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# AMELIORATING EFFECT OF FOLIAR APPLIED PROLINE ON NUTRIENT UPTAKE IN WATER STRESSED MAIZE (*ZEA MAYS* L.) PLANTS

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**Abstract**

The role of different compatible solutes in plant tolerance to drought stress is significant because they regulate multitude of metabolic processes including ion transport. The present study was therefore conducted to investigate the influence of foliar application of a potential osmoprotectant, proline, on patterns of uptake of some essential macronutrients in water stressed plants of two maize (*Zea mays*) cultivars. Two week old plants of two maize cultivars, EV-1098 and Agaiti 2002, grown under natural environmental conditions were subjected to water stress by maintaining moisture content equivalent to full field capacity (control) and 60% field capacity. Different concentrations of proline applied as a foliar spray at the seedling, vegetative and seedling + vegetative stages were: no spray, 0.1% Tween-20 solution, 30 and 60 m*M* proline in 0.1% Tween-20 solution. Water stress reduced the concentration of all four mineral nutrients in the shoots and roots of both maize cultivars. However, exogenous application of proline counteracted the adverse effects of water stress on nutrient uptake because it promoted the uptake of $K^+$, $Ca^{2+}$, N and P in both maize cultivars. Application of 30 m*M* proline concentration was more effective as compared to the other levels in up-regulating ion transport.

## Introduction

Drought is undoubtedly deleterious for plant growth (Garg *et al*., 2004; Samarah *et al*., 2004). It is known that low water availability under drought stress generally results in reduced total nutrient uptake and frequently reduces the levels of mineral nutrients in crop plants (Marschner 1995; Baligar *et al*., 2001). Plant species and genotypes within a species vary in their response to mineral uptake under drought stress (Garg, 2003). The drastic effect of drought stress observed is on the transport of nutrients to the root and on the root growth and extension (Alam, 1999).

Inorganic nutrients such as N, P, $K^+$, $Ca^{2+}$ and $Mg^{2+}$ ion play multiple essential roles in plant metabolism. Potassium plays an important role in osmoregulation, activates enzymes of respiration and photosynthesis and has a role in stomatal regulation. Calcium is the component of cell membrane and thus plays a significant role in plant growth (Lahaye & Epstein, 1971). N is a constituent of many cell components and P plays a role in cellular energy transfer, respiration and photosynthesis (Alam, 1999). Nonetheless, each of these nutrients must be maintained at an optimum concentration range for proper growth.

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Cuin & Shabala (2007) reported that compatible solutes such as glycinebetaine (GB), proline, and trehalose have a mitigating effect on $K^+$ efflux in *Arabidopsis* under stressed conditions and low concentrations of these organic osmolytes have a role in osmotic adjustment due to the accumulation of inorganic ions, as exogenous application of low concentrations of glycine betaine or proline significantly reduces the extent of the stress induced $K^+$ efflux from barley roots (Cuin & Shabala, 2005). But how proline and glycinebetaine reduce efflux of inorganic ions in drought stressed maize plants needs to be elucidated.

It is evident from different reports that exogenous application of proline induces abiotic stress tolerance in plants (Claussen, 2005; Ali *et al*., 2007). Although much attention has been paid on the role of proline in stress tolerance as a compatible osmolyte (MacCue & Hanson, 1990; Samras *et al*., 1995), little attention has been given to its role in affecting the uptake and accumulation of inorganic nutrients in plants (Okuma *et al*., 2000; Khedr *et al*., 2003). Thus, it was hypothesized that the exogenous application of proline might regulate uptake of mineral nutrients in plants subjected to water deficit conditions.

The present study was aimed to assess the role of exogenous proline in uptake of nutrients in the shoots and roots of two maize cultivars under water deficit conditions and to determine the effective concentration of proline and appropriate growth stage of maize at which exogenously applied proline could effectively alleviate the adverse effects of drought on nutrient uptake.

## Material and Methods

The present study was conducted to examine the role of foliar applied proline in ameliorating the adverse effects of drought on nutrient uptake of two maize cultivars. The work was carried out in the wire-house of the Department of Botany, University of Agriculture, Faisalabad (latitude 31°30 N, longitude 73°10 E and altitude 213 m), with 10/14 light/dark period at 600-900 μmol $m^{-2}$ $s^{-1}$ PPFD, a day/night temperature cycle of 37/25°C and mean relative humidity 65±5% during the years 2006 and 2007.

