986). A flame photometer (Jenway PFP 7) was used for the determination of potassium and calcium in both leaves and roots, while nitrogen was estimated by micro–Kjeldhal's method (Bremner, 1965) and phosphorus determined using a spectrophotometer following Jackson (1962).

Yield attributes: Yield attributes i.e., 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 computer program MSTAT-C (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 drought stress (60% of field capacity) reduced shoot fresh weight of all wheat cultivars (Table 1; Fig. 1). However, exogenous application of glycinebetaine (GB) as a pre-sowing treatment improved the shoot fresh weight of all cultivars under non-stress conditions, while under drought stress conditions a slight increase in shoot fresh weight was observed in SARC-I only. Pre-sowing seed treatment with 50 m*M* glycinebetaine was more effective than 100 m*M* glycinebetaine in enhancing shoot fresh weight of all wheat cultivars except cv. Inqlab-91 under well-watered conditions.

Shoot dry weight of all wheat cultivars reduced significantly under water deficit conditions. Exogenous application of GB as a pre-sowing seed treatment did not affect shoot dry biomass of wheat (Table 1; Fig. 1). Of various cultivars, SARC-I and S-24 were better as compared to others in this growth attribute.

Root fresh and dry masses of all wheat cultivars decreased significantly due to imposition of water stress (Table 1; Fig. 1). However, water stress-induced reduction in root fresh and dry biomass was more in cv. Bhakkar than the others. Exogenous application of glycinebetaine as a pre-sowing treatment did not ameliorate the adverse effects of drought stress on root fresh or dry biomass.

Imposition of drought stress reduced shoot length of cvs. Bhakkar and S-24, while in the others the effect was not prominent (Table 1; Fig. 1). However, exogenous application of glycinebetaine slightly increased the shoot length of only non-stressed plants of all cultivars, whereas the shoot length of stressed plants remained unchanged due to pre-sowing seed treatment with glycinebetaine.

Total leaf area per plant of all wheat cultivars except Inqlab-91 and MH-97 (Table 1; Fig. 1) decreased significantly under water deficit conditions. However, exogenous application of GB slightly increased leaf area per plant except in Bhakkar and MH-97. The effect of various levels of GB was variable. Pre-sowing with 50 m*M* GB was effective for SARC-I under non-stress and for S-24 under drought stress conditions while, in the others 100 m*M* GB was found effective.

