Pak. J. Bot., 45(1): 93-98, 2013.

DROUGHT-INDUCED MODULATION IN GROWTH AND MINERAL NUTRIENTS IN CANOLA (*BRASSICA NAPUS* L.)

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Abstract

A pot experiment was conducted to assess drought-induced modulation in growth and mineral composition of canola (*Brassica napus* L.). Four canola accessions [two drought tolerant (Dunkeld and 24177) and two sensitive (24173 and Pakola)] were grown in sandy loam soil. The plants of all four canola cultivars were subjected to three drought stress treatments i.e. normal watering (full field capacity), 75% and 50% field capacity (FC) at two growth stages (vegetative stage and flowering stage). Imposition of drought stress at different growth stages caused a significant decrease in shoot and root fresh and dry weights, shoot and root length, and shoot and root K^+, Ca^{2+}, N and P. Maximum reduction in these variables was observed at 50% FC in all canola accessions. The higher drought tolerance of Dunkeld and 24177 measured in terms of shoot fresh and dry biomass was found to be associated with higher concentrations of K^+ and N maintained in its shoot particularly at the flowering stage.

Introduction

Drought stress is considered as one of the primary factors responsible for reduced agricultural productivity (Mehmood *et al*., 2009; Ashraf, 2010), because it is often linked to other major abiotic stresses such as salinity stress, heat stress etc. Shortage of water imposes adverse effects on plants in terms of impaired growth, reduced nutrient acquisition and alterations in water status of plants (Ali & Ashraf, 2011; Shahbaz *et al*., 2011a). Dehydration of protoplasma increases the concentration of cellular electrolytes that cause substantial disruption to a variety of metabolic reactions taking place in the cell (Mahajan & Tuteja, 2005). Of a number of metabolic processes perturbed in plants due to drought stress, impairment in uptake and accumulation of essential inorganic nutrients is a common phenomenon (Akram *et al*., 2008; Shahbaz *et al*., 2011b). This nutrient deficit condition in plants under drought stress takes place due to reduced root growth, low nutrient availability in soil and reduced mineral uptake (Samarah *et al*., 2004). However, inter-specific and intra-specific variation exists in plant species in relation to nutrient uptake under water deficit conditions (Garg, 2003).

In canola (*Brassica napus* L.) considerable inter-cultivar variation for drought tolerance has been observed (Kauser *et al*., 2006). While screening a large number of canola accessions for drought tolerance (Unpublished data) accessions Dunkeld and 24177 were found to be relatively drought tolerant and 24173 and Pakola as drought sensitive. However, their response to drought stress at different growth stages has not been observed. Thus, the premier objective of the present investigation was to appraise the response to drought stress of these four canola accessions differing in drought tolerance at different growth stages, i.e. vegetative and flowering in terms of growth and accumulation of some key essential nutrients.

Materials and Methods

To assess the alteration in mineral composition of canola (*Brassical napus* L.) under water deficit conditions, four canola accessions [Dunkeld and 24177 (drought tolerant) and 24173 and Pakola (drought sensitive)] were grown in plastic pots filled with sandy loam soil. The experiment was laid out in a completely randomized design with four replications of each experimental unit. Each plastic pot (25 cm diameter and 28 cm length) was filled with equal weight sandy loam soil. Then the soil in each pot was completely saturated with normal irrigation water. When the moisture contents were at field capacity, seeds of the four canola accessions were hand sown. Thinning of plants was done 8 days after germination to maintain 4 plants per pot. Three week-old seedlings of all four canola cultivars were subjected to 3 drought stress treatments i.e. normal watering (full field capacity), 75% field capacity (FC) and 50% field capacity at 2 growth stages (vegetative stage and flowering stage). The moisture contents of droughted pots were maintained and regularly monitored by keeping the weight of each pot equal to that calculated for 75% and 50% field capacity through addition of normal irrigation water, if required on daily basis till the maturation of the crop. Two plants per replicate were harvested after 15 days of drought treatment at the flowering stage. Data for shoot and root fresh weights were recorded. These plants were then oven-dried at 65°C for 72 h for the estimation of dry weights and mineral composition.