The experiment was laid out in a completely randomized design in a factorial arrangement with four replications of each experimental unit. Equal weight plastic pots of same size were filled with equal weights (11kg) of sandy clay loam soil. These pots were then divided into two groups, each representing a specific water stress treatment (Control or 60% field capacity). Then the soil in each pot was completely saturated with normal irrigation water. When the moisture contents were at field capacity, seeds of the two maize varieties, EV-1098 and Agaiti 2002, were hand sown. Thinning of plants was done 15 days after germination to maintain 5 plants per pot. The experiment comprised two maize varieties, two water levels (control and water stress at 60% field capacity), four foliar treatments of proline (no spray, water spray, 30 and 60 mmol/L proline) and four replications per treatment.

Analysis of the soil used in the experiment was carried out in the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad. The soil used was sandy clay loam. The other contents of the soil were as follows: $CaCO_3$ 2.71%, organic matter 0.95%, available P 8.6 mg $L^{-1}$, total nitrogen content 0.73%, soluble $Cl^-$ 8.52 meq $L^{-1}$ and soluble $Ca^{2+}$+ $Mg^{2+}$ 14.30 meq $L^{-1}$.

Two water stress treatments [field capacity, (control) and 60% field capacity] were started four weeks after seed germination. The moisture contents of droughted pots were maintained and regularly monitored by keeping the weight of each pot equal to that calculated for 60% field capacity through addition of normal irrigation water if required on daily basis till the maturation of the crop.

Proline of Sigma Aldrich, Japan was used for the present study. There were three levels of proline (0, 30 and 60 mmol/L) which were applied as a foliar spray at the seedling, vegetative, and seedling + vegetative stages. To ensure penetration of proline into leaf tissues Tween-20 (0.1%) was used as a surfactant for the foliar spray.

Two plants per replicate were harvested after 15 days of last foliar application of proline and data for shoot and root fresh weights were recorded. These plants were then oven dried at 65ºC for 72 h after which time dry weight was recorded.

**Determination of inorganic elements in shoots and roots:** The dried ground material (0.1 g) of leaf or root was digested with 2 mL Sulphuric acid-hydrogen peroxide mixture according to the method of Wolf (1982). Potassium and calcium in the digests were determined using a flame photometre (Jenway, PFP-7). Nitrogen was estimated by the Kjeldahl method. Phosphorus and $Mg^{2+}$ in the extracts were determined following Allen *et al*., (1986).