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 glycinebetaine as a seed treatment did not significantly affect net CO_2 assimilation rate. Of all cultivars, Bhakkar was the lowest of all cultivars 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 glycinebetaine 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	98.91***	4.416***	0.864***	0.282***
Drought (D)	1	678.4***	16.68***	10.29***	1.149***
Glycinebetaine (GB)	2	21.02***	0.042ns	0.075ns	0.040ns
Cvs x D	4	11.13***	0.504ns	0.217*	0.020ns
Cvs x GB	8	10.92***	0.246ns	0.309***	0.021ns
D x GB	2	31.92***	0.370ns	0.321*	0.015ns
Cvs x D x GB	8	20.75***	0.448ns	0.220*	0.057**
Error	60	1.419	0.223	0.080	0.0165033
		Shoot length	Total leaf area	Grain weight	100-grain weight
Cultivars (Cvs)	4	229.61***	208883.5***	1.692***	0.657ns
Drought (D)	1	1676.2***	207112.9**	31.69***	36.74***
Glycinebetaine (GB)	2	248.0***	165352.5***	0.790***	0.016ns
Cvs x D	4	132.8**	83736.4**	0.857***	0.320ns
Cvs x GB	8	34.17ns	39759.6ns	0.333***	0.511ns
D x GB	2	208.5**	1405.97ns	0.599***	1.136*
Cvs x D x GB	8	40.03ns	100694.3***	0.245***	0.406ns
Error	60	27.36	20860.7	0.043	0.352
		A	*E*	g_s	*A/E*
Cultivars (Cvs)	4	79.84***	17.12***	82866.3***	23.03***
Drought (D)	1	1077.9***	120.4***	880803.5***	12.66ns
Glycinebetaine (GB)	2	17.98ns	3.506*	5552.3ns	7.799ns
Cvs x D	4	4.290ns	3.530**	17496.1ns	13.16**
Cvs x GB	8	26.15*	1.706*	12606.9ns	5.132ns
D x GB	2	33.19*	0.078ns	4342.9ns	3.447ns
Cvs x D x GB	8	38.38**	1.627*	20894.5*	11.04**
Error	60	10.45	0.743	9155.5	3.304
		Shoot N	Shoot K^+	Shoot Ca^{2+}	Shoot P
Cultivars (Cvs)	4	43.41ns	74.51**	425.73ns	25.75***
Drought (D)	1	4.268ns	196.5**	2856.1**	5.789ns
Glycinebetaine (GB)	2	2.809ns	51.43ns	88.31ns	0.070ns
Cvs x D	4	66.88*	40.99ns	925.4*	6.001*
Cvs x GB	8	13.37ns	33.67ns	203.2ns	2.586ns
D x GB	2	25.97ns	6.878ns	30.53ns	7.213*
Cvs x D x GB	8	20.33ns	20.20ns	506.2ns	2.104ns
Error	60	25.76	17.07	297.6	2.232
		Root N	Root K^+	Root Ca^{2+}	Root P
Cultivars (Cvs)	4	16.75ns	10.59ns	307.9ns	5.950ns
Drought (D)	1	16.38ns	11.38ns	193.6ns	0.170ns
Glycinebetaine (GB)	2	9.513ns	3.433ns	228.5ns	1.621ns
Cvs x D	4	21.35ns	3.794ns	207.7ns	15.05*
Cvs x GB	8	28.60ns	10.04ns	565.3*	5.165ns
D x GB	2	8.898ns	7.078ns	497.6ns	13.41ns
Cvs x D x GB	8	19.03ns	4.078ns	312.6ns	3.083ns
Error	60	16.37	7.978	226.1	4.796