Determination of mineral elements: The dried ground material i.e., shoot and root (0.1 g) was taken in digestion flasks and 2 mL of the digestion mixture prepared after Wolf (1982) were added. The flasks were incubated overnight at room temperature. After heating the flasks containing plant material at 150ºC, 0.5 mL of H_2O_2 (35%) was poured down the sides of the digestion flasks. Then the tubes were placed in a digestion block and heated at 250°C until fumes produced. The above step was repeated until the cooled digested material became colorless". The volume of the extract was maintained up to 50 mL in volumetric flasks. The extract was filtered and used for the determination of N, P, K^+ and Ca^{2+}.

Determination of cations (K^+ and Ca^{2+}): Potassium (K^+) and calcium (Ca^{2+}) contents were determined using a flame photometer (Jenway PFP 7).

Estimation of nitrogen: Nitrogen was estimated by micro–Kjeldhal's method (Bremner, 1965). The digested plant material (5 mL) was taken in Kjeldhal's tubes. The tubes were placed on the Kjeldhal's ammonia distillation unit and 5 mL of 40% NaOH added to each tube. Boric acid solution (5mL) was taken in a conical flask with a few drops of mixed indicator. When the distillate was approximately 40 mL, the distillation stopped. The distillate was cooled for a few minutes and titrated it with 0.01 *N* standard H_2SO_4 till the solution turned pink. A blank was run for the complete procedure.

N was estimated by the following formula:
N %age = (V_2-V_1) x *N* x 0.014 x 100/W
The V_1 and V_2 in the above equation show the volume of standard H_2SO_4 required to titrate blank solution and sample solution, respectively, whereas *N* shows the normality of the H_2SO_4 used and W the weight of sample.

Phosphorus estimation: Phosphorus (P) was determined spectrophotometrically. The extracted material (2 mL) was dissolved in 2 mL of Barton's reagent and total volume made to 50 mL. These samples were kept for 30 min before analyzing the phosphorus.

Statistical analysis

Data for different parameters were analyzed statistically by adopting two-way analysis of variance technique based on completely randomized design with 4 replications according to Steel & Torrie (1986). The least significance difference test (LSD) was used for appraising the significant difference between the mean values (Snedecor & Cochran, 1980).

Results

Shoot fresh and dry weights of all canola accessions decreased significantly due to imposition of different drought stress regimes applied at different growth stages. The maximum reduction in shoot biomass in all accessions was observed at 50% FC at both growth stages. On comparing different growth stages it was found that application of drought stress at the vegetative stage caused more reduction in shoot fresh and dry weights of all canola accessions as compared to that at the flowering stage. Of different canola accessions the maximum reduction in shoot biomass due to drought stress was recorded in Pakola and minimum in Dunkeld at all drought stress levels at both growth stages (Fig. 1).

Root fresh and dry weights of all canola accessions reduced significantly due to different levels of drought stress at different growth stages. Both drought levels were found to be equally effective in reducing root fresh and dry weights of all canola accessions applied at both growth stages. On comparing different canola accessions it was found that the maximum reduction in root fresh and dry weights due to drought stress at both growth stages was recorded of Pakola as compared to the other accessions (Fig. 1).

A significant reduction in shoot length of all canola accessions was observed due to imposition of different levels of drought stress at different growth stages. This reduction in shoot length of all canola accessions was more prominent at 50% FC. Of all canola accessions the maximum reduction in shoot length was observed in accession Pakola as compared with the other accessions. However, root length of all canola accessions increased with a decrease in soil field capacity at different growth stages. The maximum increase in root length due to drought stress applied at different growth stages was observed at 50% soil field capacity. On comparing different canola accessions it was observed that more increase in root length due to drought stress imposed at different growth stages was observed in accession 24173 as compared with the other canola accessions (Fig. 1).

