Journal of Applied Botany and Food Quality 84, 192 - 199 (2011)

[1]Department of Botany, University of Agriculture, Faisalabad Pakistan

Is foliar-applied glycinebetaine effective in mitigating the adverse effects of drought stress on wheat (*Triticum aestivum* L.)?

M. Shahbaz[1]*, Y. Masood[1], S. Perveen[1], M. Ashraf[1]

(Received April 11, 2011)

Summary

Drought stress is a serious threat to crop growth and development. Exogenous application of various compatible solutes is an effective means to lessen the adverse drought effects on plants. To explore the effectiveness of exogenously applied GB as foliar spray in mitigating the harmful effect of drought on wheat crop, an experiment was conducted using five wheat cultivars (SARC-I, Inqlab-91, MH-97, Bhakkar and S-24) and three levels (0, 50 and 100 m*M*) of GB applied as foliar spray under well-watered and water-stressed (60% field capacity) conditions. Growth and yield attributes, gas exchange characteristics, and root and shoot N, P, and K^+ were determined in the wheat cultivars. Drought stress significantly reduced shoot and root fresh and dry biomass, shoot length, leaf area, grain yield, photosynthetic (*A*) and transpiration rates (*E*), stomatal conductance (g_s), and shoot and root P and K^+ contents. However, foliar-applied GB mitigated the adverse effects of drought stress by enhancing plant biomass, shoot length, transpiration rate, root P, and N contents and in shoot only K^+ in both cultivars under stress conditions, while its effect was not prominent on leaf area per plant, water use efficiency (WUE), and shoot P and N, and root K^+. The cultivar response to varying GB levels was variable. Overall, foliar-applied GB @ 50 m*M* showed better performance in reducing the adverse effects of drought stress on wheat crop under water deficit conditions. Cultivars SARC-I and Inqlab-91 were better as compared to the others in their response to foliar-applied GB under water deficit conditions.

Introduction

Environmental factors play important roles in plant growth and development. Any adverse change in these factors negatively affects physiological and biochemical processes of plants (Garg, 2010). For example, scarcity of water (drought) causes adverse effects on crop yield and quality (Blum, 2005; Neumann, 2008; Abd El-Rhman, 2010). Under drought stress conditions, alteration in various morphological, physiological and biochemical attributes occur, that help plants to protect themselves against the stress (Demiral and Türkan, 2006; Nawaz and Ashraf, 2010; Ashraf, 2010). Of various, adaptations, accumulation of compatible solutes is an effective phenomenon which plays a significant role in improving stress tolerance (Chen et al., 2000; Munns, 2005). Of these solutes, glycinebetaine (GB) is an effective osmoprotectant which accumulates in a number of plants under drought stress (Zhang et al., 2009) thereby playing a vital role in drought tolerance of plants (Yang et al., 2007; Mahmood et al., 2009; Khan et al., 2009). GB stimulates a multitude of physiological phenomena including net CO_2 assimilation rate (Wang et al., 2010). It protects the quaternary structure of proteins, maintains enzyme activity, stabilizes membrane structure of PSII complex, prevents oxidative damage to membranes and enhances antioxidative defense system under osmotic stress (Ma et al., 2006; Raza et al., 2007; Yang et al., 2007; Chen and Murata, 2008; Hassine et al., 2008; Hossain and Fujita, 2010). The accumulation of glycinebetaine depends not only on type of plant species (Moghaieb et al., 2004), plant varieties (Cha-Um et al., 2007; Hassine et al., 2008), plant organelles (Zhu et al., 2003), but also on environmental factors, such as salinity (Longstreth et al., 2004; Saneoka et al., 2001; Girija et al., 2002), drought (Zhang et al., 2008), alkaline stress (Cui et al., 2008), and extreme temperatures (Zhang et al., 2008), etc.

Exogenous application of a variety of compatible organic solutes has been advocated as one of the prospective means of improving plants stress tolerance (Raza et al., 2007; Ashraf et al., 2008; Mahmood et al., 2009). It has been widely reported that exogenous application of GB regulates some key physiological attributes in plants subjected to stress conditions (Mahmood et al., 2009). For example, GB protects photosynthetic machinery (Zhao et al., 2001; Allakhverdiev et al., 2003) by preventing photoinhibition (Ma et al., 2006), partially preserves the net photosystem-II efficiency (Demiral and Turkan, 2006), and enhances the tolerance of photosynthetic apparatus of wheat plants subjected to various stress conditions (Cherian et al., 2006; Wang et al., 2010a; 2010b). However, mechanism involved in GB protection of this machinery is still unclear and thus needs to be investigated.

Glycinebetain biosynthesis has been reported to increase in most crop species under water deficit conditions (Martinez et al., 2005; Hessine et al., 2009), while in others the natural synthesis of GB is considerably lower than the required level to protect the plants from the stress conditions (Subbarao et al., 2001). However, exogenous application of glycinebetaine is known to have beneficial effects on growth and final yield of a number of crops, particularly those which do not normally accumulate significant amount of GB under these stresses including drought stress (Raza et al., 2006; 2007; Athar et al., 2009; Wang et al., 2010a; 2010b; Cha-Um and Kirdmanee, 2010). For example, foliar spray of glycinebtaine mitigated the unpleasant drought stress effects on sunflower achene weight (Iqbal et al., 2005), improved gas exchange characteristics and biomass production in sunflower (Iqbal et al., 2009), and increased dry matter, grain yield and osmolytes in maize (Zhang et al., 2009). In view of Naidu et al. (1998) even under mild field stress conditions, crop yield could be improved from 10 to 50% with foliar application of GB. Effectiveness of exogenous application of GB depends on the type of species, developmental stage of plant, application level and the number of application, etc. (Ashraf and Foolad, 2005; 2007; Ashraf et al., 2008).

Keeping in the view the role of glycinebtaine in plants under water deficit conditions, we hypothesize whether or not exogenously applied GB as foliar spray alter morpho-physiological and yield responses of various wheat cultivars under water deficit conditions. The chief objective of the current study was to assess whether foliarly-applied GB could alleviate the adverse effects of drought on growth, gas exchange attributes and mineral nutrition of wheat plants.

