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EXOGENOUSLY APPLIED PROLINE AT DIFFERENT GROWTH STAGES ENHANCES GROWTH OF TWO MAIZE CULTIVARS GROWN UNDER WATER DEFICIT CONDITIONS

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Abstract

Exogenous application of potential osmoprotectants such as proline is an important shotgun approach to alleviate adverse effects of abiotic stresses on plants. However, information about the effects of exogenously applied proline in counteracting the adverse effects of water stress on crops is scanty. An experiment was therefore conducted to assess the ameliorative effect of exogenously applied proline on growth and photosynthetic capacity of two maize cultivars grown under water deficit conditions. Four-week old plants of 2 maize cultivars, viz., EV-1098 and AGAITI 2002 were subjected to water stress by maintaining moisture content equivalent to 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 growth and photosynthetic capacity of both maize cultivars. However, exogenous application of proline counteracted the adverse effects of water stress on growth of both maize cultivars. Although proline induced improvement in growth of water stressed maize plants was almost similar at all growth stages, application of 30 mM proline proved to be more effective in inducing water stress tolerance as compared to the other level. Photosynthetic rate of water stressed plants of both maize cultivars was also enhanced due to foliar applied proline which was positively associated with sub-stomatal CO_2 (C_i,) and stomatal conductance (g_s) as well as photosynthetic pigments. Overall, foliar applied proline ameliorated the adverse effects of water stress on growth and photosynthetic capacity of two maize cultivars.

Introduction

Of various abiotic stresses known in nature, drought stress poses a major threat to crop production because water is essential at every stage of plant growth from seed germination to plant maturation (Chaves *et al.*, 2003; Athar & Ashraf, 2005), so any degree of water imbalance may produce deleterious effects on crop growth, but it depends upon the nature of crop species (El-Far & Allan, 1995). Keeping in view the considerable demand for food, crop improvement for drought stress tolerance is of prime importance. However, understanding about the biochemical and physiological basis of water stress tolerance in plants is vital to select and breed plants for improving crop water stress tolerance (Boyer, 1982; Chaves *et al*., 2003). Long ago, Turner (1979) described some mechanisms of water stress tolerance in plants such as drought escape, avoidance and tolerance to low water potential. However, in fact, all these plant strategies depend on certain specific plant adaptations to water deficit conditions (Turner, 1979; 1982; Chaves *et al*., 2003). In view of Serraj & Sinclair (2002) osmotic adjustment is one of the major physiological phenomena vital for sustaining growth of plants under osmotic stress.

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It has been widely reported that plants accumulate a variety of compatible solutes such as proline and betaine, as an adaptive mechanism of tolerance to salinity and drought (Rhodes & Hanson, 1993; Hasegawa *et al*., 2000; Ashraf & Harris, 2004; Ashraf & Foolad, 2007). These compatible solutes protect and stabilize 3D structure of proteins and photosynthetic apparatus (Papageorgiou & Murata, 1995), regulate cellular osmotic adjustment (Wyne Jones *et al*., 1977; Subbarao *et al*., 2001) and detoxify reactive oxygen species (ROS) (Bohnert & Jensen, 1996; Ashraf & Foolad, 2007) in response to abiotic stresses. Upon relief from stress these solutes are metabolized and are considered as an important energy source for recovery from stress (Hare & Cress, 1997). Although much attention has been paid on the role of proline in stress tolerance as a compatible osmolyte (Csonka, 1981; Yancey *et al*., 1982; Le-Rudulier *et al*., 1984; MacCue & Hanson, 1990; Samaras *et al*., 1995), little attention has been given on its role in other biochemical and physiological processes responsible for stress tolerance in plants (Nanjo *et al*., 1999; Okuma *et al*., 2000; Khedr *et al*., 2003).

Although it is evident from different reports that exogenous application of proline induces abiotic stress tolerance in plants, there are some reports that reveal that high concentrations of proline may be harmful to plants, including inhibitory effects on growth or deleterious effects on cellular metabolisms (Nanjo *et al*., 2003). The available information from different studies suggests that optimal concentrations of proline may be species or genotype dependent, which needs to be determined before recommending its commercial use as to improve stress tolerance of a particular crop. Thus, in view of the above mentioned reports, it was hypothesized that influence of varying levels of exogenously applied proline may vary in alleviating the inhibitory effects of drought on growth of maize, the most important cereal after wheat and rice. Since there are some reports that water stress tolerance of maize (*Zea mays* L) varies with the change in developmental stages (Westgate, 1994), it is necessary to determine the appropriate growth stage at which exogenous application of proline may be the most effective in promoting growth under stressful environments. Thus, the present study was aimed 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 maize.