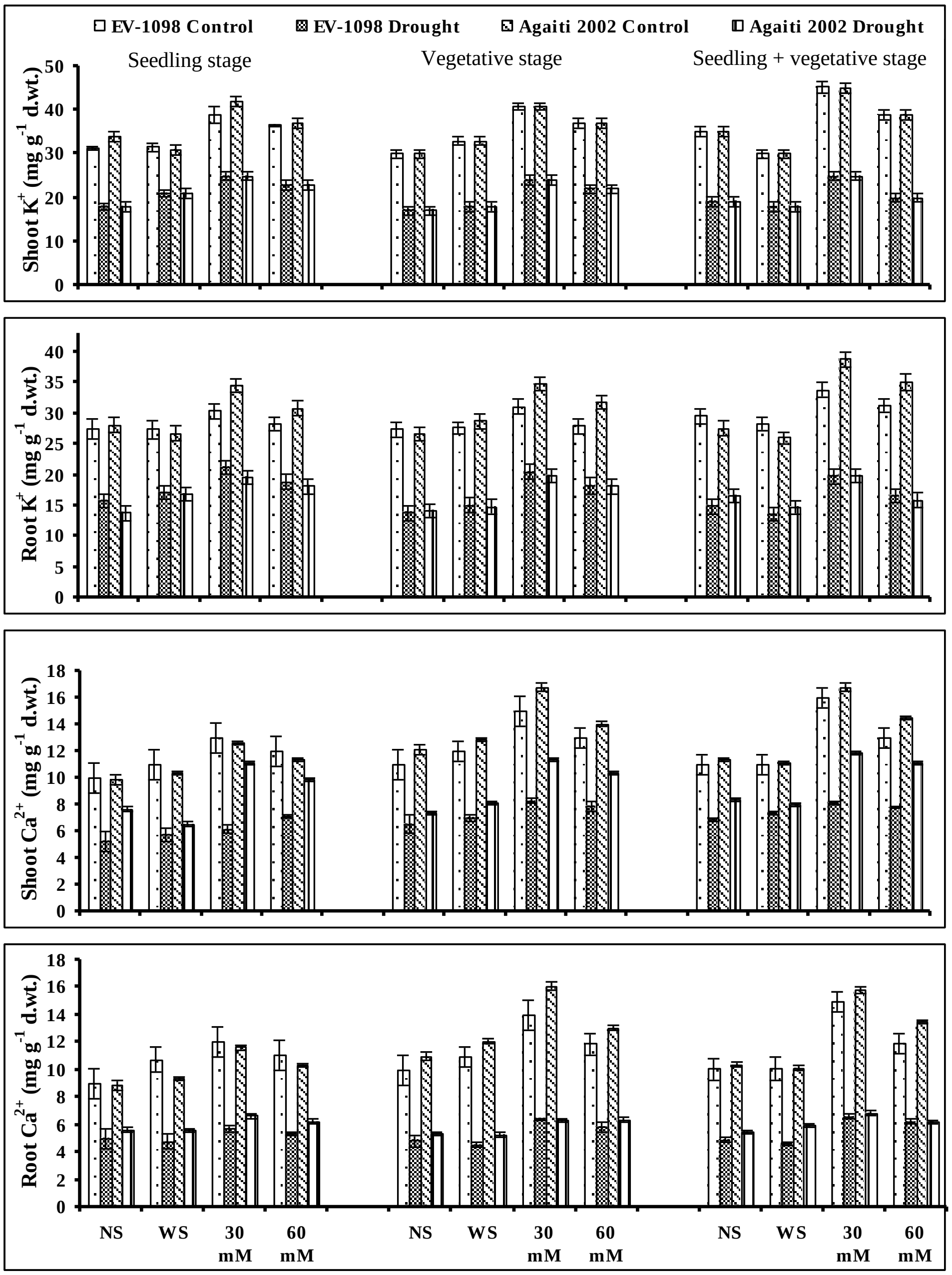
**Statistical analysis of data:** The data for all variables were subjected to analysis of variance using a COSTAT computer package (Cohort Software, Berkeley, California). The mean values were compared with the least significance difference test following Snedecor & Cochran (1980).

## Results

Potassium in the shoots of two maize cultivars was decreased significantly with the imposition of water stress at different growth stages. Exogenous application of proline at all growth stages significantly increased the shoot $K^+$ both under stress and non-stress conditions in both maize cultivars. However, this increase was more obvious when proline was applied @ 30 m*M* and the maximum increase was observed when proline applied at the seedling+vegetative stage (Fig. 1).

Root $K^+$ was also decreased in both maize cultivars under water deficit conditions. Different concentrations of foliar applied proline at different growth stages significantly increased the $K^+$ concentration in the roots of both maize cultivars. However, 30 m*M* proline concentration was more effective in increasing root $K^+$ in the maize plants and this effect was more pronounced when it was applied at the seedling + vegetative stage (Fig. 1).

Shoot $Ca^{2+}$ was also decreased in the plants of both maize cultivars due to water stress. Different concentrations of foliar applied proline at different growth stages significantly increased the $Ca^{2+}$ concentration in the shoots of both maize cultivars under stress and non stress conditions (Fig. 1). However, 30 m*M* proline concentration applied at different growth stages was more effective than the other proline levels, but this effect of proline on $Ca^{2+}$ concentration was more obvious when it was applied at the vegetative stage. Significantly higher concentrations of $Ca^{2+}$ in the shoots were observed in cv. Agaiti 2002 as compared to cv. EV-1098 under water stress conditions.




NS = Non spray; WS = Water spray; 30, 60 m*M* = Proline spray with 30 and 60 m*M*

Fig. 1. $K^+$ and $Ca^{2+}$ concentrations (mg $g^{-1}$ d. wt.) in shoots and roots of two maize cultivars as influenced by the exogenous application of different concentrations of proline at different growth stages under controlled and water stress conditions. ($n$ = 4 ± S.E)

Imposition of water stress significantly decreased $Ca^{2+}$ in the roots of both cultivars of maize. Both cultivars did not differ significantly in root $Ca^{2+}$. Slightly higher values of $Ca^{2+}$ were observed in cv. Agaiti 2002 as compared to cv. EV-1098 under both stress and non-stress conditions. Different concentrations of exogenously applied proline significantly increased root $Ca^{2+}$ in both maize cultivars and this increase in root $Ca^{2+}$ was more when it was applied @ 30 m*M* (Fig. 1).