* Corresponding author

Materials and methods

A pot experiment was conducted to assess the influence of GB as foliar spray on the growth, physiological and biochemical attributes of wheat (*Triticum aestivum* L.) under non-stress (control) and stress (drought) conditions. There were five wheat cultivars (SARC-I, Inqlab- 91, MH-97, Bhakkar and S-24) obtained from Ayub Agriculture Research Institute, Faisalabad (Inqlab-91, MH-97, Bhakkar), Department of Plant Breeding and Genetics (SARC-I) and from the Department of Botany, University of Agriculture, Faisalabad (S-24). The study was carried out in the net-house under natural conditions. During to course of experiment day and night average temperatures were 31.2 ± 5 °C and 26.5 ± 4 °C, respectively, relative humidity was from 32.6 to 64.7%, and day-length from 11 to 12 h. The experiment was laid out in a completely randomized design with three replications of each experimental unit. Equal weight plastic pots (diameter 20 cm and 24 cm depth) were filled with 9 kg dry sandy loam soil. Soil in each pot was completely saturated with normal irrigation water. When the moisture contents were at field capacity, 12 healthy seeds (surface sterilized with 1% sodium hypochloride for 5 minutes) of each cultivar were hand sown. Thinning of wheat plants was done to maintain 6 plants per pot after 15 days of germination.

Two levels of water i.e., (well-watered (control) and irrigation at 60% field capacity (drought) were started after 6 weeks of seed sowing. The moisture contents of drought pots were regularly monitored and maintained by keeping weight of every pot equal to that calculated for 60% field capacity through watering with normal irrigation water if required on daily basis till the maturation of the crop. Three levels of glycinebetaine (GB) (M. wt. = 117.15 of Sigma Aldrich) i.e., 0, 50 and 100 m*M* were applied after four weeks of drought treatment (10-weeks of seed sowing) @ 32 mL per pot as foliar spray (Tween-20 (0.1%) was used as a surfactant). Data for various growth attributes, gas exchange characteristics and mineral nutrients were collected after 3-weeks of glycinebetaine application. Two uniform plants from each pot were uprooted carefully, washed with distilled water and recorded their mean shoot and root fresh weights, shoot length, and leaf area per plant. The samples were oven dried at 65 °C for 72 hours and then their dry biomass was computed.

Gas exchange characteristics: An IRGA (infrared gas analyzer, model LCA-4; Analytical Development Company, Hoddesdon, England) was used to measure gas exchange attributes including photosynthetic (A) and transpiration (E) rates, stomatal conductance (gs), etc. All attributes were measured from 10.00 to 13.00 hours with the following leaf chamber specifications: air flow per unit leaf area 410.3 mmol m^{-2} s^{-1}, atmospheric pressure 99.5 kPa, chamber water vapor pressure from 7.0 to 9.1 mbar, maximum *PAR* at leaf surface was up to 1620 μmol m^{-2} s^{-1}, leaf temperature from 26.3 to 30.5 °C, 21.4 to 23.8 °C ambient temperature, and ambient CO_2 concentration was 350 μmol mol^{-1}.

Determination of mineral elements: The (0.1g) dried ground material of leaves or roots tissues was digested according to the method described by Allen et al. (1986). Potassium ion was determined by a flame photometer (Jenway PFP 7), while nitrogen was measured according to micro-Kjeldhal's method (Bremner, 1965) and phosphorus by spectrophotometer according to Jackson (1962).

Yield attributes: Yield parameters i.e., grain yield per plant, and 100-grain weight were determined at maturity.

Statistical analysis: Computer program MSTAT-C (MSTAT Development Team 1989) was used for the analysis of variance of the data. The least significance difference test (LSD) was used to compare mean values of each attribute according to Snedecor and Cochran (1980).

Results

Drought stress (60% of field capacity) markedly reduced shoot fresh and dry weights of all five wheat cultivars (Tab. 1; Fig. 1). However, foliar-applied glycinebetaine (GB) enhanced shoot fresh and dry biomass of some cultivars under drought stress conditions. For example, positive effect of GB was observed in Inqlab-91, Bhakkar, and slightly in SARC-1, while in MH-97 and S-24 the influence of GB was non-significant. Overall, 50 m*M* GB level was more effective as compared to 100 m*M* in causing growth improvement. SARC-1 and Inqlab-91 also showed better response in shoot dry weight to exogenous-applied 50 m*M* GB under normal watering conditions.

Root fresh and dry weights of all wheat cultivars reduced significantly under different water stress (Tab. 1; Fig. 1). Foliar-applied 50 m*M* GB significantly improved root fresh and dry weights in SARC-1, Inqlab-91 and MH-97 under well-watered conditions and, in Inqlab-91 under water deficit conditions. Overall, SARC-I and Inqlab-91 showed better performance under well-watered and water deficit conditions in terms of root biomass.

Shoot length of all wheat cultivars decreased significantly under drought regimes (Tab. 1; Fig. 1). However, foliar spray of GB slightly increased the shoot length of all cultivars under both drought regimes. Total leaf area per plant of all wheat cultivars decreased markedly due to water deficit conditions (Tab. 1; Fig. 1). Foliar-applied GB did not mitigate the unpleasant effects of drought stress in terms of leaf area per plant.

Drought stress significantly reduced grain yield per plant, however, 100-grain weight was not affected markedly (Tab. 1; Fig. 1). The exogenous application of GB slightly improved the grain yield of all cultivars under non-stress conditions, while under water deficit conditions, the interaction between GB and drought stress was non-significant.

Drought stress significantly reduced the photosynthetic rate (A) of all five wheat cultivars (Tab. 1; Fig. 2). Response of all cultivars to foliar applied GB was variable under well-watered and drought stress conditions. In MH-97 GB enhanced net photosynthetic rate under well-watered conditions while in S-24 under drought conditions. In all remaining cultivars, GB did not alter net CO_2 assimilation rate effectively. Transpiration rate of all wheat cultivars decreased due to imposition of drought stress conditions. While, GB application showed a contradictory role in this attribute. Transpiration rate in cvs. MH-97 and Bhakkar increased, while that in S-24 decreased with the application of GB under different water stress regimes (Tab. 1; Fig. 2).