Material and Methods

In the present investigation, exogenous application of proline was used to minimize the crop yield losses caused by water stress. Hence, the present study provides an important information on physiological and biochemical roles of exogenously applied proline in drought tolerance of maize. 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 an average 10/14 h light/dark period at 600-900 µmol m^{-2} s^{-1} PPFD, a day/night temperature cycle of 37/25°C and the relative humidity 65±5% during the year 2006 and 2007. The experiment was laid out in a completely randomized design in a factorial arrangement with four replications of each experimental unit. The experiment comprised two maize cultivars, EV-1098 and AGAITI 2002, two water regimes (control and water stressed at 60% field capacity), four foliar spray of proline (no spray, water spray, 30 and 60 mM proline in 0.1% Tween 20 solution) and four replications per treatment. Equal weight plastic pots were taken and filled with equal weights of sandy clay loam soil. These pots were then divided into two groups of each representing a specific water stress treatment. 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 cultivars were hand sown. Two weeks after emergence, plants were thinned to 5 plants per pot.

Analysis of soil used in the experiment was carried out in the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad by using hygrometer method (Dewis & Freitas, 1970) and the soil on percentage basis was comprised of 60% sand, 30% clay and 10% silt. The soil on textural basis 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, total nitrogen content 0.73%, soluble Cl^- 8.52 meq L^- and soluble Ca^{2+}+ Mg^{2+}, 14.30 meq L^{-1}.

Water stress treatments (field capacity (control) and 60% field capacity) were started four weeks after plant emergence. 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 by adding normal irrigation water if required on daily basis till the maturation of the crop. Proline (no spray, 0 or water spray [0.1% Tween-20 solution], 30 and 60 mM proline in 0.1% Tween 20 solution) were applied as a foliar spray at the seedling, vegetative and seedling + vegetative stages. 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 65°C for 72 h after which dry weights were recorded.

Chlorophyll contents: The chlorophyll ‘a’ and ‘b’ were determined according to the method of Arnon (1949). Fresh leaves (0.1 g) were cut and extracted over night with 80% acetone at 0-4°C. The extracts were centrifuged at 10,000 x *g* for 5 minutes. Absorbance of the supernatant was read at 645, 663 and 480 nm using a spectrophotometer (Hitachi-U2001, Tokyo, Japan).

Gas exchange parameters: Measurements of gas exchange attributes were made on the 2nd intact leaf from top of each plant using an ADC LCA-4 portable infrared gas analyzer (Analytical Development, Hoddesdon, UK). These measurements were made from 10.30 a.m. to 12.30 p.m. with the following specifications/adjustments: leaf surface area, 11.25 cm^2; ambient temperature, 45±3°C; ambient CO_2 concentration, 352 μmol mol^{-1}; temperature of leaf chamber varied from 37.2 to 47.2°C; leaf chamber gas flow rate (U), 251 μmol s^{-1}; molar flow of air per unit leaf area (Us) 221.06 mol m^{-2} s^{-1}; RH of the chamber ranged from 25.4 to 41.2 %; *PAR* (Q_{leaf}) at leaf surface during noon was maximum up to 918 μmol m^{-2} s^{-1}, ambient pressure 98.8 kPa.

Statistical analysis of data: The data 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

Imposition of water stress reduced shoot fresh and dry weights of both maize cultivars (Fig. 1a, 1b). However, exogenous application of proline as a foliar spray at different growth stages improved the shoot fresh and dry weights of both maize cultivars. Foliar spray with 30 or 60 m*M* proline at all growth stages enhanced the shoot fresh and dry weights of both cultivars under non-stress or water stress conditions. However, in non-stressed plants of Agaiti-2002 foliar spray with 30 m*M* proline caused a maximum increase in shoot fresh and dry weights.

Root fresh and dry masses of both maize cultivars decreased significantly due to the imposition of water stress (Fig. 1c, 1d). However, water stress-induced reduction in root fresh and dry biomass was more in cv. EV-1098 than that in Agaiti-2002. Foliar application of proline increased the root biomass of non-stressed or water stressed plants of both cultivars, but this increasing effect of exogenous proline application was very prominent under non-stressed conditions. Furthermore, 30 m*M* proline was more effective in counteracting the adverse effects of water stress in both maize cultivars.