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

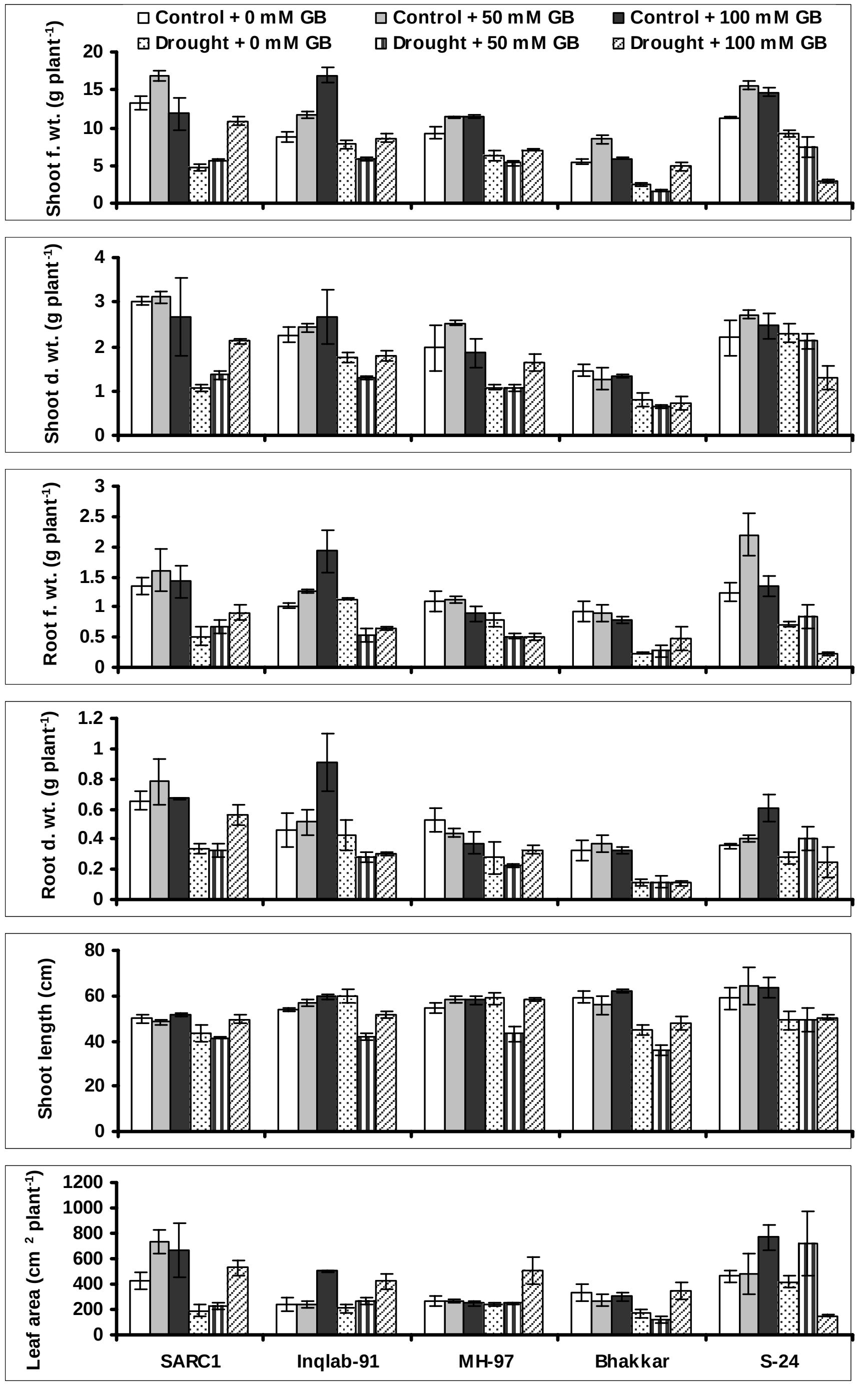

Fig. 1. Growth attributes of wheat plants (raised from glycinebetaine pre-treated grains) subjected to control or drought stress conditions for 63 days.