Stomatal conductance in all wheat cultivars was markedly higher under normal watering as compared to that under drought stress conditions (Tab. 1; Fig. 2). Foliar-applied GB did not alter this attribute under well-watered or water deficit conditions. There was a slight difference among the cultivars in stomatal conductance under normal watering with high value being in SARC-1 as compared to the others. Water use efficiency was higher in all cvs under drought regimes as compared to that under non-stress. Of different cultivars, S-24 showed better performance in WUE due to GB application under drought stress conditions (Tab. 1; Fig. 2).

Sub-stomatal internal CO_2 concentration and *Ci/Ca* ratio showed a non-significant behavior under drought stress conditions (Tab. 1; Fig. 2). Of different cultivars, SARC-1 showed high value for sub-stomatal CO_2 concentration at 100 m*M* GB under drought stress

Tab. 1: Mean square values from analyses of variance of data for growth, yield, gas exchange and mineral nutrients of different cultivars of wheat (*Triticum aestivum* L.) when 71-d old well-watered and water-stressed plants were subjected for 21-d to various levels of foliar-applied glycinebetaine.

Source of variation	d.f.	Shoot f. wt.	Shoot d. wt.	Root f. wt.	Root d. wt.	Shoot length
Glycinebetaine (GB)	2	109. 3ns	0. 241*	1. 278***	0. 036*	348. 1***
Drought (D)	1	978. 3***	10. 48***	10. 16***	0. 555***	1291. 8***
Cultivars (Cvs)	4	13. 34ns	0. 053ns	0. 682***	0. 106***	69. 50***
GB x D	2	206. 9*	1. 517***	0. 208ns	0. 043**	25. 90***
GB x Cvs	8	63. 42ns	0. 928***	0. 707***	0. 07***	58. 66***
D x Cvs	4	48. 63ns	0. 588***	0. 804***	0. 044***	14. 87***
D x GB x Cvs	8	74. 25ns	0. 758***	0. 545***	0. 018*	48. 08***
Error	60	64. 83	0. 055	0. 076	0. 008	2. 05

Source of variation	d.f.	Leaf area per plant	Grain yield per plant	100-seed weight	*A*	*E*
Glycinebetaine (GB)	2	10869. 2*	0. 397ns	0. 397ns	0. 608ns	7. 27***
Drought (D)	1	867025. 7***	85. 79***	0. 121ns	5269. 0***	76. 40***
Cultivars (Cvs)	4	125620. 2***	2. 71***	0. 546ns	53. 84***	2. 14***
GB x D	2	3402. 5ns	0. 023ns	0. 061ns	51. 03***	5. 33***
GB x Cvs	8	21917. 3***	0. 84*	0. 493ns	25. 01***	1. 35***
D x Cvs	4	69483. 0***	2. 31***	0. 367ns	50. 74***	1. 48***
D x GB x Cvs	8	14754. 8***	0. 92**	0. 422ns	11. 94***	0. 795***
Error	60	2202. 4	0. 317	0. 315	0. 77	0. 165

Source of variation	d.f.	g_s	*A/E*	C_i	C_i/C_a	Shoot P
Glycinebetaine (GB)	2	752. 7ns	64. 12***	26. 26ns	0. 007ns	31. 1***
Drought (D)	1	20091. 3***	25. 70**	13603. 8**	0. 233***	87. 52***
Cultivars (Cvs)	4	1798. 14*	32. 95***	3764. 9*	0. 04***	0. 197ns
GB x D	2	215. 24ns	5. 13ns	170. 4ns	0. 011ns	0. 736ns
GB x Cvs	8	1650. 29ns	28. 55***	2436. 3ns	0. 025***	0. 491ns
D x Cvs	4	3599. 63**	31. 91***	5622. 4**	0. 018*	2. 512***
D x GB x Cvs	8	1419. 80ns	7. 52*	1614. 9ns	0. 014*	1. 023*
Error	60	813. 95	2. 76	1429. 7	0. 055	0. 448

Source of variation	d.f.	Shoot N	Shoot K^+	Root P	Root N	Root K^+
Glycinebetaine (GB)	2	111. 61**	166382 ***	0. 626*	11. 32ns	263. 40***
Drought (D)	1	35. 84ns	4885380 ***	1. 048**	164. 02***	302. 5**
Cultivars (Cvs)	4	70. 34**	45795.1 ***	0. 126ns	15. 35*	35. 13ns
GB x D	2	5. 22ns	217785 ***	1. 361***	20. 32*	601. 45***
GB x Cvs	8	12. 28ns	18691. 1***	0. 452**	6. 295ns	88. 83*
D x Cvs	4	53. 45*	20106. 6***	0. 496*	3. 43ns	113. 61*
D x GB x Cvs	8	5. 13ns	27563. 2***	0. 035ns	9. 662*	169. 60***
Error	60	14. 71	5689. 3	0. 143	4. 52	33.611

*, **, *** = significant at 0.05, 0.01, and 0.001 levels, respectively.
ns = non-significant

conditions. The response of all the remaining four cultivar was almost not uniform in this attribute.

Water deficit conditions significantly reduced shoot and root P contents, however, under drought regimes, foliar-applied GB improved root P but not those of shoot P contents (Tab 1; Fig. 3). Drought had a significant reducing effect on root N contents while shoot N contents did not change significantly under water deficit condition (Tab. 1; Fig. 3). Foliar application of GB did not improve shoot N contents but slightly increased root N contents. The interaction among cultivars for foliar application of GB was non-