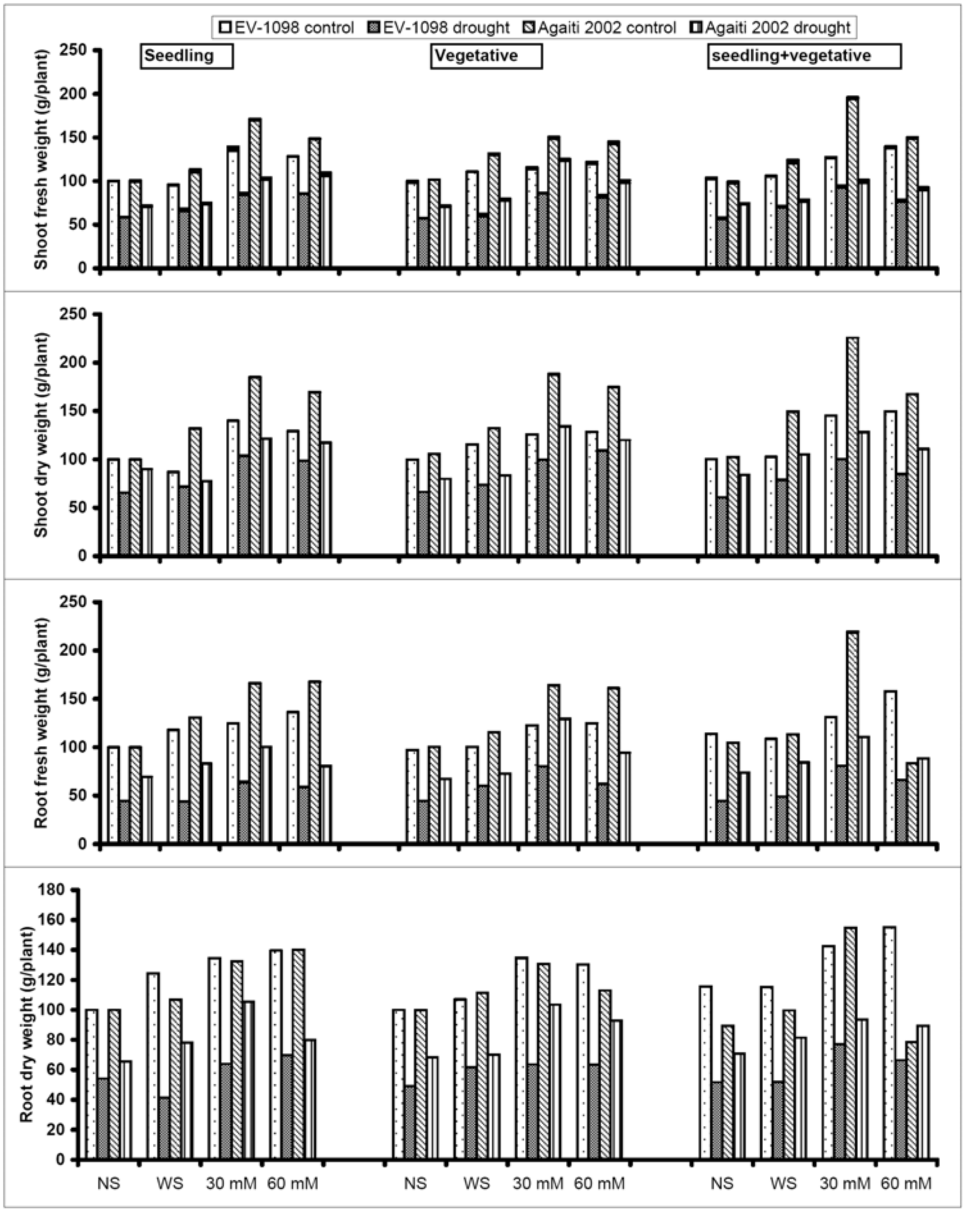
Water stress caused a significant reduction in photosynthetic rate of both cultivars (Fig. 2a). Both maize cultivars did not differ significantly in photosynthetic rate under non-stress or water stress conditions. Although exogenous application of both levels of proline at all growth stages significantly enhanced the photosynthetic rate of both cultivars under control or water stress conditions, 30 m*M* proline proved to be more effective in improving photosynthetic rate in both cultivars.

Transpiration rate of both cultivars was markedly suppressed due to water stress (Fig. 2b). Both cultivars did not differ in transpiration rate under water stress conditions. However, externally applied proline increased the transpiration rate of water stressed plants of both cultivars. Furthermore, proline-induced enhancement in transpiration rate was minimal in water stressed plants of EV-1098 when 60 mM proline applied at the seedling and vegetative stages.

A marked reduction in stomatal conductance in both maize cultivars was observed due to water stress (Fig. 2c). Both cultivars did not differ in this gas exchange attribute. Although the foliar application of proline significantly improved stomatal conductance at all growth stages under stressed and non-stressed conditions, 30 m*M* proline applied at the seedling and vegetative stages was more effective in enhancing stomatal conductance in water stressed plants of both cultivars. Imposition of water stress significantly ($p<0.001$) reduced the internal CO_2 concentration of both maize cultivars at all growth stages (Fig. 2d). However, foliary applied proline significantly enhanced the internal CO_2 concentration in both maize cultivars.