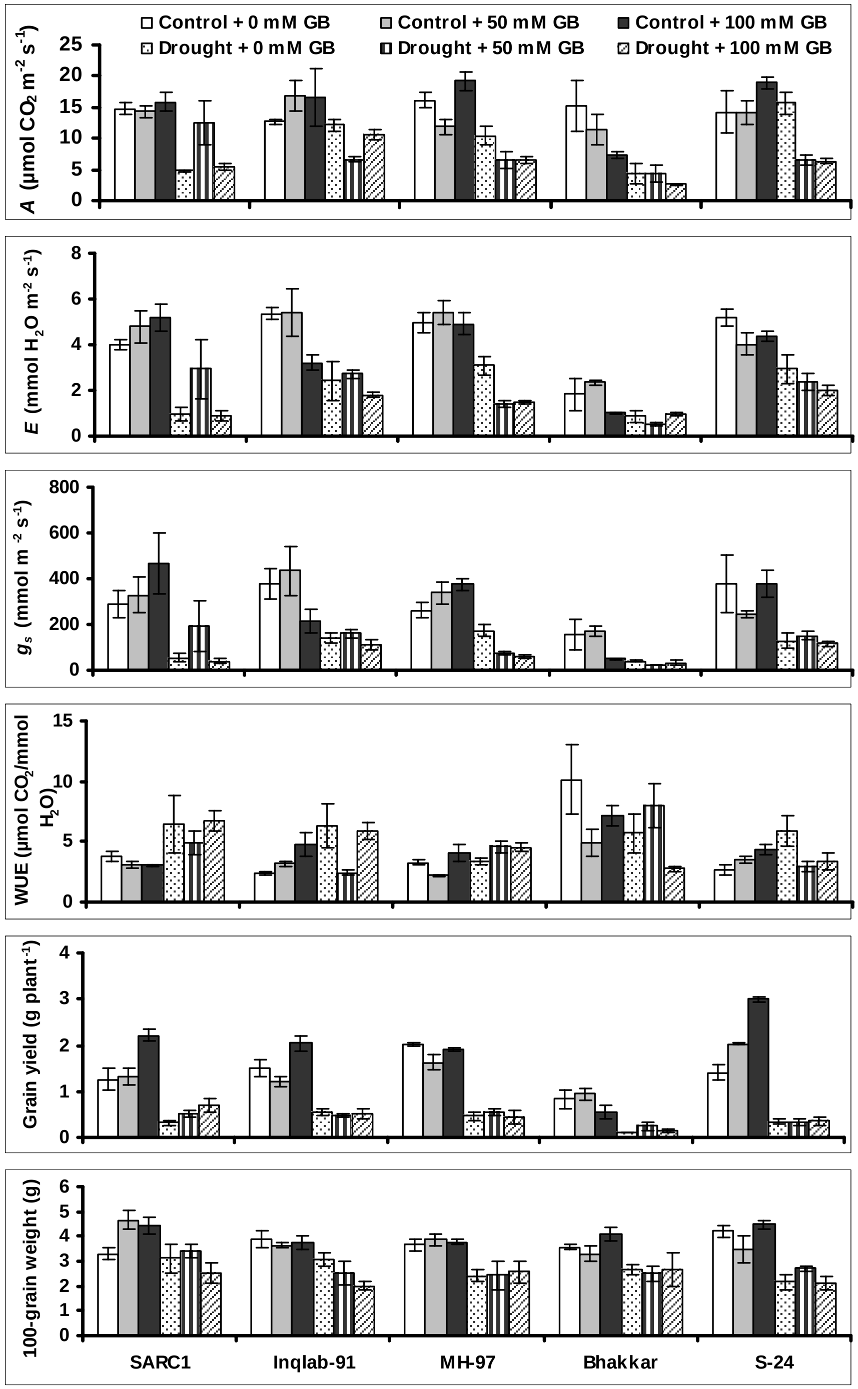

Fig. 2. Gas exchange and yield attributes of wheat plants (raised from glycinebetaine pre-treated grains) subjected to control or drought stress conditions for 63 days.

Transpiration rate of all wheat cultivars decreased markedly due to water deficit conditions. Cultivars differed significantly in this attribute, while; the effect of exogenous application of GB was variable under stress or non-stress conditions. Under water deficit conditions, the effect of 50 m*M* GB was positive in promoting transpiration in SARC-I, while in the remaining cultivars the effect of GB was not prominent (Table 1; Fig. 2).

A marked reduction in stomatal conductance in all wheat cultivars was observed due to water stress (Table 1; Fig. 2), however, exogenous application of GB as a seed treatment did not improve stomatal conductance of either cultivar.

Effect of water deficit conditions was non-significant on water use efficiency of all wheat cultivars and the effect of exogenous GB as a seed treatment was also non-significant on this attribute (Table 1; Fig. 3).

Imposition of water stress reduced grain yield per plant and 100-grain weight in all five wheat cultivars (Table 1; Fig. 2). However, pre-sowing seed treatment with GB slightly improved the grain yield of all cultivars. Improvement in yield due to 100 m*M* GB was prominent in non-stressed plants of SARC-I, Inqlab-91 and S-24, while in others there was no effect of GB levels under both stress and non-stress conditions. The effect of pre-sowing GB treatment was not prominent in terms of 100-seed weight.