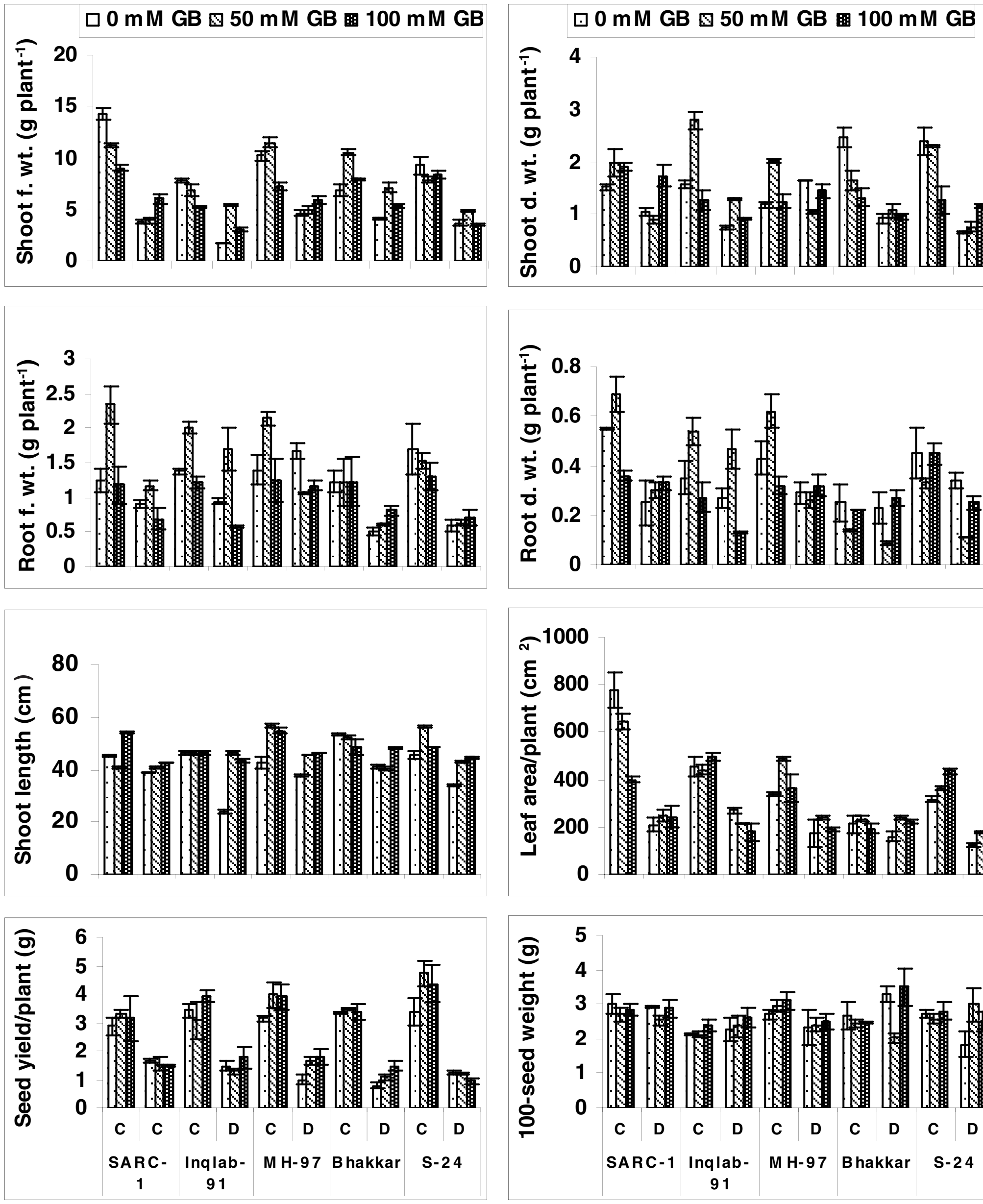

Fig. 1: Growth and yield attributes of wheat (*Triticum aestivum* L.) when 71-day old well-watered and water-stressed plants were subjected for 21 days to various levels of foliar- applied glycinebetaine. (C = control; D = drought)

significant for both shoot and root N contents.

Water deficit conditions significantly reduced shoot and root K^+ contents. Foliar-applied GB slightly improved shoot K^+ under water deficit conditions in all wheat cultivars except SARC-1, while the reverse was true for root K^+. Of various GB levels, 100 m*M* GB was more effective in increasing shoot K^+ under water deficit conditions (Tab. 1; Fig. 3).

Discussion

Drought stress triggers a series of biochemical and physiological processes in plants, which impose adverse effects on plant growth and biomass production (Gomes and Prado, 2010). In the present study, water stress reduced the growth of all wheat cultivars which is in agreement with some earlier investigations in which growth of different crops was markedly decreased due to water stress, e.g., in sugar beet (Bloch et al., 2006), wheat (Passioura, 2006), and maize (Ashraf et al., 2007). However, foliar-applied GB proved to be effective in enhancing shoot fresh and dry biomass of all cultivars under drought regimes. The beneficial role of GB in promoting shoot and root fresh biomass in wheat under drought stress has already been reported (Mahmood et al., 2009). Genetic variation for drought tolerance is reported in various crops like grass species (Ashraf et al., 1986), and maize cultivars (Ashraf et al., 2007; Zhang et al., 2009). Similarly, in our study of the wheat cultivars examined, SARC-1, Inqlab-91 and MH-97 were relatively better under drought stress. Generally, water stress is known to reduce the growth of plants by reducing the photosynthetically active leaf area, one of the most important factors affecting crop productivity (Dubey, 1997). Similarly, in our experiment leaf area was decreased under water deficit conditions, while foliar-applied GB did not reduce the bad effect of drought stress in terms of leaf area which did not agree to the findings of Mahmood et al. (2009)