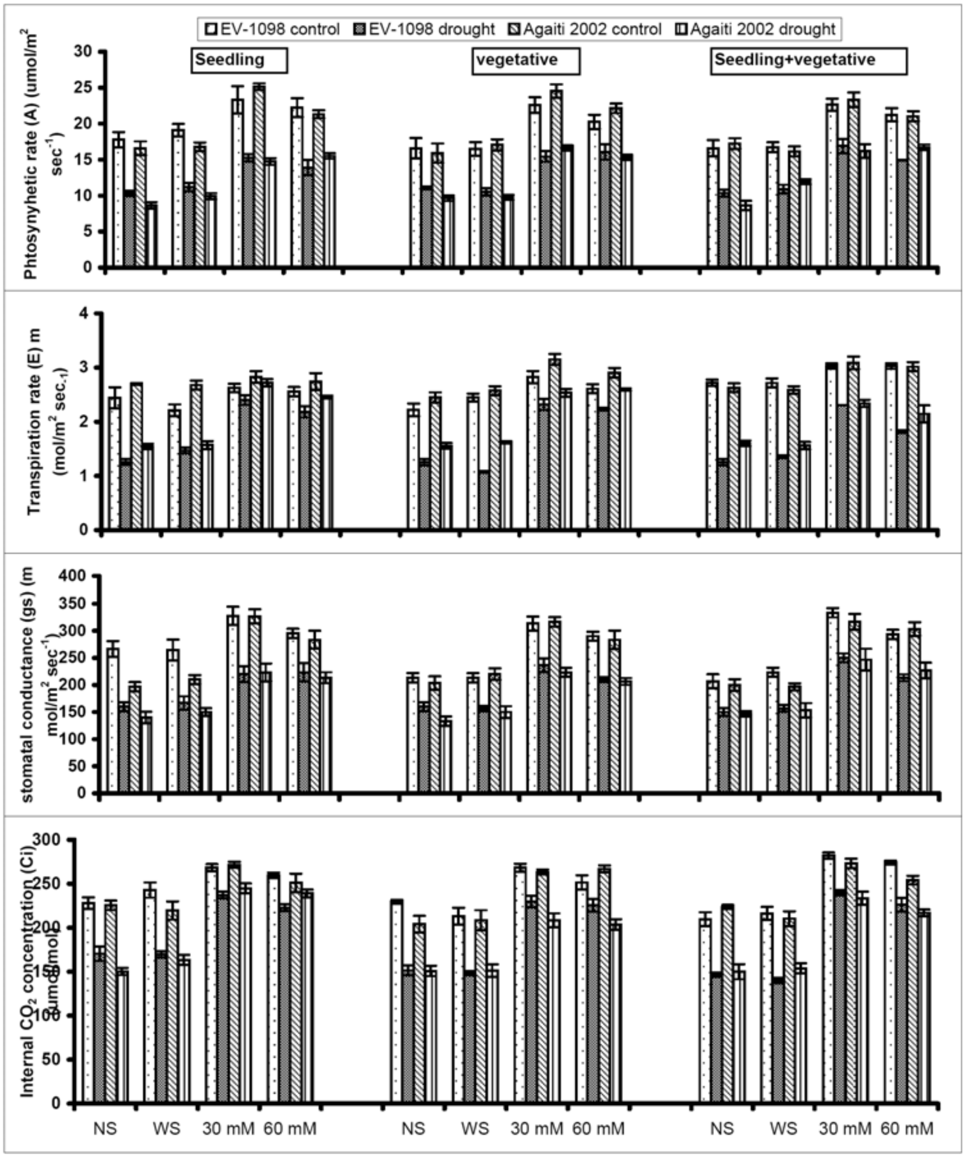
Although water use efficiency of both maize cultivars (Fig. 3a) was significantly reduced due to water stress, foliary applied proline at the seedling stage improved WUE in water stressed plants of EV-1098 only. Water stress reduced the C_i/C_a ratio in both cultivars, but exogenous application of proline significantly improved C_i/C_a ratio under both normal and water stressed conditions (Fig. 3b).

Imposition of water stress significantly reduced the chlorophyll '*a*', '*b*' and total chlorophyll contents in the leaves of both cultivars. However, exogenous application of proline improved chlorophyll '*a*', '*b*' and total chlorophyll contents in water stressed plants of both cultivars.



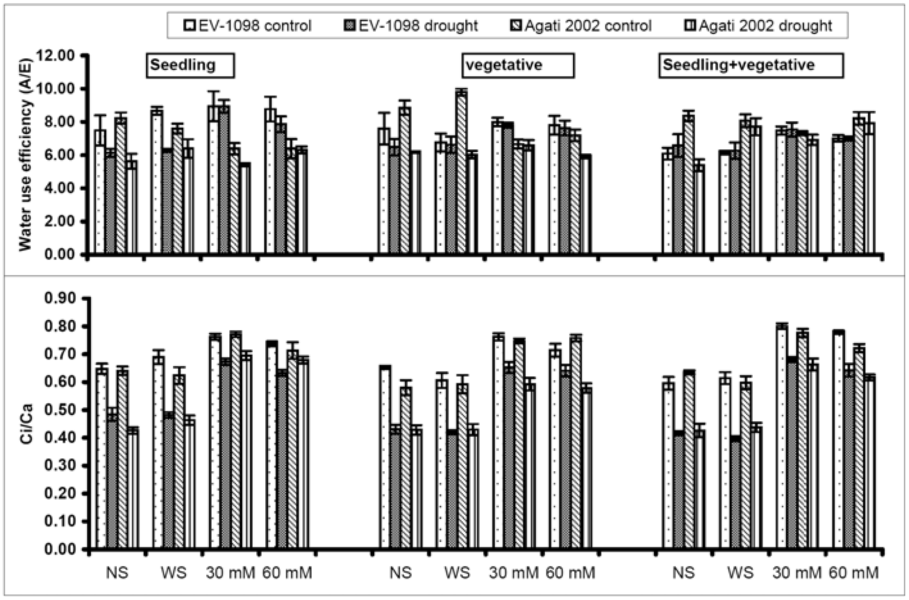
NS = Non spray; WS = Water spray; 30, 60 m*M* = Proline spary with 30 and 60 m*M*