Water deficit conditions caused a non-significant effect on shoot N and P while, a significant increasing effect on shoot K^+ and Ca^{2+}. Variability in cultivars was observed only in shoot K^+ and P while in shoot N and Ca^{2+}, the cultivars did not differ significantly (Table 1; Fig. 3). Exogenous application of GB did not affect leaf mineral nutrients.

Imposition of drought stress did not affect the root N, K^+, Ca^{2+} and P. Furthermore, cultivar difference was also not significant. Pre-sowing seed treatment with GB did not alter the levels of these four nutrients in either cultivar under either water stress regime (Table 1; Fig. 4).

Discussion

Growth of all wheat cultivars was found to be adversely affected by water deficit conditions in the present study. Drought-induced decrease in growth has already been reported in different crops e.g., pearl millet (Ashraf *et al*., 2001), okra (Ashraf *et al.,* 2002), wheat (Waseem *et al*., 2006), grasses (Akram *et al*., 2007), maize (Ali *et al*., 2007; Jabeen *et al*., 2008). However, pre-sowing seed treatment with glycinebetaine mitigated the adverse effects of drought stress on all wheat cultivars but the response to exogenous GB was found to be cultivar specific. In view of existing literature it is evident that the effect of GB could be positive or negative on plant growth e.g., exogenous application of GB improved growth and yield in tobacco and *Glycine max* (Agboma *et al*., 1997c), wheat (Borojevic *et al*., 1980) and *Gossypium hirsutum* (Naidu *et al*., 1998; Gorham *et al*., 2000). However, in contrast, in wheat and *G. hirsutum,* non-significant effect of exogenous GB was reported (Agboma *et al*., 1997a; Meek *et al*., 2003). In the present study, pre-sowing seed treatment with GB improved the plant biomass of the wheat cultivars and GB level 50 m*M* was found to be more effective than 100 m*M* in promoting wheat growth.

Water deficit conditions generally reduce the net CO_2 assimilation rate and transpiration rate (Akram *et al*., 2007). In general, net CO_2 assimilation rate is reduced due to stomatal closure and injury of photosynthetic apparatus. Due to stomatal closure, plants have to face the problem of CO_2 deficiency, while prolonged drought stress can damage the photosynthetic apparatus (Makela *et al*., 1999). In this study, water deficit significantly reduced the net CO_2 assimilation rate, transpiration rate and stomatal conductance of all wheat cultivars. Application of GB as a pre-sowing seed treatment was found to be ineffective in altering the gas exchange characteristics of wheat plants, although reports are available which show the positive effect of exogenously applied GB on different crops e.g., pea and tunip (Makela *et al*., 1998), tobacco (Ma *et al*., 2006) and tomato (Makel *et al*., 1999).