Shoot and root K^+ and Ca^{2+} contents of all canola accessions decreased significantly due to imposition of different levels of drought stress applied at different growth stages. The maximum reduction in these nutrients both in shoots and roots due to drought stress at different growth stages was recorded at 50% FC. All canola accessions did not differ significantly with reference to these nutrients in shoots and roots under drought stress applied at different growth stages except shoot K^+ contents which were found maximum in Dunkeld and 24177 and minimum in accession Pakola under drought stress particularly at the flowering stage (Fig. 2).

Like shoot and root K^+ and Ca^{2+} contents, shoot and root P and N contents also decreased significantly due to different levels of drought stress applied at different growth stages. The maximum reduction in these nutrients was observed at 50% FC at both growth stages. The accessions did not differ significantly in relation to these nutrient contents under water deficit conditions applied at both growth stages except shoot N which significantly higher in Dunkeld and 24177 particularly at the flowering stage (Figs. 2).

Discussion

Of different deleterious environmental factors, drought is the major abiotic factor that adversely affects plant growth and metabolism leading to reduced final yield (Akram *et al*., 2007; Ali & Ashraf, 2011; Shahbaz *et al*., 2011b). Plants experience drought stress due to high rate of transpiration or due to low supply of water to roots. A number of reports show that even a temporary water stress can cause a substantial growth reduction of economically important crops (Ashraf & Mehmood, 1990; Pinheiro *et al*., 2005; Kamran *et al*., 2009). However, water stress either temporarily or permanently adversely affects a number of morphological processes like shoot and root biomass and their lengths. Water is essential during the whole life of plant growth from seed germination to final growth stage. Water stress at any stage of plant growth and development reduces the final crop yield (Dicken & Wright, 2008). So any degree of water imbalance at any crop growth stage adversely affects the crop growth and development. However, the deleterious effects of shortage of water on crop growth may be more obvious at some particular growth stage depending upon the nature of a crop cultivar or species (Suralta & Yamauchi, 2008; Arshad *et al*., 2008). These adverse effects of drought stress on plant biomass production are due to inhibited cell expansion (Arshad *et al*., 2008), alterations in plant metabolism (Ashraf & O'Leary, 1996; Lawlor & Cornic, 2002; Chimenti *et al*.,

2006), and reduction in the activities of different metabolic enzymes (Hong & Ji-yun, 2007; Xu *et al*., 2008). Similarly, in the present study different levels of water stress applied at vegetative and flowering stage of plants of different accessions of canola decreased the plant biomass production of all canola accessions but the accessions Dunkeld followed by 24177 was found to be better as compared with the other accessions. The more reduction in biomass production was recorded when drought stress was applied at the vegetative stage in all canola accessions. This differential response of different canola accessions to different levels of drought stress at different growth stage on plant biomass production might be due to their differential genetic potential to drought stress at different growth stages. Such variations in plant biomass production under drought stress have already been reported in different cultivars of oilseed rape (Abedi & Pakniyat, 2010).