Fig. 1. Shoots and roots fresh and dry weights (g/plant) of two maize cultivars as influenced by exogenous application of different concentrations of proline at different growth stages under controlled and water stressed conditions.



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

Fig. 2. Photosynthetic attributes of two maize cultivars as influenced by exogenous application of different concentrations of proline at different growth stages under controlled and water stressed conditions.

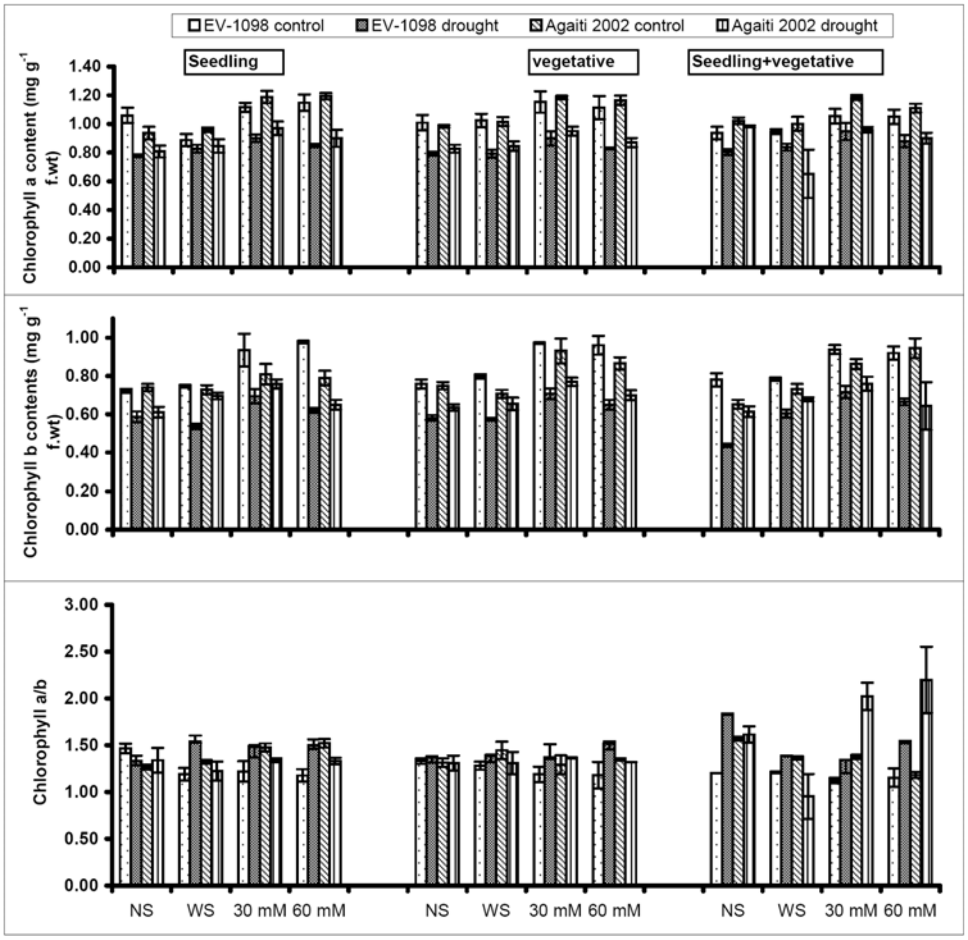


NS = Non spray; WS = Water spray; 30, 60 m*M* = Proline spary with 30 and 60 m*M*

Fig. 3. Water use efficiency and C_i/C_a ratio of two maize cultivars as influenced by exogenous application of different concentrations of proline at different growth stages under controlled and water stressed conditions.