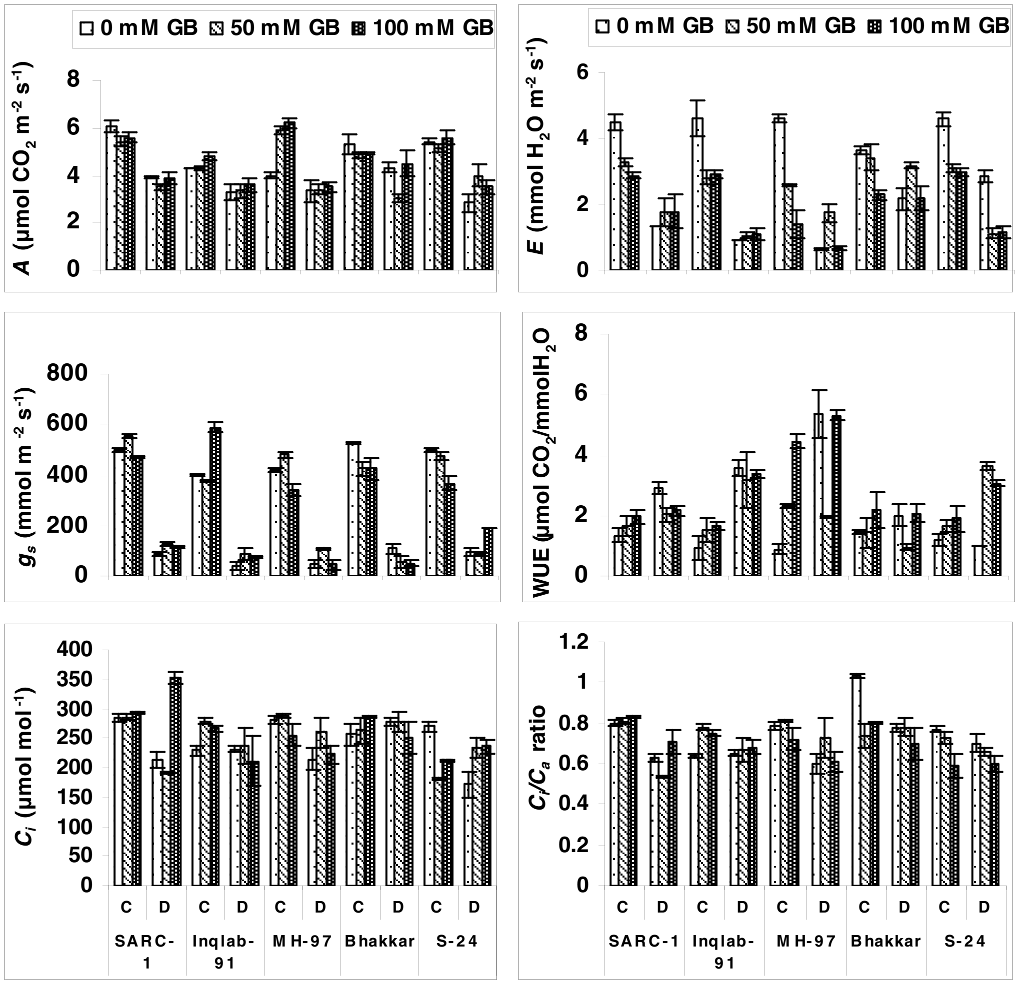

Fig. 2: Gas exchange characteristicsYield attributes and mineral nutrients of wheat (*Triticum aestivum* L.) when 71-day old well-watered and water-stressed plants were subjected for 21 days to various levels of foliar-applied glycinebetaine. (C = control; D = drought)

who reported an increase in leaf area by exogenous application of GB.

Enhancement in dry weight and grain yield by exogenous GB application has been earlier reported in maize under drought stress, but not under well-watered conditions (ZHANG et al., 2009). Cultivar S911 (drought sensitive) of maize was more responsive to exogenous applied GB compared to drought tolerant cultivar S9 (ZHANG et al., 2009). In our study, a marked reduction was observed in grain yield per plant under drought stress. However, foliar-applied GB slightly increased the grain yield of all cultivars under normal watering, but not under drought stress.

The net photosynthetic and transpiration rates, stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) in most plants decrease under drought stress (PEREZ-PEREZ et al., 2009; WANG et al., 2010). However, over-accumulation of GB increased rate of photosynthesis in wheat under drought and other stress conditions (WANG et al., 2010). In our study, drought stress caused a significant reduction in photosynthetic rate of all wheat cultivars. However, the cultivars differed in their response to foliar-applied GB under well watered and drought stress conditions. For example, under water deficit conditions, photosynthetic rate (A) is slightly increased in all the wheat cultivars while, under well-watered conditions, it markedly increased in MH-97, and slightly in Inqlab-91 and S-24 as compared to SARC-1 and Bhakkar. It has been reported that exogenously-applied GB enhances net CO_2 assimilation rate under drought stress conditions particularly in plants which naturally accumulate low levels of GB (MAKELA et al., 1999; LOPEZ et al., 2002) as compared to those with higher levels of endogenous GB (GORHAM et al., 2000; MEEK et al., 2003). IQBAL et al. (2009) reported that sunflower line with high endogenous GB level showed higher stomatal conductance. In our experiment, stomatal conductance was higher in the well-watered plants of all cultivars as compared to that under water deficit conditions. Foliar application of GB showed a variable role in transpiration rate and water use efficiency. Foliar-applied GB increased transpiration rate under drought stress, while WUE under well watered conditions. MAHMOOD et al. (2009) reported that exogenous application of GB proved ineffective in altering the gas exchange characteristics in wheat. However, in contrast in our study, of all cultivars, SARC-1 was better than the other cultivars particularly at 100 m*M* GB. These results reinforce the statement that effectiveness of GB application varies on the basis of species type and level of GB used (ASHRAF and FOOLAD, 2005; 2007; ASHRAF et al., 2008).

Under long-term exposure to water deficit conditions a significant decrease in inorganic P concentration in younger and older leaves occur in sugar beet (CHOLUJ et al., 2008). In our experiment, water deficit conditions reduced both shoot and root P contents in all wheat cultivars. However, under drought stress foliar-applied GB improved root P than that of shoot. Drought stress in our study decreased root N but not shoot N in all wheat cultivars. These