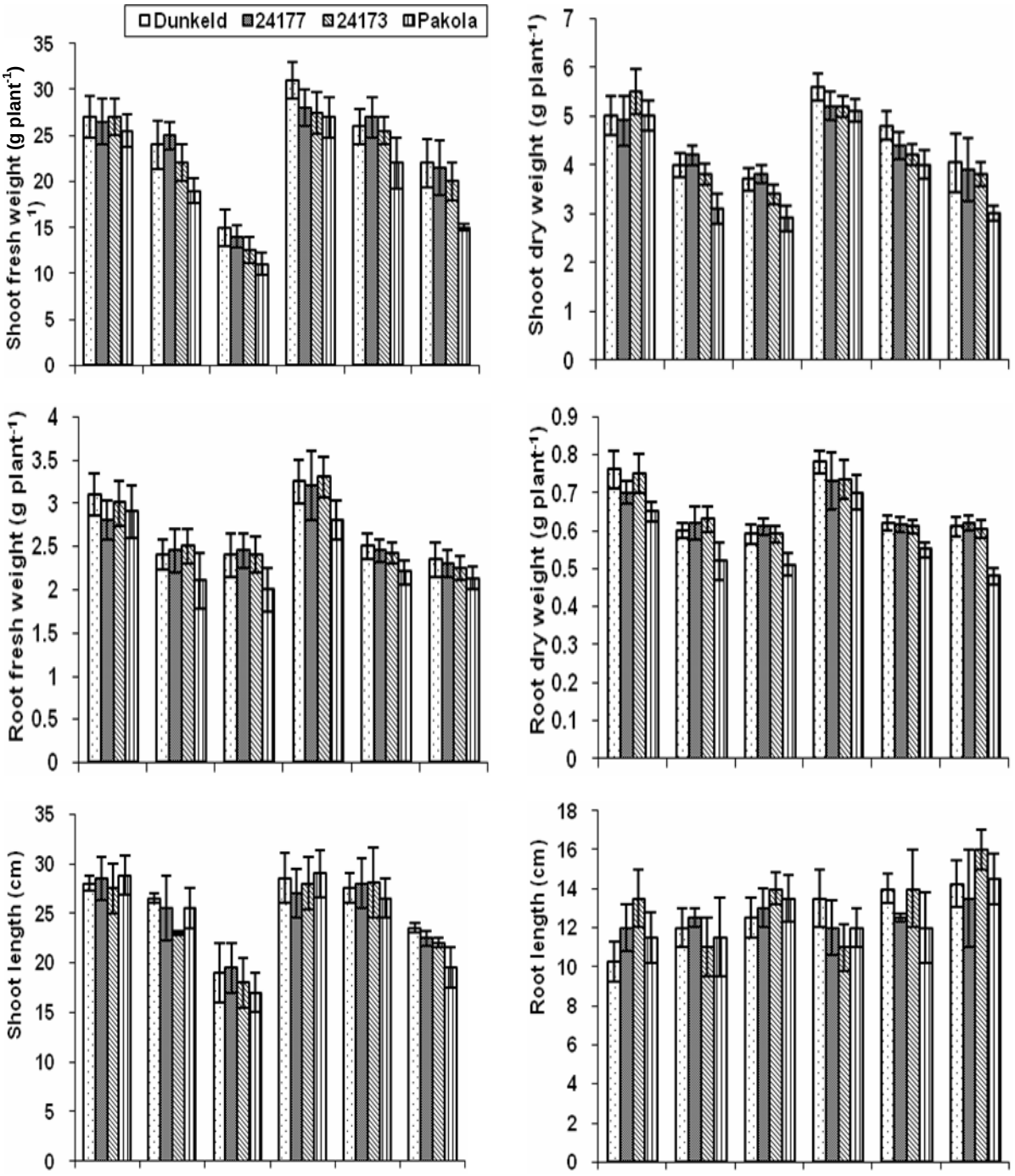

Fig. 1. Growth attributes of different accessions of canola (*Brassica napus* L.) as affected by different levels of water stress when applied at different growth stages (mean ± S.E.).