Discussion

In the present study, water stress caused a significant reduction in growth of both maize cultivars. However, exogenous application of proline counteracted the adverse effects of low water availability on the growth of both maize cultivars. These findings of the present study are similar to some earlier studies in which it has been shown that exogenous application of proline alleviates the adverse effects of water stress on the growth and/or yield of different crops e.g., rice (Kavi-Kishore *et al*., 1995) and halophyte *Allenrolfea occidentalis* (Chrominski *et al*., 1989). In view of some earlier reports it is suggested that exogenously applied might have caused enhanced endogenous proline accumulation under water stress conditions which not only protects enzymes, 3D structures of proteins and organelle membranes, but it also supplies energy for growth and survival thereby helping the plant to tolerate stress (Chandrashekar *et al*., 1996; Hoque *et al*., 2007; Ashraf & Foolad, 2007). Thus, exogenous application of proline may be an efficient approach to ameliorate the adverse effects of water stress as has been observed in the present study. However, effectiveness of proline applied as a foliar spray depends on the type of species, plant developmental stage, time of application and concentration (Ashraf & Foolad, 2007). For example, in this experiment, improvement in growth of both maize cultivars was much evident at 30 m*M* proline under water stress conditions. Likewise, in rice, exogenous application of 30 m*M* proline proved to be beneficial when applied at the seedling stage (Roy *et al*., 1993), 20-30 m*M* was effective in mung bean (*Vigna radiate*) cell cultures for the mitigation of adverse effects of stress and 10 m*M* was effective for tobacco suspension cells under stress (Okuma *et al*., 2000). Thus, the response of different levels of externally applied proline under adverse environmental conditions is species specific (Ashraf & Foolad, 2007).



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

Fig. 4. Chlorophyll '*a*' and '*b*' contents and chlorophyll *a*/*b* of two maize cultivars as influenced by exogenous application of different concentrations of proline at different growth stages under controlled and water stressed conditions.

Water-deficit conditions significantly reduces considerably photosynthetic capacity of plants by stomatal closure or through metabolic impairments such as damaging proteins associated with PSII and PSI and chlorophyll (Lawlor & Cornic, 2002; Athar & Ashraf, 2005). In this study, water stress-induced reduction in photosynthesis was ameliorated in both maize cultivars by exogenous application of proline. Furthermore, foliar application of proline to water stressed plants of both maize cultivars caused an increase in stomatal conductance and sub-stomatal CO_2 with an increase in net CO_2 assimilation rate. These results suggest that the increase in photosynthesis was primarily due to increase in stomatal conductance which caused higher CO_2 diffusion inside the leaf thus favoring higher photosynthetic rate (Sharkey *et al*., 2007). Thus, foliar applied proline enhanced the photosynthetic capacity of both maize cultivars under water stress conditions. There are number of reports which show that either stomatal or metabolic impairment is a major limitation to photosynthesis (Chaves, 1991; Lawlor, 1995; Cornic & Massacci, 1996; Lawlor & Cornic, 2002; Chaves *et al*., 2003). However, recently a consensus has been developed that diffusion of CO_2 due to stomatal closure is a predominating factor in reducing photosynthesis under water-stress situations, although under severe water stress conditions metabolic limitations occur (Flexas *et al*., 2004; Athar & Ashraf, 2005; Ennahli & Earl, 2005). In view of these reports and the results from the present study, it is suggested that photosynthetic capacity of water stressed plant can be enhanced by reducing stomtal limitations, particularly in a situation where stomatal limitation is a predominating limiting factor for photosynthesis.

Exogenously applied proline caused relative more enhancement in *A* than in stomatal conductance or transpiration rate in water stressed maize cultivars, which resulted in higher water use efficiency. These results indicate that foliar applied proline caused adjustment in maize plants between carbon uptake and water loss through transpiration as has earlier been suggested by Raven (2002).

Drought induced reduction in photosynthesis can also be attributable to decrease in chlorophyll content (Athar & Ashraf, 2005; Baker *et al*., 2007). In the present study, photosynthetic pigments like chlorophyll 'a' and 'b' decreased in both maize cultivars due to water stress, which is in agreement with some previous studies on different crops e.g., *Vicia faba* (Gadallah, 1999), wheat (Waseem *et al*., 2006), canola (Kauser *et al*., 2006), maize (Ashraf *et al*., 2007). However, application of proline increased the photosynthetic pigments in both maize cultivars under water stress conditions. Furthermore, in the present study, a close association between proline-induced increase in photosynthetic pigments and photosynthetic rate and growth of both maize cultivars under water stress conditions has been observed. A similar relationship between growth or net CO_2 assimilation rate and photosynthetic pigments has already been observed in different crop species under different abiotic stresses e.g., in maize under waterlogged conditions (Ashraf & Rehman, 1999), wheat under saline conditions (Raza *et al*., 2006), canola under water stress conditions (Kauser *et al*., 2006) and some trees under hypoxic conditions (Kozlowski, 1982). Similarly, a positive association between photosynthetic rate and growth has also been found which is in agreement with some earlier studies e.g., in cotton (Faver *et al*., 1997), maize (Shuting *et al*., 1997) and wheat (Raza *et al*., 2006; Arfan *et al*., 2007). Thus, foliar application of proline enhanced growth of water stressed maize plants by enhancing photosynthetic capacity which support the arguments made by Nátr & Lawlor (2005) that different situations under different scenarios can be tried to enhance the final biological or economical yield by increasing the rate of photosynthesis.
By summarizing all the results, it is clear that foliar application of proline was effective in ameliorating the adverse effects of water stress on growth of both maize cultivars. Moreover, beneficial effect of proline applied as a foliar spray was due to its promotive effects on photosynthetic capacity by overcoming stomatal limitations, enhancing biosynthesis of photosynthetic pigments, or protecting photosynthetic pigments from water stress-induced degradation.