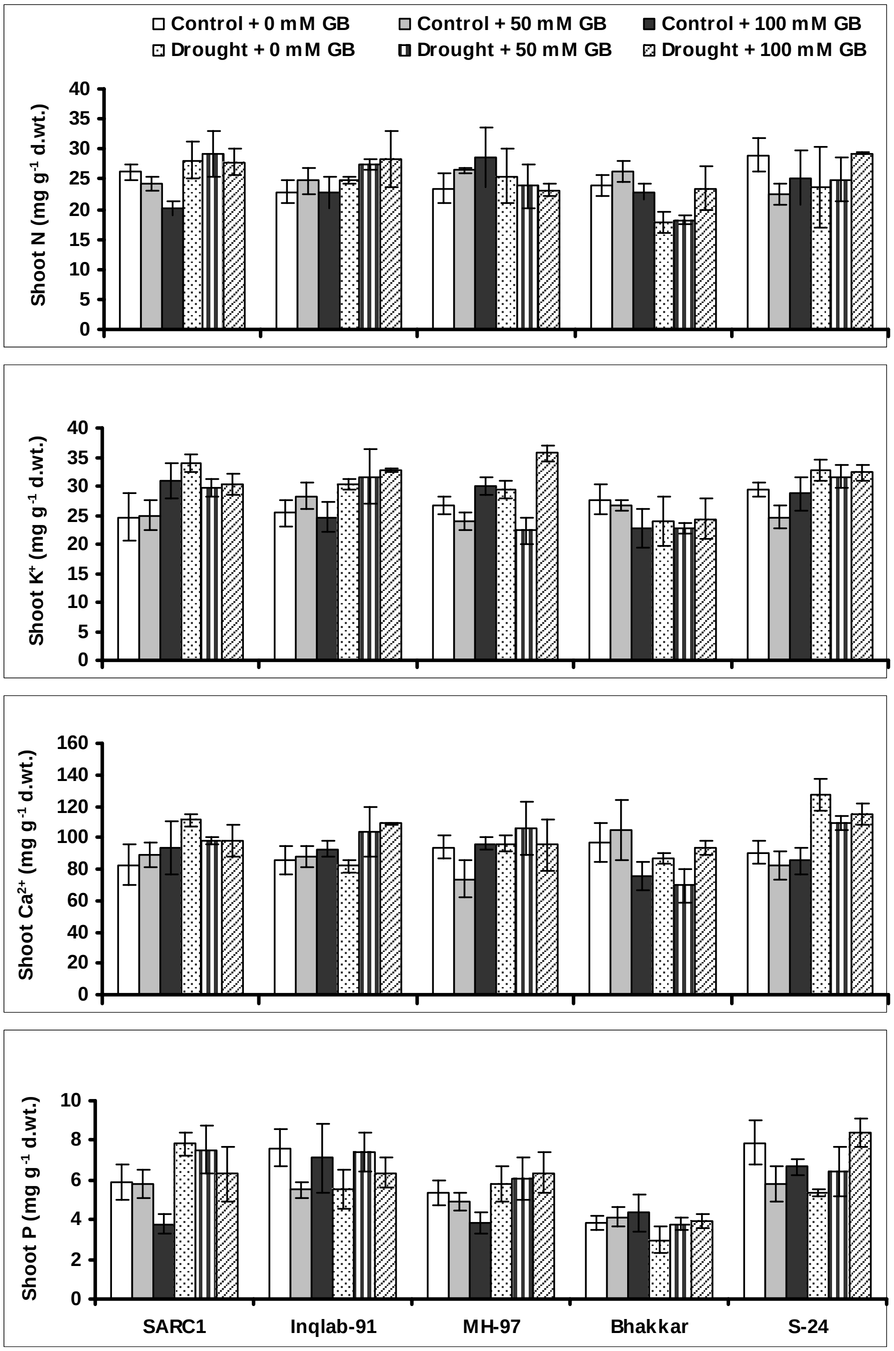

Fig. 3. Leaf mineral nutrients of wheat plants (raised from glycinebetaine pre-treated grains) subjected to control or drought stress conditions for 63 days.