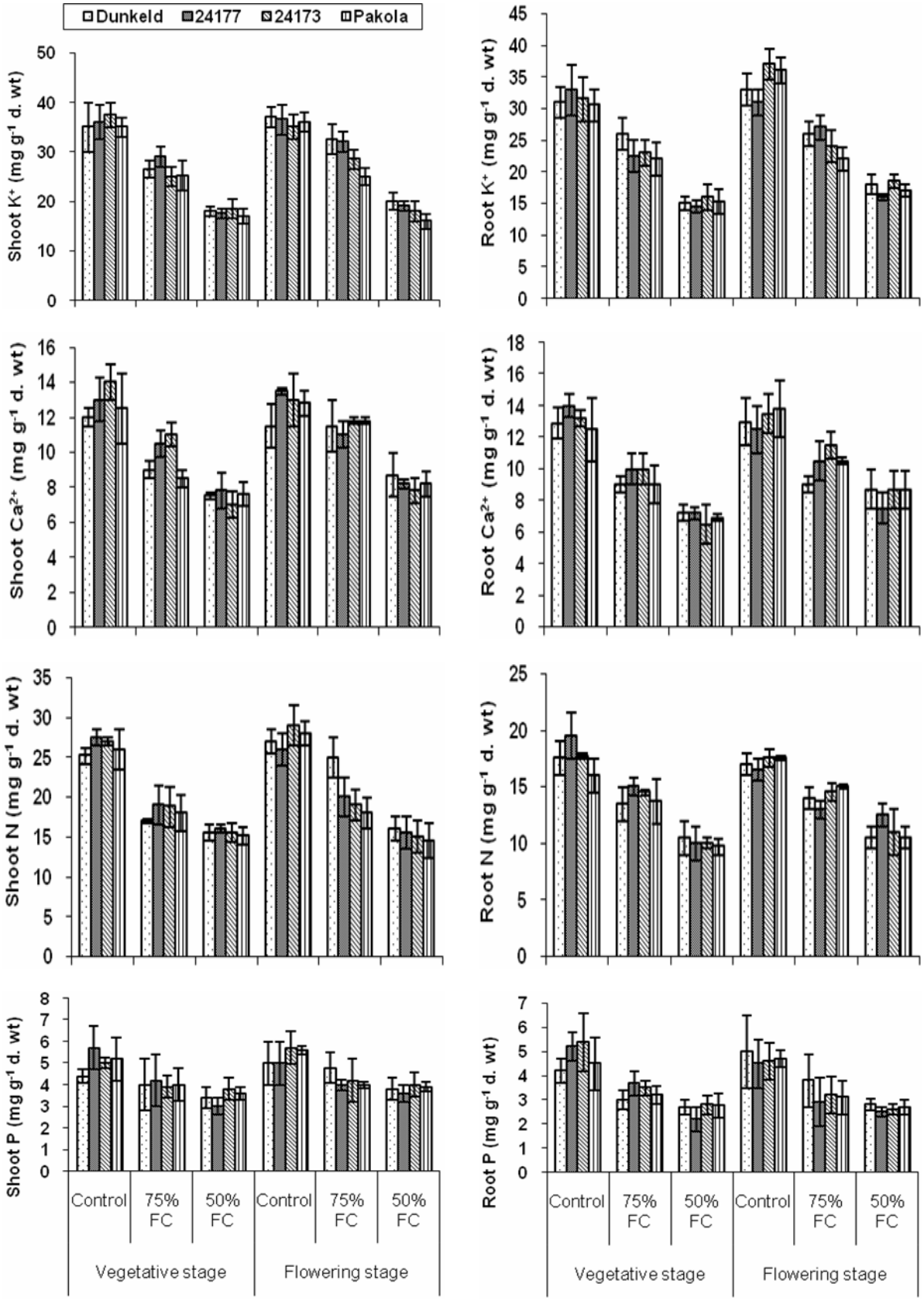

Fig. 2. Shoot and root mineral nutrients of different accessions of canola (*Brassica napus* L.) as affected by different levels of water stress when applied at different growth stages (mean ± S.E.).

In the present study, water stress resulted in a significant reduction in accumulation of essential nutrients (K^+, Ca^{2+}, N, and P) both in roots and shoots. This reduced uptake was positively associated with reduced plant growth. The more reduction in this regard was observed in accessions 24173 and Pakola as compared with the other two drought tolerant cultivars. This reduction in nutrient uptake may be due to their less solubility that results in altered physiological processes including low absorption and uptake of nutrients in plants grown under such conditions (Garg, 2003; Fageria *et al*., 2002). This decrease in absorption of nutrients in plants generally results due to a substantial decrease in transpiration rate, reduced active transport as well as membrane permeability (Baligar *et al*., 2001; Gunes *et al*., 2006) that result in diminished tissue nutrient concentration (Garg, 2003; McWilliams, 2003). However, in a number of reports, it has been observed that under water deficit conditions plant species and cultivars within a species differ in absorbing nutrients from soil and transporting them to roots and then from roots to shoot (Ali *et al*., 2008). Normally, the decrease in nutrient uptake under water deficit conditions takes place due to a reduction in transpiration rate (Ali *et al*., 2008; Jabeen *et al*., 2008). The similar results were observed in the present study under water deficit conditions.

