

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 mM 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 CO₂ (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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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^{-1} and soluble $\text{Ca}^{2+} + \text{Mg}^{2+}$, 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 $\mu\text{mol mol}^{-1}$; temperature of leaf chamber varied from 37.2 to 47.2°C; leaf chamber gas flow rate (U), 251 $\mu\text{mol s}^{-1}$; molar flow of air per unit leaf area (U_s) 221.06 $\text{mol m}^{-2} \text{s}^{-1}$; RH of the chamber ranged from 25.4 to 41.2 %; $PAR (Q_{\text{leaf}})$ at leaf surface during noon was maximum up to 918 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 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 mM 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 mM 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 mM 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 mM 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 mM 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.

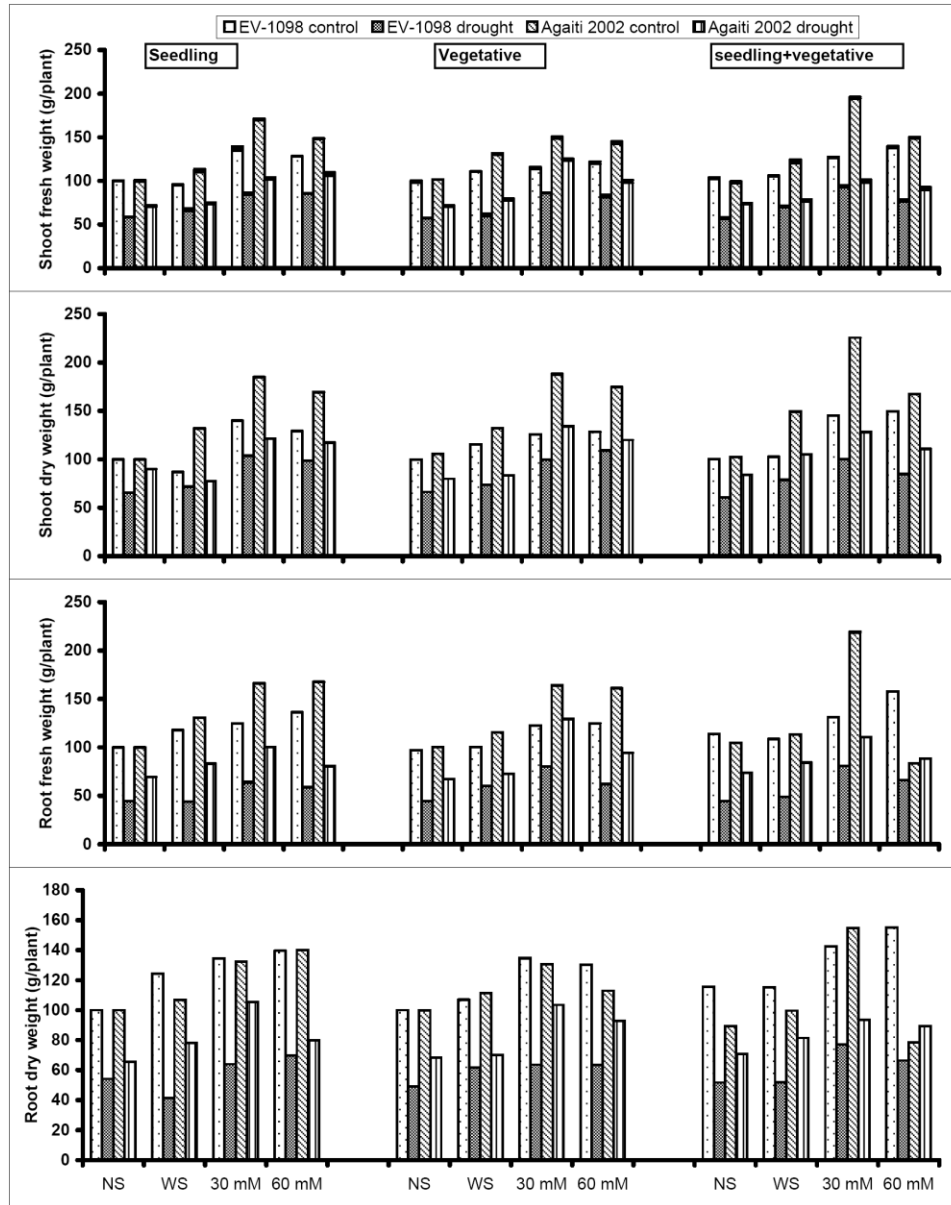
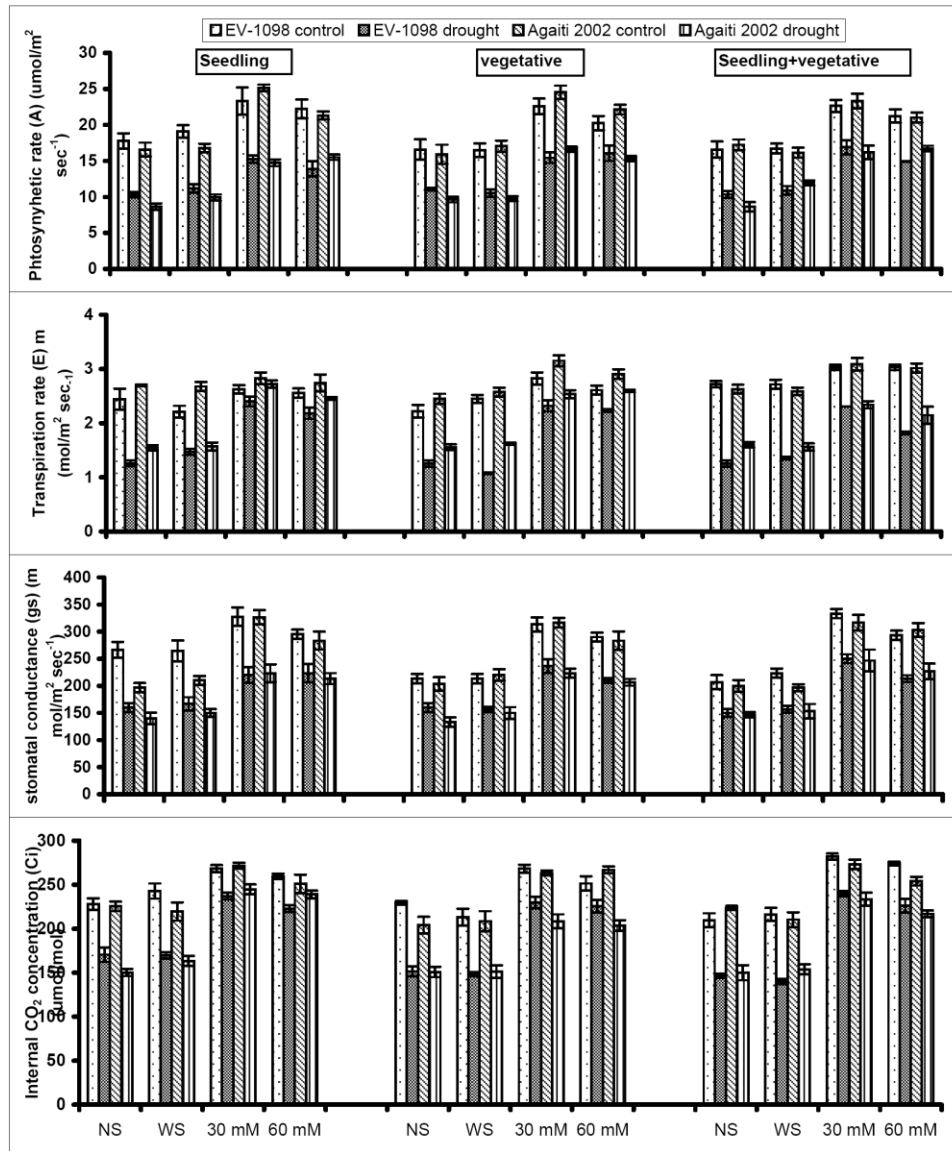
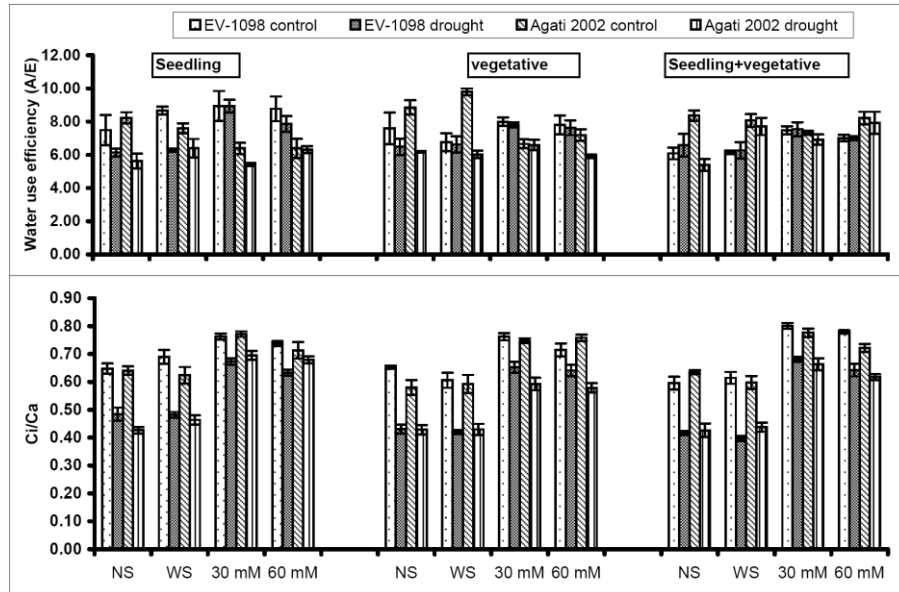


Fig. 1. Shoots and roots fresh and dry weights (g/plant) of two maize cultivars as influenced by exogenous application of different concentrations of proline at different growth stages under controlled and water stressed conditions.