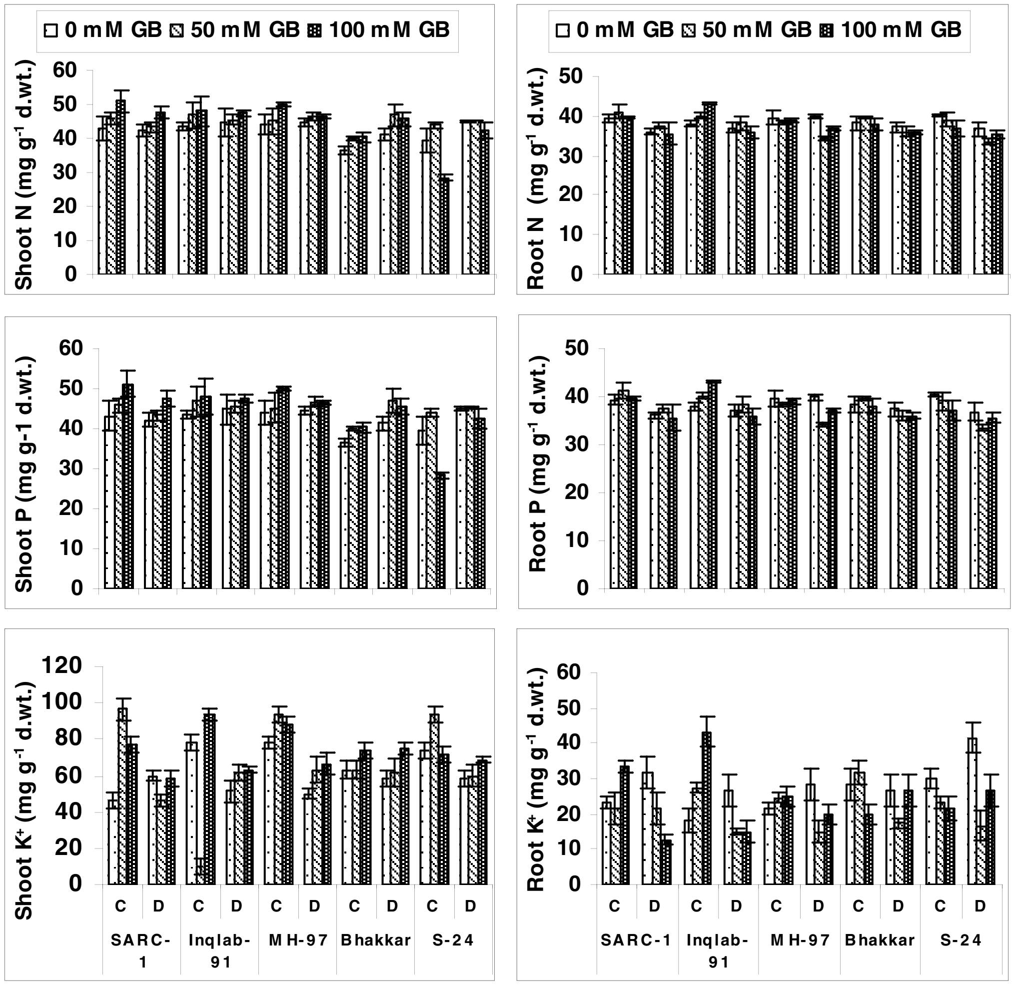

Fig. 3: Mineral nutrients of wheat (*Triticum aestivum* L.) when 71-day old well-watered and water-stressed plants were subjected for 21 days to various levels of foliar-applied glycinebetaine. (C = control; D = drought)

results can be explained with the help of an earlier report in which leaf drought was reported to decrease plant N uptake and availability of leaf N (ZHEN and ZHOU, 2006). Earlier SARWAR et al. (1991) examined the effect of drought regimes on different wheat varieties and found a marked increase in N concentration under drought stress conditions. However, in our study, foliar application of GB did not improve shoot N, but slightly increased root N. Likewise, MAHMOOD et al. (2009) found that exogenously-applied GB did not alter the leaf mineral nutrients in wheat.

Overall, drought stress decreased the growth, yield components, gas exchange characteristics and mineral nutrients of all five genetically diverse wheat cultivars. Of all cultivars, SARC-1 and Inqlab-91 showed relatively better performance in terms of plant biomass, net photosynthetic rate and stomatal conductance under well watered and drought stress conditions. Foliar-applied GB was found to be effective in enhancing various growth attributes and grain yield as well as the levels of some key metabolites of all wheat cultivars. Of various GB levels, 50 m*M* was found to be more efficient than the other GB levels for promoting wheat growth under water deficit conditions.

References

ABD EL-RHMAN, I.E., 2010: A study on some treatments which mitigate drought effects on barrani grapevines cv. J. Appl. Sci. Res. 6, 704-711.

ALLAKHVERDIEV, S.I., HAYASHI, H., NISHIYAMA, Y., IVANOV, A.G., ALIEV, J.A., KLIMOV, V.V., MURATA, N., CARPENTIER, R., 2003: Glycinebetaine protects the D1/D2/Cyt*b559* complex of photosystem II against photo-induced and heat-induced inactivation. J. Plant Physiol. 160, 41-49.

ALLEN, S.E., GRIMSHAW, H.M., ROWLAND, A.P., 1986: Chemical analysis. In: Moore P.D., Chapman, S.B. (ed.), Methods in Plant Ecology. 2nd edition, 258-344.

ASHRAF, M., MCNEILLY, T., BRADSHAW, A.D., 1986: Tolerance of sodium chloride and its genetic basis in natural populations of four grass species. New Phytol. 103, 725-734.

ASHRAF, M., 2010: Inducing drought tolerance in plants: some recent advances. Biotechnol. Adv. 28, 169-183.

ASHRAF, M., FOOLAD, M.R., 2005: Pre-sowing seed treatment-a shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. Adv. Agron. 88, 223-271.

ASHRAF, M., FOOLAD, M.R., 2007: Role of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 59, 206-216.

ASHRAF, M., ATHAR, H.R., HARRIS, P.J.C., KWON, T.R., 2008: Some prospective strategies for improving crop salt tolerance. Adv. Agron. 97, 45-110.

ASHRAF, M., NAWAZISH, S., ATHAR, H.R., 2007: Are chlorophyll fluorescence and photosynthetic capacity potential physiological determinants of drought tolerance in maize (*Zea mays* L.)? Pak. J. Bot. 39, 1123-1131.

ATHAR, H.R., ASHRAF, M., WAHID, A., JAMIL, A., 2009: Inducing salt tolerance in canola (*Brassica napus* L.) by exogenous application of glycinebetain and proline; Response at the initial growth stages. Pak. J. Bot. 41, 1311-1319.

Bloch, D., Hoffmann, C.M., Märländer, B., 2006: Impact of water supply on photosynthesis, water use and carbon isotope discrimination of sugar beet genotypes. Europ. J. Agron. 24, 218-225.