In conclusion, water deficit conditions reduced growth and mineral composition of all four canola accessions. Maximum reduction was observed at 50% FC and at vegetative growth stage. Of various canola accessions, drought tolerant accession Dunkeld was superior while Pakola inferior among all the accessions. The superiority of Dunkeld over the other cultivars in terms of drought tolerance was found to be due to its potential of high accumulation of K^+ and N in the shoot particularly at the flowering stage.

Acknowledgements

The authors gratefully acknowledge the Higher Education Commission, Pakistan for providing funds for current study through the NRPU grant no. 20-1479.

References

Abedi, T. and H. Pakniyat. 2010. Antioxidant enzyme changes in response to drought stress in ten cultivars of oilseed rape *Brassica napus* L. *Czech J. Genet. Plant Breed*., 46: 27-34.

Akram, N.A., M. Shahbaz and M. Ashraf. 2007. Relationship of photosynthetic capacity and proline accumulation with the growth of differently adapted populations of two potential grasses (*Cynodon dactylon* (L.) Pers. and *Cenchrus ciliaris* L.) to drought stress. *Pak. J. Bot*., 39(3): 777-786.

Akram, N.A., M. Shahbaz and M. Ashraf. 2008. Nutrient acquisition in differentially adapted populations of *Cynodon dactylon* (L.) Pers. and *Cenchrus ciliaris* L. under drought stress. *Pak. J. Bot.*, 40(4): 1433-1440.

Ali, Q. and M. Ashraf. 2011. Induction of drought tolerance in maize *Zea mays* L. due to exogenous application of trehalose: growth, photosynthesis, water relations and oxidative defence mechanism. *J. Agron. Crop Sci*., DOI: 10.1111/j.1439-037X.2010.00463.x

Ali, Q., M. Ashraf, M. Shahbaz and H. Humera. 2008. Ameliorating effect of foliar applied proline on nutrient uptake in water stressed maize (*Zea mays* L.) plants. *Pak. J. Bot*., 40(1): 211-219.

Arshad, M., B. Shaharoona and T. Mahmood. 2008. Inoculation with Pseudomonas spp. containing ACC-deaminase partially eliminates the effects of drought stress on growth, yield, and ripening of pea (*Pisum sativum* L.). *Pedosphere*, 18: 611-620.

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

Ashraf, M. and J.W. O'Leary. 1996. Effect of drought stress on growth, water relations and gas exchange of two lines of sunflower differing in degree of salt tolerance. *Int. J. Plant Sci*., 157: 729-732.

Ashraf, M. and S. Mehmood. 1990. Response of four *Brassica* species to drought stress. *Environ. Exp. Bot*., 30: 93-100.

Baligar, V.C., N.K. Fageria and Z.L. He. 2001. Nutrient use efficiency in plants. *Commun. Soil Sci. Plant Anal*., 32: 921-950.

Bremner, J.M. 1965. Total nitrogen and inorganic forms of nitrogen. In: *Methods of Soil Analysis.* Vol. 2, (Ed.): C.A Black, Amer. Soc. Agron., Madison, Wisconsin, pp. 1149-1237.

Chimenti, C.A., M. Marcantonio and A.J. Hall. 2006. Divergent selection for osmotic adjustment results in improved drought tolerance in maize (*Zea mays* L.) in both early growth and flowering phases. *Field Crops Res*., 95: 305-315.

Dickin, E. and D. Wright. 2008. The effects of winter water logging and summer drought on the growth and yield of winter wheat *Triticum aestivum* L. *Eur. J. Agron*., 28: 234-244.

Fageria, N.K., V.C. Baligar and R. B. Clark. 2002. Micronutrients in crop production. *Adv. Agron*., 77: 185-67.