Imposition of water stress significantly reduced N contents in the shoots and roots of both maize cultivars. A significant effect of foliar applied proline on shoot N contents was observed under stress and non-stress conditions (Fig. 2). A maximum increase in N contents was observed when it was applied @ 30 m*M* under stress and non-stress conditions in both maize cultivars.

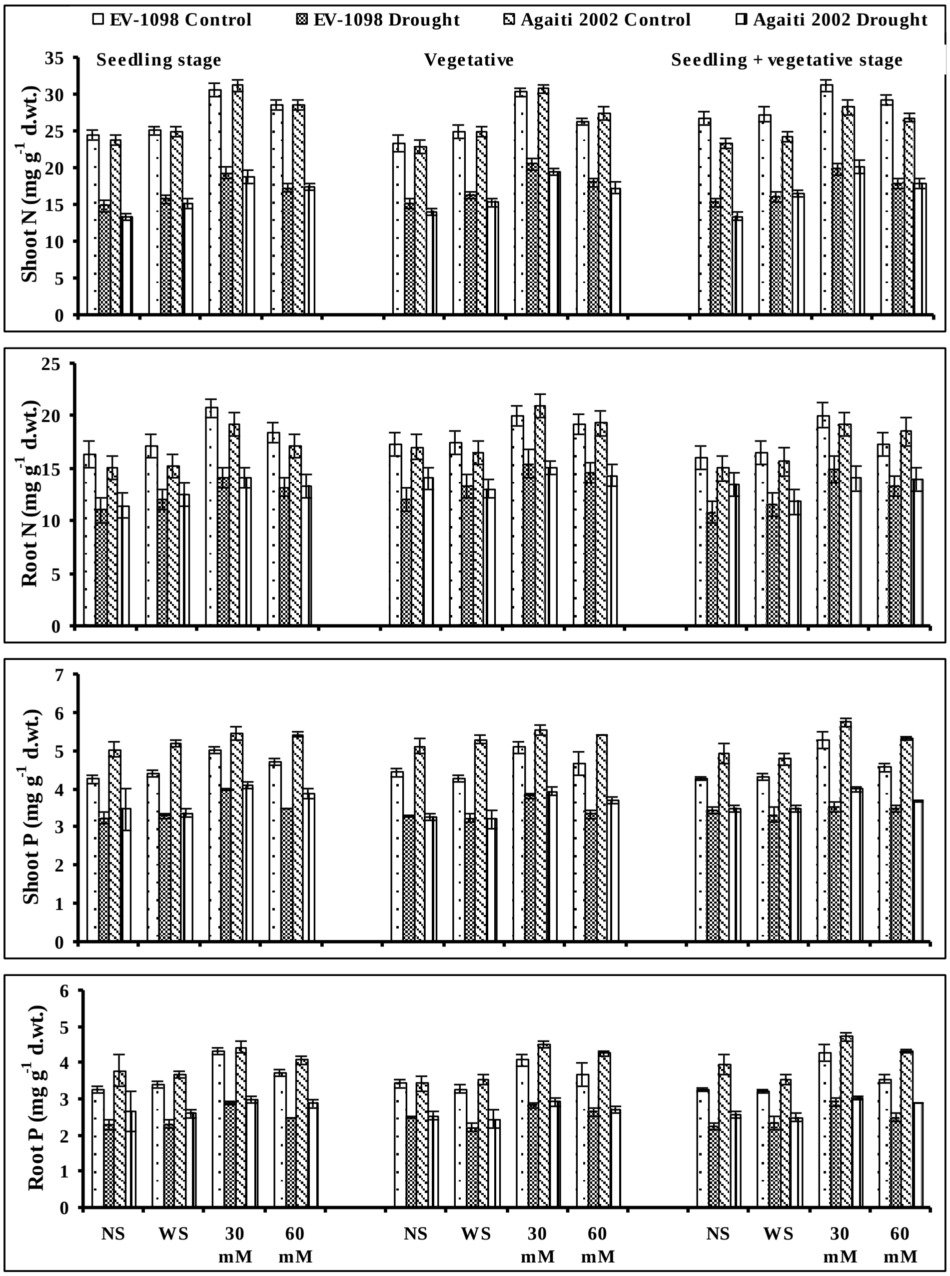
Shoot and root phosphorus was decreased significantly with the imposition of water stress. Significantly higher values of P were observed in the shoots of cv. Agaiti 2002 than those in cv. EV-1098 both under stress and non-stress conditions. Foliar applied proline at all growth stages under stress or normal conditions significantly increased shoot P contents and the maximum increase was observed when it was applied @ 30 m*M* (Fig. 1).