NS = Non spray; WS = Water spray; 30, 60 mM = Proline spray with 30 and 60 mM

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.

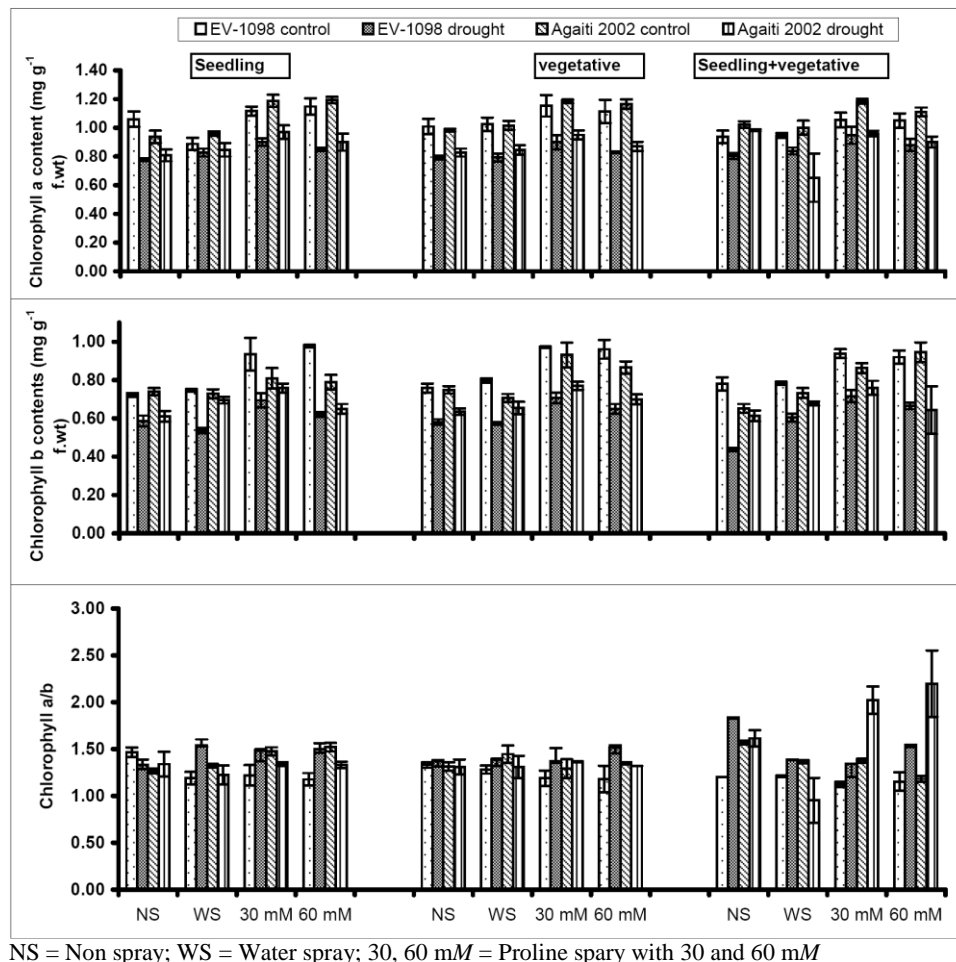


NS = Non spray; WS = Water spray; 30, 60 mM = Proline spary with 30 and 60 mM

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 mM proline under water stress conditions. Likewise, in rice, exogenous application of 30 mM proline proved to be beneficial when applied at the seedling stage (Roy *et al.*, 1993), 20-30 mM was effective in mung bean (*Vigna radiate*) cell cultures for the mitigation of adverse effects of stress and 10 mM 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 mM = Proline spary with 30 and 60 mM

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 CO₂ with an increase in net CO₂ assimilation rate. These results suggest that the increase in photosynthesis was primarily due to increase in stomatal conductance which caused higher CO₂ 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 CO₂ 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 stomatal 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 (Kausar *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 CO₂ 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 (Kausar *et al.*, 2006) and some trees under hypoxic conditions (Kozłowski, 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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