Garg, B.K. 2003. Nutrient uptake and management under drought: Nutrient-moisture interaction. *Curr. Agric*., 27: 1-8.

Gunes, A., N. Cicek, A. Inal, M. Alpaslan, F. Eraslan, E. Guneri and T. Guzelordu. 2006. Genotypic response of chickpea *Cicer arietinum* L. cultivars to drought stress implemented at pre-and post-anthesis stages and its relations with nutrient uptake and efficiency. *Plant Soil Environ*., 528: 368-376.

Hong, W. and J. Ji-yun. 2007. Effects of zinc deficiency and drought on plant growth and metabolism of reactive oxygen species in maize (*Zea mays* L.). *Agric. Sci. China*, 6(8): 988-995.

Jabeen, F., M. Shahbaz and M. Ashraf. 2008. Discriminating some prospective cultivars of maize *Zea mays* L. for drought tolerance using gas exchange characteristics and proline contents as physiological markers. *Pak. J. Bot*., 40(6): 2329-2343.

Kamran, M., M. Shahbaz, M. Ashraf and N.A. Akram. 2009. Alleviation of drought-induced adverse effects in spring wheat (*Triticum aestivum* L.) using proline as pre-sowing seed treatment. *Pak. J. Bot*., 41(2): 621-632.

Kauser, R., H.R. Athar and M. Ashraf. 2006. Chlorophyll fluorescence: A potential indicator for rapid assessment of water stress tolerance in canola (*Brassica napus* L.). *Pak. J. Bot*., 38(5): 1501-1509.

Lawlor, D.W. and G. Cornic. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ*., 25: 275-294.

Mahajan, S. and N. Tuteja. 2005. Cold, salinity and drought stresses: an overview. *Arch. Biochem. Biophys*., 444: 139-158.

Mahmood, T., M. Ashraf and M. Shahbaz. 2009. 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? *Pak. J. Bot*., 41(3): 1291-1302.

McWilliams, D. 2003. Drought strategies for cotton, cooperative extension service circular 582, college of agriculture and home economics. New Mexico State University, USA.

Pinheiro, H.A., F.M. DaMatta, A.R.M. Chaves, M.E. Loureiro and C. Ducatti. 2005. Drought tolerance is associated with rooting depth and stomatal control of water use in clones of *Coffea canephora*. *Ann. Bot*., 96: 101-108.

Samarah, N., R. Mullen and S. Cianzio. 2004. Size distribution and mineral nutrients of soybean seeds in response to drought stress. *J. Plant Nutr*., 27: 815-835.

Shahbaz, M., M. Iqbal and M. Ashraf. 2011a. Response of differently adapted populations of blue panic grass (*Panicum antidotale* Retz.) to water deficit conditions. *J. Appl. Bot. Food Qual*., 84(2): 134-141.

Shahbaz, M., Y. Masood, S. Perveen and M. Ashraf. 2011b. Is foliar-applied glycinebetaine effective in mitigating the adverse effects of drought stress on wheat (*Triticum aestivum* L.)? *J. Appl. Bot. Food Qual*., 84(2): 192-199.

Snedecor, G.W. and W.G. Cochran. 1980. Statistical Methods, 7th edition. Iowa State University Press, Ames, IowaIA.

Steel R.G.D. and J.H. Torrie. 1986. Principles and procedures of statistics. McGraw Hill Book Co., Inc. New York, NY.

Suralta, R.R. and A. Yamauchi. 2008. Root growth, aerenchyma development, and oxygen transport in rice genotypes subjected to drought and water logging. *Environ. Exp. Bot*., 64: 75-82.

Wolf, B. 1982. A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. *Comm Soil Sci Plant Anal*., 13: 1035-1059.

Xu, D.Q., J. Huang, S.Q. Guo, X. Yang, Y.M. Bao, H.J. Tang and H.S. Zhang. 2008. Over-expression of a TFIII-type zinc finger protein gene ZFP252 enhances drought and salt tolerance in rice (*Oryza sativa* L.). *FEBS Lett*., 582: 1037-1043.

(Received for publication 30 June 2011)