The increasing or decreasing trend of $Mg^{2+}$ concentration in the shoots and roots due to the imposition of water stress was not obvious in both maize cultivars. Both cultivars differed significantly in shoot or root $Mg^{2+}$ concentration (Fig. 3). Slightly higher concentrations of $Mg^{2+}$ were observed in the shoots and roots of cv. Agaiti 2002 as compared to those in EV-1098. Exogenous application of different concentrations of proline at different growth stages had a slight increasing effect on $Mg^{2+}$ concentration in shoots and roots under normal and water stress conditions. The maximum increase was observed when proline was applied @30 m*M*.

## Discussion

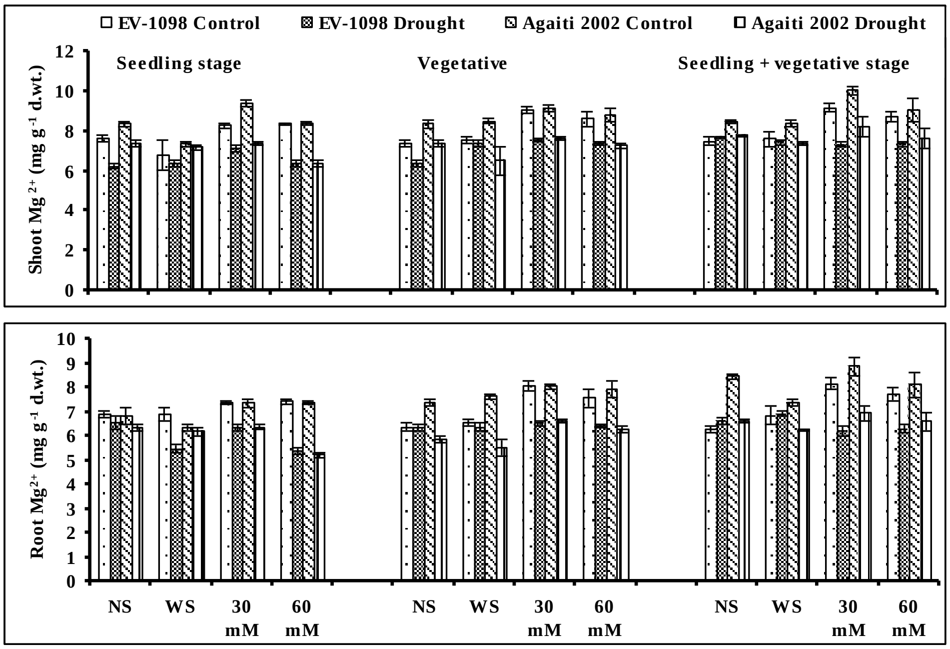
Drought induced osmotic stress triggers a wide range of adverse effects in plants ranging from poor growth, imbalanced ion transport (Lutts *et al.*, 1996; Bajji *et al*., 2000), considerable decrease in transpiration rate and impaired membrane permeability (Levit, 1980) resulting in a reduced absorbing capacity of crop plants (Alam, 1999). However, plant species and genotypes within a species vary in their response to mineral uptake under water stress (Garg, 2003).