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References

Arfan, M., H.R. Athar and M. Ashraf. 2007. Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress, *J. Plant Physiol*., 164:685-694.

Arnon, D.T. 1949. Copper enzyme in isolated chloroplasts, polyphenaloxidase in *Beta vulgaris*. *Plant Physiol*. 24: 1-15.

Ashraf, M. and H. Rehman. 1999. Interactive effects of nitrate and long-term water logging on growth, water relations and exchange properties of maize (*Zea mays* L). *Plant Sci*., 144: 35-43.

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

Ashraf, M., S. Nawazish and H.R. Athar. 2007. Are chlorophyll fluorescence and photosynthetic capacity, potential physiological determinants of drought tolerance in maize (*Zea mays* L.) *Pak. J. Bot*., 39 (4): In Press.

Ashraf, M. and P.J.C. Harris. 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci*., 166: 3-16.

Athar, H.R. and M. Ashraf. 2005. Photosynthesis under drought stress. In: *Handbook of Photosynthesis*. (Ed.): M. Pessarakli, CRC Press, Taylor and Francis Group, NY, pp 793-804.

Baker, N.R., J. Harbinson and D.M. Kramer. 2007. Determining the limitations and regulation of photosynthetic energy transduction in leaves. *Plant Cell Environ*., 30: 1107-1125.

Bohnert, H.J. and R.G. Jensen. 1996. Strategies for engineering water stress tolerance in plants. *Tren. Biotech*., 14: 89-97

Boyer, J.S. 1982. Plant productivity and environment potential for increasing crop plant productivity, genotypic selection. *Science*, 218: 443-448.

Chandrashekar, K.R. and S. Sandhyarani. 1996. Salinity induced chemical changes in *Crotalaria striata* Dc. Plants. *Indian J. Plant Physiol*., 1: 44-48.

Chaves, M.M, J.P. Maroco and J.S. Pereira. 2003. Understanding plant responses to drought - from genes to the whole plant. *Funct. Plant Biol*., 30: 239-264.

Chaves, M.M. 1991. Effects of water deficits on carbon assimilation. *J. Exp. Bot*., 42: 1-16.

Chrominski, A., S. Halls, D.J. Weber and B.N. Smith. 1989. Proline affectsACCto ethylene conversion under salt andwater stresses in the halophyte *Allenrolfea occidentalis*. *Environ. Exp. Bot*., 29: 359-363.

Cornic, G. and A. Massacci. 1996. Leaf photosynthesis under drought stress. In: NR. Baker. *Photosynthesis and the Environment*. (Ed.): N.R. Baker. Dordrecht, the Netherlands: Kluwer Academic, 347-366.

Csonka, L.N. 1981. Proline over-production results in enhanced osmoregulation in *Salmonella typhimurium*. *Mol Gen Gent*., 182: 82-86.

Dewis, J. and F. Freitas. 1970. *Physical methods of soil and water analysis*. FAO Soil Bull. No. 10, Rome, pp. 39-51.

El-Far, I.A. and A.Y. Allan. 1995. Responses of some wheat cultivars to sowing methods and drought at different stages of growth. *Assuit J. Agric. Sci*., 26(1): Pp. 267-277.

Ennahli, S. and H.J. Earl. 2005. Physiological limitations to photosynthetic carbon assimilation in cotton under water stress. *Crop Sci*., 45: 2374-2382.

Faver, K.L., T.J. Gerik and R.G. Percy. 1997. Modern Pima cotton cultivars have higher photosynthetic capacity than obsolete varieties. *The Proceedings of Beltwide Cotton Conference, New Orleans*. LA. USA, 2: 1499-I502.

Flexas, J., J. Bota and J. Cifre. 2004. Understanding down regulation of photosynthesis under water stress, future prospects and searching for physiological tools for irrigation management. *Ann. Appl. Biol*., 144: 273-283.

Gadallah, M.M.A. 1999. Effects of proline and glycine betaine on *Vicia faba* responses to salt stress. *Biol. Plant*., 42: 249-257.

Hare, P.D. and W.A. Cress. 1997. Metabolic implications of stress induced proline accumulation in plants. *Plants Growth Regul*., 21: 79-102.

Hasegawa, P.M., R.A. Bressan, J.K. Zhu and H.J. Bohnert. 2000. Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., 51: 463-499.

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 antioxidant enzyme activities. *J. Plant Physiol*., 164: 553-561

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.

Kavi-Kishor, P.B., Z. Hong, G.H. Miao, C.A.A. Hu and D.P.S. Verma. 1995. Overexpression of D1-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. *Plant Physiol*., 108: 1387-1394.

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

Kozlowski, T.T. 1982. Water supply and tree growth. II. Flooding, *Forest. Abst*., 43: 145-161.