Blum, A., 2005: Drought resistance, water-use efficiency, and yield potential-are they compatible, dissonant, or mutually exclusive? Aust. J. Agric. Res. 56, 1159-1168.

Bremner, J.M., 1965: Total nitrogen and inorganic form of nitrogen. In: Black, C.A. (eds.), Method of Soil Analysis. Am. Soc. Agron. Madison, Wisconsin, 1149-1237.

Cha-um, S., Kirdmanee, C., 2010: Effect of glycinebetaine on proline, water use, and photosynthetic efficiencies, and growth of rice seedlings under salt stress. Turk. J. Agric. Forestry 34, 517-527.

Cha-um, S., Supaibulwatana, K., Kirdmanee, C., 2007: Glycinebetaine accumulation, physiological characterizations and growth efficiency in salt-tolerant and salt-sensitive lines of indica rice (*Oryza sativa* L. ssp. *indica*) in response to salt stress. J. Agron. Crops Sci. 193, 157-166.

Chen, W.P., Li, P.H., Chen, T.H.H., 2000: Glycinebetaine increase chilling tolerance and reduces chilling-induced lipid peroxidation in *Zea mays* L. Plant Cell Environ. 23, 609-618.

Chen, T.H., Murata, N., 2008: Glycinebetaine: an effective protectant against abiotic stress in plants. Trends Plant Sci. 13, 499-505.

Cherian, S., Reddy, M., Ferreira, R., 2006: Transgenic plants with improved dehydration-stress tolerance: progress and future prospects. Biol. Plant. 50, 481-495.

ChoLuj, D., Karwowska, R., Ciszewska, A., Jasinska, M., 2008: Influence of long-term drought stress on osmolyte accumulation in sugar beet (*Beta vulgaris* L.) plants. Acta Physiol. Plant. 30, 679-687.

Cui, X.H., Fu-Shun, H., Chen, H., Chen, J., Wang, X.C., 2008: Expression of the *Vicia faba VfPIP* gene in *Arabidopsis thaliana* plants improves there drought resistance. J. Plant Res. 121, 207-214.

Demiral, T., Türkan, I., 2006: Exogenous glycinebetaine affects growth and proline accumulation and retards senescence in two rice cultivars under NaCl stress. Environ. Exp. Bot. 56, 72-79.

Dubey, R.S., 1997: Photosynthesis in plants under stressful conditions. In: Pessarakli, M., (ed.), Handbook of Photosynthesis. Marcel Dekker, New York, NY. ISBN 0-8247-9708-6, 859-875.

Garg, G., 2010: *In Vitro* screening of *Catharanthus roseus* L. cultivars for salt tolerance using physiological parameters. Int. J. Environ. Sci. Dev. 1, 2010-2064.

Girija, C., Smith, B.N., Swamy, P.M., 2002. Interactive effects of sodium chloride and calcium chloride on the accumulation of proline and glycinebetaine in peanut (*Arachis hypogaea* L.). Environ. Exp. Bot. 47, 1-10.

Gomes, F.P., Prado, C.H.B.A., 2010: Coconut palm: ecophysiology under water deficit conditions. In: Damatta, F.M., (ed.), Ecophysiology of tropical tree crops. Nova Science Publishers Inc., New York, 151-179.

Gorham, J., Jokinen, K., Malik, M.N.A., Khan, I.A., 2000: Glycinebetaine treatment improves cotton yields in field trails in Pakistan. Proc. World Cotton Res. Conf-II, Athens, Greece, 624-627.

Hassine, A.B., Ghanem, M.E., Bouzid, S., Lutts, S., 2008: An inland and a coastal population of the Mediterranean xero-halophyte species *Atriplex halimus* L. differ in their ability to accumulate proline and glycinebetaine in response to salinity and water stress. J. Exp. Bot. 59, 1315-1326.

Hessine, K., Martínez, J.P., Gandour, M., Albouchi, A., Soltani, A., Abdelly, C., 2009: Effect of water stress on growth, osmotic adjustment, cell wall elasticity and water-use efficiency in *Spartina alterniflora*. Environ. Exp. Bot. 67, 312-319.

Hossain, M.A., Fujita, M., 2010: Evidence for a role of exogenous glycinebetaine and proline in antioxidant defense and methylglyoxal detoxification systems in mung bean seedlings under salt stress. Physiol. Mol. Biol. Plants 16, 19-29.

Iqbal, N.M., Ashraf, M., Ashraf, M.Y., 2009: Influence of exogenous glycine betaine on gas exchange and biomass production in sunflower (*Helianthus annuus* L.) under water limited conditions. J. Agron. Crop Sci. 195, 420-426.

Iqbal, N., Ashraf, M.Y., Ashraf, M., 2005: Influence of water stress and exogenous glycinebetaine on sunflower achene weight and oil percentage. Int. J. Sci. Tech. 2, 155-160.

Jackson, M.L., 1962: Soil chemical analysis. Contable Co. Ltd. London.

Khan, M.A., Shirazi, M.U., Khan, M.A., Mujtaba, S.M., Islam, E., Mumtaz, S., Shereen, A., Ansari, R.U., Ashraf, M.Y., 2009: Role of proline, K/Na ratio and chlorophyll content in salt tolerance of wheat (*Triticum aestivum* L.). Pak. J. Bot. 41, 633-638.

Longstreth, D.J., Burrow, G.B., Yu, G., 2004: Solutes involved in osmotic adjustment to increasing salinity in suspension cells of *Alternanthera philoxeroides* Griseb. Plant Cell Tissue Organ Cult. 78, 225-230.

Lopez, C.M.L., Takahashi, H., Yamazaki, S., 2002: Plant water relations of kidney bean plants treated with NaCl and foliarly applied glycinebetaine. J. Agron. Crop Sci. 188, 73-80.

Ma, Q.Q., Wang, W., Li, Y.H., Li, D.Q., Zou, Q., 2006: Alleviation of photoinhibition in drought-stressed wheat (*Triticum aestivum* L.) by foliar-applied glycinebetaine. J. Plant Physiol. 163, 165-175.

Mahmood, T., Ashraf, M., Shahbaz, M., 2009: Does exogenous application of glycinebetain as a pre-sowing seed treatment improve growth and regulate some key physiological attributes in wheat plants grown under water deficit conditions. Pak. J. Bot. 41, 1291-1302.