Plants accumulate different types of organic and inorganic solutes in the cytosol to lower osmotic potential thereby maintaining cell turgor (Rhodes & Samaras, 1994). Of these solutes, proline is the most widely studied because of its considerable importance in the stress tolerance (Delauney & Verma, 1993) There are a number of reports that exogenous application of proline increases its endogenous levels in plant tissues subjected to water stress conditions (Ali *et al*., 2007; Ashraf & Foolad, 2007; Haque *et al*., 2007) which contribute to osmotic adjustment in plant tissues (Bajji *et al*., 2000). Thus, exogenous application of proline may be an efficient means of ameliorating the adverse effects of water stress on plants as has been observed in the present study. However, the effectiveness of proline applied as a foliar spray in triggering growth depends on the type of species, plant developmental stage, time of application and concentration (Ashraf & Foolad, 2007). In our previous studies on maize (Ali *et al*., 2007) it was reported that exogenous application of different concentrations of proline had an ameliorating effect on different photosynthetic parameters including transpiration rate and stomatal conductance and maximum increase was observed at 30 m*M* proline




NS = Non spray; WS = Water spray; 30, 60 m*M* = Proline spray with 30 and 60 m*M*

Fig. 2. N and P concentrations (mg $g^{-1}$ d. wt.) in shoots and roots of two maize cultivars as influenced by the exogenous application of different concentrations of proline at different growth stages under controlled and water stress conditions. ($n = 4 \pm$ S.E)




NS = Non spray; WS = Water spray; 30,60 m*M* = Proline spray with 30 and 60 m*M*

Fig. 3. $Mg^{2+}$ concentration (mg $g^{-1}$ d. wt.) in shoots and roots of two maize cultivars as influenced by the exogenous application of different concentrations of proline at different growth stages under controlled and water stress conditions. ($n$ = 4 ± S.E)

concentration. In the present study, the results for mineral nutrients (N, P, $K^+$, $Ca^{2+}$ and $Mg^{2+}$) analyzed in the present study clearly indicate that water stress decreased all four nutrients in the shoots and roots of both maize cultivars, which may have been due to decrease in transpiration rate and stomatal conductance (Pessarakli, 1999). However, exogenously applied different concentrations of proline at different growth stages enhanced the accumulation of all these macronutrients under water stress conditions and 30 m*M* proline concentration was found to be more effective as compared to the other concentrations as this concentration has already been found more effective in increasing transpiration rate in our previous studies (Ali *et al*., 2007). While describing the role of compatible solutes, Cuin & Shabalah (2007) reported that solutes like glycinebetaine, proline, mannitol, trehalose or inositol significantly reduced $K^+$ efflux from the cell and maintains cytosolic $K^+$ homeostasis possibly through the enhanced activity of $H^+$-ATPase. This in turn controls voltage-dependent outward-rectifying $K^+$ channels and created the electrochemical gradient necessary for secondary ion transport processes (Cuin & Shabala, 2005).

Water and different nutrients exist together in plant tissues in close association, because nutrient ions are dissolved in the soil solution and nutrient uptake by plants depends on water flow through the soil-root-shoot continuum (Keller, 2005). Leaf transpiration creates the tension necessary for the roots to absorb the soil solution containing essential nutrients (Keller, 2005). In grasses, increase in the shoot N under water stress conditions was found to be due to the increased accumulation of proline occurring due to increased transpiration and stomatal aperture (Singh *et al*., 1973; Tanguiling, 1987). Similarly, there was a substantial increase in leaf P and N in tomato with increase in transpiration after release from water stress, however all these reports support the results of the present study.

The results of the present study would suggest that foliar application of proline was effective in ameliorating the adverse effects of water stress on both maize cultivars by promoting the uptake and accumulation of essential nutrients such as N, P and $K^+$ and 30 m*M* concentration of proline was found to be more effective as compared to the other concentrations tested in the present study.

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### References

Alam, S.N. 1999. Nutrient uptake by plants under stress conditions. In: *Handbook of Plant and Water Stress*. Pessarakli, M. 2nd Edition. University of Arizona. Tucsan Arizona. U.S.A.

Ali, Q., M. Ashraf and H.R. Athar. 2007. Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. *Pak. J. Bot.*, 39(4): 1133-1144.

Allen, S.E., H.M. Grimshaw and H.P. Rowland. 1986. Chemical analysis. In: *Methods in Plant ecology*. (Ed.): P.D. Moore and S.P. Chapman. Oxford Blackwell Scientific Publications. pp. 285-334.

Ashraf, M. and M.R. Foolad. 2007. Roles of glycinebetaine and proline in improving plant abiotic stress tolerance. *Env. Exp. Bot*., 59: 206-216.