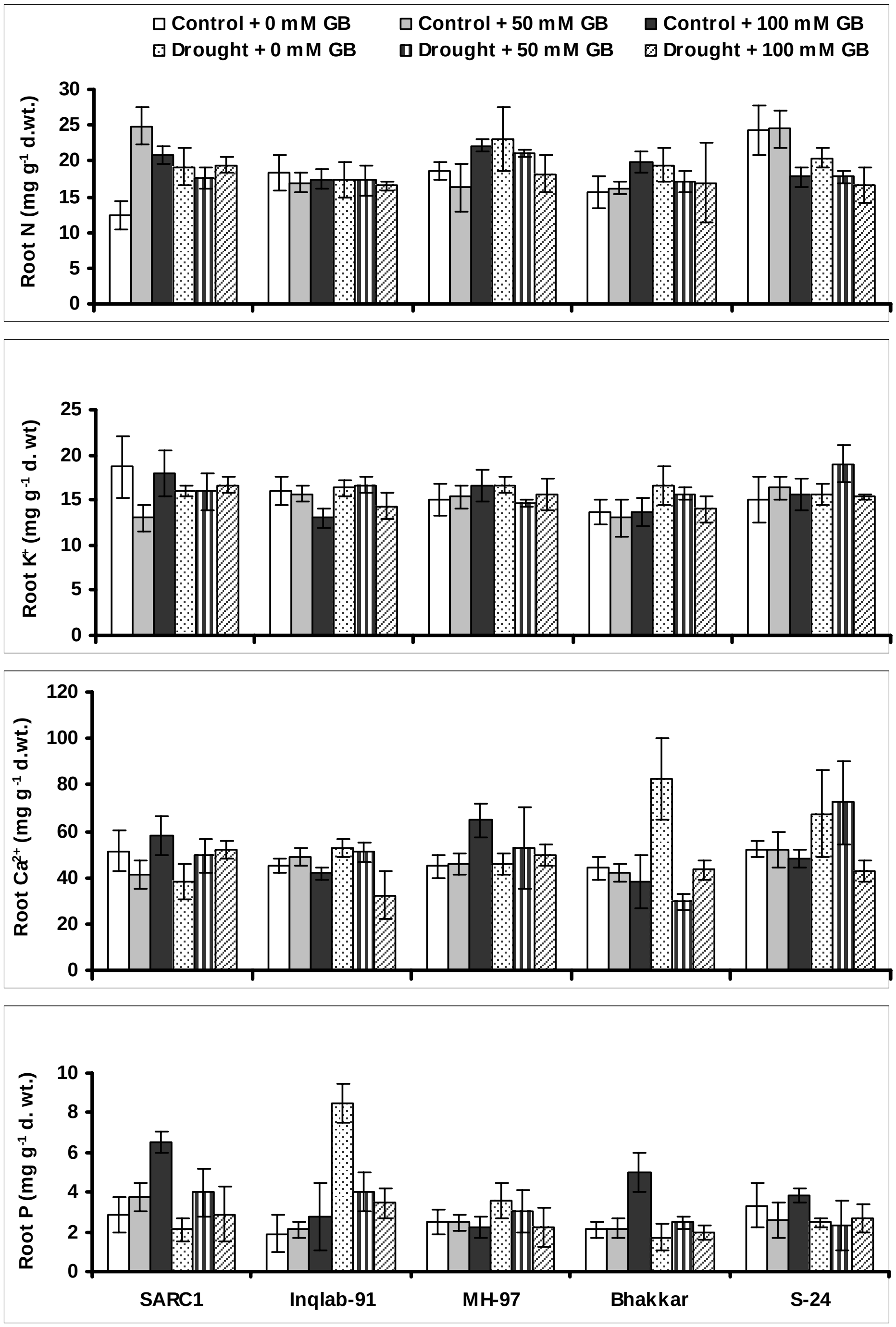

Fig. 4. Root mineral nutrients of wheat plants (raised from glycinebetaine pre-treated grains) subjected to control or drought stress conditions for 63 days.

In the present study, water deficit conditions did not affect shoot N and P contents while, a significant increasing effect on shoot K^+ and Ca^{2+} was observed. However, exogenous application of GB did not alter the leaf mineral nutrients. Drought stress also did not affect the root N, K^+, Ca^{2+} and P. Pre-sowing seed treatment with GB again was found to be ineffective in altering the levels of these four nutrients in either cultivar under either water stress regime. These findings are not in accordance with Ali *et al*., (2007) in which they observed reduction in mineral elements of maize under water deficit conditions.

In conclusion, water deficit reduced the plant growth, gas exchange characteristics and grain yield of all five wheat cultivars. Exogenous application of GB as a pre-sowing seed treatment only improved the plant biomass. However, this increase in plant biomass could not be related to net CO_2 assimilation rate. Of different GB levels used for pre-sowing seed treatment, 50 m*M* was found to be better than the other levels in affecting growth of wheat cultivars.

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