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.

Lawlor, D.W. 1995. The effects of water deficit on photosynthesis. In: *Environment and Plant Metabolism Flexibility* and *Acclimation*. (Ed.): N. Smirnoff. BIOS, Oxford, pp. 129-160.

Lawlor, D.W. 2002. Limitation to photosynthesis in water stressed leaves: stomata versus metabolism and the role of ATP. *Ann. Bot*., 89: 1-15.

Le-Rudulier, D., A.R. Strom, A.M. Dandekar, L.T. Smith and R.C. Valentine.1984. Molecular biology of osmoregulation. *Science*, 224: 1064-8.

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

Nanjo, T., M. Fujita, M. Seki, M. Kato, S. Tabata and K. Shinozaki. 2003. Toxicity of free proline revealed in an *Arabidopsis* TDNA- tagged mutant deficient in proline dehydrogenase. *Plant Cell Physiol*., 44: 541-548.

Nanjo, T., M. Kobayashi, Y. Yoshiba, Y. Kakubari, K. Yamaguchi-Shinozaki K. and K. Shinozaki. 1999. Antisense suppression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. *FEBS Lett*., 461: 205-210.

Natr, L. and D.W. Lawlor. 2005. Photosynthetic plant productivity. In: *Hand Book of Photosynthesis*, 2nd edition, (Ed.): M. Pessarakli. C.R.C. Press, New York, USA, pp. 501-524.

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

Papageorgiou, G.C. and N. Murata. 1995. The unusually strong stabilizing effects of glycine betaine on the structure and function of the oxygen-evolving Photosystem II complex. *Photosynth Res*., 44: 243-252.

Raven, J.A. 2002. Selection pressures on stomatal evolution. *New Phytol*., 153: 371-386.

Raza, S.H., H.R. Athar and M. Ashraf. 2006. Influence of exogenously applied glycinebetaine on the photosynthetic capacity of two differently adapted wheat cultivars under salt stress. *Pak. J. Bot*., 38: 341-352.

Rhodes, D. and A.D. Hanson. 1993. Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., 44: 357-384.

Roy, D., N. Basu, A. Bhunia and S. K. Banerjee. 1993. Counteraction of exogenous l-proline with NaCl in salt-sensitive cultivar of rice. *Biol. Plant*., 35: 69-72.

Samaras, Y., R.A. Bressan, L.N. Csonka, M.G. Garcı´a-Rı´os, 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.

Serraj, R. and T.R. Sinclair. 2002. Osmolyte accumulation: can it really help increase crop yield under drought conditions. *Plant Cell Environ*., 25: 333-341.

Sharkey, T.D., J.B. Carl, D.F. Graham and E.L. Singsaas. 2007. Fitting photosynthetic carbon dioxide response curves for C_3 leaves. *Plant Cell Environ.*, 30: 1035-1040.
Shuting, D., G. Rongqi, H. Changltao, W. Qunying, W. Koogjun. 1997. Study of canopy photosynthesis properties and high yield potential after anthesis in maize. *Acta Agron Sin*., 23(3): 318-325.
Snedecor, G. W. and W. G. Cochran. 1980. *Statistical methods*. 7th Edition. Iowa State University Press, Ames, IOWA.
Subbarao, G.V., R.M. Wheeler, L.H. Levine and G.W. Stutte. 2001. Glycinebetaine accumulation, ionic and water relations of red-beet at contrasting levels of sodium supply. *J. Plant Physiol*., 158: 767- 776.
Turner, N.C. 1979. Drought resistance and adaptation to water deficits in crop plants In: (Eds.): H. Mussell and R. C. Staples. - (Name of the book) Wiley: New York. pp. 343-372.
Turner, N.C. 1981. Designing crops for dryland Australia: can the desert help us? *J. Aust. Inst. Agric. Sci.*, 47, 29-34.
Turner, N.C. 1982. The role of shoot characteristics in drought resistance of crop plants. In: *Drought Resistance in Crops with emphasis on Rice*. International Rice Research Institute: Los Baños. pp. 115-134.
Waseem, M., H.R. Athar and M. Ashraf. 2006. Effect of salicylic acid applied through rooting medium on drought tolerance of wheat. *Pak J. Bot*., 38(4): 1127-1136.
Westgate, M.E. 1994. Water status and development of the maize endosperm and embryo during drought. *Crop Sci*., 34: 76-83.
Wyn Jones, R.G, R. Storey, R.A. Leigh, N. Ahmad and A. Pollard. 1977. A hypothesis on cytoplasmic osmoregulation. In: *Regulation of Cell Membrane Activities in Plants*. (Eds.): E. Marr & O. Ciferri. Elsevier/North-Holland Biomedical Press, Amsterdam, pp 121–136
Yancey, P.H., M.B. Clark, S.C. Hands, R.D. Bowlus and G.N. Somero. 1982. Living with water stress: evaluation of osmolyte systems. *Science,* 217: 1214-1222.

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