Bajji, M., S. Lutts, J.M. Kinet. 2000. Physiological changes after exposure to and recovery from polyethylene glycol-induced water deficit in callus cultures issued from durum wheat (*Triticum durum* Desf.) cultivars differing in drought resistance. *J. Plant Physiol*., 156: 75-83.

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

Claussen, W. 2005. Proline as a measure of stress in tomato plants. *Plant Sci.* 168:241-246

Cuin, T.A. and S. Shabala. 2005. Exogenously supplied compatible solutes rapidly ameliorate NaCl-induced potassium efflux from barley roots. *Plant Cell Physiol*., 46:1924-33.

Cuin, T.A. and S. Shabala. 2007. Compatible solutes reduce ROS-induced potassium efflux in Arabidopsis roots. *Plant Cell Environ.*, 30 (7): 875-85

Delauney, A.J. and D.P.S. Verma. 1993. Proline biosynthesis and osmoregulation in plants. *Plant J*., 4: 215-223.

Garg, B.K. 2003. Nutrient uptake and management under drought: nutrient-moisture interaction. *Curr. Agric*., 27(1/2): 1-8

Garg, B.K., U. Burman and S. Kathju. 2004. The influence of phosphorus nutrition on the physiological response of moth bean genotypes to drought. *J. Plant Nutr. Soil Sci*., 167: 503-508.

Hoque, M.A., E. Okuma, M.N.A. Banu, Y. Nakamura, Y. Shimoishi and N. Murata. 2007. Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidants enzyme activity. *J. Plant Physiol*., 164: 553-561.

Keller, M. 2005. Deficit irrigation and vine mineral nutrition. *Am. J. Enol. Vitic*., 56(3):267-283.

Khedr, A.H.A., M.A. Abbas, A.A.A. Wahid, W.P. Qauick and G.M. Abugadallah. 2003. proline induces the expression of salt stress responsive proteins and may improve the adaptation of *Pancratium maritimum* L., to alt stress. *J. Exp. Bot.,* 54: 2553-62.

Lahaye, P.A and E. Epstein. 1971. Calcium and salt tolerance in bean plants. *Plant Physiol*., 25:213.

Levitt, J. 1980. *Responses of plants to environmental stresses*. 2nd Ed. New York. Academic Press.

Lutts, S., J.M. Kinet and J. Bouharmont. 1996. Effects of various salts and of mannitol on ion and proline accumulation in relation to osmotic adjustment in rice (O*ryza sativa L*.) callus cultures. *J. Plant Physiol*., 149: 186-195.

Marschner, H. 1995. *Mineral Nutrition of Higher Plants*. 2nd Ed. Acad. Press, London.

McCue, K.F. and A.D. Hanson. 1990. Drought and salt tolerance: towards understanding and application. *Trends Biotechnol.*, 8: 358-362.

Okuma, E., K. Soeda, M. Tada and Y. Murata. 2000. Exogenous proline mitigates the inhibition of growth of Nicotiana tobacum cultured cells under saline conditions. *Siol Sci*. *Plant Nutr*., 46: 257-63.

Pessarakli, M. 1999. *Handbook of Plant and Crop Stress*. 2nd Edition. University of Arizona. Tucson Arizona. U.S.A

Rhodes, D. and Y. Samaras. 1994. Genetic control of osmoregulation in plants. In *cellular and molecular physiology of cell volume regulation*. Strange, K. Boca Raton: CRC Press. pp. 347-361.

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.

Samaras, Y., R.A. Bressan, L.N. Csonka, M.G. Garci a-Rios, D. Paino, M. Urzo and D.Rhodes. 1995. Proline accumulation during drought and salinity, In: *Environment and Plant metabolism*. (Ed.): N. Smirnoff. *Bios Scientific publishers, oxford*, pp. 161-187.

Singh, T.N., L.G. Paleg and D. Aspinall. 1973. Stress metabolism. 1. Nitrogen betabolism and growth in barley plants during water stress. *Aust. J. Biol. Sci.*, 26: 45.

Snedecor, G.W. and Cochran. 1980. S*tatistical methods*.7th Edition. Iowa State University Press, Ames, IOWA.

Tanguiling, V.C., E.B. Yambao, J.C.O. Toole and S.K. De Datta. 1987. Water stress effects on leaf elongation, leaf water potential, transpiration, and nutrient uptake of rice, maize and soybean. *Plant Soil*, 103: 155.

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

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