Makela, P., Kontturi, M., Pheu, E., Somersalo, S., 1999: Photosynthetic response of drought and salt-stressed tomato and turnip rape plants to foliar-applied glycinebetaine. Physiol. Plant. 105, 45-50.

Martínez, A.T., Speranza, M., Ruiz-Dueñas, F.J., Ferreira, P., Camarero, S., Guillén, F., Martínez, M.J., Gutiérrez, A., Del Río, J.C., 2005: Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. Int. Microbiol. 8, 195-204.

Meek, C., Oosterhuis, D., Gorhuam, J., 2003: Does foliar applied glycine betaine affect endogenous betaine levels and yield in cotton? Crop Manage DOI: 10/1094/ CM-2003-0804-02-RS (Online).

Moghaieb, R.E.A., Saneoka, H., Fujita, K., 2004: Effect of salinity on osmotic adjustment, glycinebetaine accumulation and the betaine aldehyde dehydrogenase gene expression in two halophytic plants, *Salicornia europaea* and *Suaeda maritima*. Plant Sci. 166, 1345-1349.

MSTAT Development Team, 1989: MSTAT user's guide: A microcomputer program for the design management and analysis of agronomic research experiments. Michigan State Univ. East Lansing, USA.

Munns, R., 2005: Genes and salt tolerance: bringing them together. New Phytol. 167, 645-663.

Naidu, B.P., Cameron, D.F., Konduri, S.V., 1998: Improving drought tolerance of cotton by glycinebetaine application and selection. Proceedings of the Australian Agronomy Conference, Australian Society of Agronomy.

Nawaz, K., Ashraf, M., 2010: Exogenous application of glycinebetaine modulates activities of antioxidants in maize plants subjected to salt stress. J. Agron. Crop. 196, 28-37.

Neumann, P.M., 2008: Coping mechanisms for crop plants in drought-prone environments. Ann. Bot. 101, 901-907.

Passioura, J., 2006: Increasing crop productivity when water is scarce from breeding to field management. Agric. Water Manage. 80, 176-196.

Pérez-Pérez, J.G., Robles, J.M., Tovar, J.C., Botía, P., 2009: Response to drought and salt stress of lemon 'Fino 49' under field conditions: water relations, osmotic adjustment and gas exchange. Sci. Hortic. 122, 83-90.

Raza, S.H., Athar, H.R., Ashraf, M., 2006: Influence of exogenously applied glycinebetaine on the photosynthetic capacity of two differently adapted wheat cultivars under salt stress. Pak. J. Bot. 38, 341-351.

Raza, S.H., Athar, H.R., Ashraf, M., Hameed, A., 2007: Glycinebetaineinduced modulation of antioxidant enzymes activities and ion accumulation in two wheat cultivars differing in salt tolerance. Environ. Exp. Bot. 60, 368-376.

SANEOKA, H., ISHIGURO, S., MOGHAIEB, R.E., 2001: Effect of salinity and abscisic acid on accumulation of glycinebetaine and betaine aldehyde dehydrogenase mRNA in sorghum leaves (*Sorghum bicolor*). J. Plant Physiol. 158, 853-859.

SARWAR, M., FIRKINS, J.L., EASTRIDGE, M.L., 1991: Effect of replacing neutral detergent fiber of forage with soy hulls and corn gluten feed for dairy heifers. J. Dairy Sci. 74,106-108.

SNEDECOR, G.W., COCHRAN, W.G., 1980: Statistical Methods, 7th edition. Iowa State University Press, Ames, IowaIA

SUBBARAO, G.V., WHEELER, R.M., LEVINE, L.H., STUTTE, G.W., 2001: Glycinebetaine accumulation, ionic and water relations of red-beet at contrasting levels of sodium supply. J. Plant Physiol. 158, 767-776.

WANG, G.P., LI, F., ZHANG, J., ZHAO, M.R., HUI, Z., WANG, W., 2010a: Overaccumulation of glycine betaine enhances tolerance of the photosynthetic apparatus to drought and heat stress in wheat. Photosynthetica 48, 30-41.

WANG, G.P., ZHANG, X.Y., LI, F., LUO, Y., WANG, W., 2010b: Over-accumulation of glycinebetaine enhances tolerance to drought and heat stress in wheat leaves in the protection of photosynthesis. Photosynthetica 48, 117-126.

WANG, G.P., HUI, Z., LI, F., ZHAO, M.R., ZHANG, J., WANG, W., 2010: Improvement of heat and drought photosynthetic tolerance in wheat by over accumulation of glycinebetaine. Plant Biotech. Rep. 4, 213-222.

YANG, X.H., WEN, X.G., GONG, H.M., LU, Q.T., YANG, Z.P., TANG, Y.L., LIANG, Z., LU, C.M., 2007: Genetic engineering of the biosynthesis of glycinebetaine enhances thermotolerance of photosystem II in tobacco plants. Planta 225, 719-733.

ZHANG, H., ZHANG, S., YANG, J., ZHANG, J., WANG, Z., 2008: Postanthesis moderate wetting drying improves both quality and quantity of rice yield. Agron. J. 100, 726-734.

ZHANG, L.X., LI, S.X., LIANG, Z.S., 2009: Differential plant growth and osmotic effects of two maize (*Zea mays* L.) cultivars to exogenous glycinebetaine application under drought stress. Plant Growth Regul. 58, 297-305.

ZHAO, Z., CHEN, G., ZHANG, C., 2001: Interaction between reactive oxygen species and nitric oxide in drought-induced abscisic acid synthesis in root tips of wheat seedlings. Aust. J. Plant Physiol. 28, 1055-1061.

ZHEN, Z.X., ZHOU, G.S., 2006: Combined effects of water stress and high temperature on photosynthesis, nitrogen metabolism and lipid peroxidation of a perennial grass *Leymus chinensis*. Planta 224, 1080-1090.

ZHU, M.Y., AHN, S.J., MATSUMOTO, H., 2003: Inhibition of growth and development of root border cells by Al. Physiol. Plant. 117, 359-367.

Address of the author:
E-mail: shahbazmuaf@yahoo.com