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Application of proline to root medium is more effective for amelioration of photosynthetic damages as compared to foliar spraying or seed soaking in maize seedlings under short-term drought

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Abstract: Exogenous proline (PRO) at low concentrations can enhance drought stress tolerance in different application modes such as application to rooting medium, foliar spray, and seed soaking. However, there is no information about which application mode is more effective for increasing the drought tolerance. Comparative effects of 1, 10, and 20 mM PRO applications through three application modes to hydroponically grown seedlings were examined under short-term drought stress in maize seedlings. Effects on leaf water potential, membrane damage, chlorophyll content, proline level, and gas exchange parameters such as net photosynthetic rate (Pn), transpiration rate (E), stomatal conductance (gs), and substomatal CO_2 concentration (Ci) were compared. Results indicated that PRO pretreatments raised the water potential, chlorophyll content, Pn, E, gs, and Ci but lowered the malondialdehyde content in the three application modes as compared to the untreated plants. Of the three different modes of PRO pretreatment, rooting medium treatment at 1 mM concentration was also more effective in alleviating stress-induced damages in maize seedlings. Moreover, effectively applied PRO increased the maximum quantum efficiency of PS II, quantum yield of PS II photochemistry, photochemical quenching, and electron transport rate but decreased nonphotochemical quenching of chlorophyll fluorescence under short-term drought stress. In conclusion, exogenous PRO was markedly more effective in the root-treated mode than in foliar spray or seed soaking mode, suggesting that PRO had a different ameliorating effect in different application modes. Proline application in an effective mode can induce photochemical efficiency under short-term drought in maize.

Key words: Application mode, drought, maize, membrane damage, photosynthesis, proline treatment

1. Introduction

Plants are often exposed to various abiotic stress factors during their life cycle, including drought, high or low temperatures, toxic metal ions, and UV radiation. These abiotic stress factors limit the growth and development of the plants to varying degrees depending on the severity of the stress (Chaves et al., 2009). Drought stress deteriorates membranes, which adversely affects a number of metabolic reactions occurring within the cell (Ashraf and Foolad, 2007). Drought also causes stomatal closure that reduces stomatal conductance (g_s) and net photosynthetic rate (Pn) in plants (Ali and Ashraf, 2011). It is known that either stomatal or metabolic impairment is a major limitation to photosynthesis (Athar and Ashraf, 2005). Changes in the contents of photosynthetic pigments can also affect the photosynthetic activities of plants. Chlorophyll plays a main role in photosynthesis, but degradation of chlorophylls under drought stress inhibits the net photosynthetic rate in major crops (Sairam et al., 1998; Anjum et al., 2011). Drought stress may damage the oxygen-evolving complex of photosystem II and PSII reaction centers (Subrahmanyam et al., 2006). Chlorophyll fluorescence is measured to understand the function of the photosynthetic apparatus under drought stress (Fracheboud and Leipner, 2003). Some stress factors can cause a greater decline in the effective quantum yield of PSII and electron transport rate (ETR). However, drought-tolerant genotypes can protect PSII activity under stress (Batra et al., 2014). On the other hand, protective compound treatments are widely used to increase the stress tolerance in tolerant or susceptible plant genotypes (Ali et al., 2007; Shahbaz et al., 2013).

One of the protective compounds applied to plants to increase stress tolerance is proline (PRO). Proline is not only an osmolyte but also a metal chelator, an antioxidant compound, and a signal molecule during stress (Hayat

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et al., 2012). Besides that, it can modulate the functions of mitochondria, influence plant growth, and induce the expression of some genes involved in abiotic stress tolerance (Szabados and Savaure, 2009). In addition to its major roles, PRO also plays an extremely important role in buffering cellular redox potential under abiotic stress conditions (Ali et al., 2013). PRO accumulation under various abiotic stress conditions is connected with stress tolerance in plants (Nanjo et al., 1999), and its concentration is generally higher in tolerant genotypes than in stress-sensitive ones (Ashraf and Foolad, 2007). It has been recorded that a proper concentration of PRO was involved in the osmotic potential of some plants under stress. Therefore, PRO application to plants under abiotic stress is an important approach to mitigate the hazardous effects of the stress. Many reports have depicted that exogenous PRO could play an important role in increasing plant tolerance against abiotic stress factors (Ali et al., 2007, 2013; Ashraf and Foolad, 2007; Athar et al., 2008; Shahbaz et al., 2013). These reports included the responses of plants, especially under salt stress (Hoque et al., 2007; Shahbaz et al., 2013). To the best of our knowledge, there are not enough records on ameliorating the effects of PRO application under drought stress. Furthermore, although PRO application improved the adverse effects of drought stress in plants (Ali et al., 2007, 2013), its application was performed just as a foliar application (Ali et al., 2007, 2013; Moustakas et al., 2011) or in the rooting medium (Kamran et al., 2009) under drought stress in plants. The effectiveness of PRO applied via seed soaking mode to alleviate stress injuries is not well known. Namely, there is not enough information about its tolerance-enhancing properties in different application modes under short-term drought in maize seedlings.

The stress tolerance of plants could improve if PRO were supplied exogenously at a low concentration, but it could be toxic if supplied exogenously at a higher concentration (Hayat et al., 2012). For instance, Ali et al. (2007) recorded that 30 mM PRO was the most effective concentration for improvement in growth of water-stressed maize seedlings subjected to water deficit conditions, while higher concentrations of exogenous PRO (40 or 50 mM) suppressed the growth of the seedlings. Therefore, more research is needed to detect the most effective PRO concentration and its application mode under different stress conditions.

In our study, we applied PRO to maize seedlings by three different modes: presowing seed treatment, rooting medium, and foliar spray. After PRO application the seedlings were subjected to short-term drought stress. We first sought to investigate the effects of PRO pretreatment on water status, chlorophyll content, and gas exchange parameters in maize seedlings subjected to short-term drought stress. Therefore, we aimed to determine which application mode is more effective for increasing drought stress tolerance. Our second aim was to measure the changes in chlorophyll fluorescence parameters to elucidate in detail the ameliorating effect of exogenous PRO on photosynthetic damages in the most effective PRO application mode and at the most effective application concentration.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of *Zea mays* L. cultivar Safak were obtained from the Black Sea Agricultural Research Institute, Samsun, Turkey. For surface sterilization, the seeds were treated with 0.1% $HgCl_2$ for 3 min. After that, the seeds were washed with sterilized distilled water three or four times. For seed priming, some seeds were soaked in solutions of 0, 1, 10, or 20 mM PRO for 10 h. After that, all seeds were germinated in vermiculite. Seven days after emergence, seedlings were transferred to Hoagland nutrient solution and grown in a growth chamber at 22 ± 1 °C, 60 ±/10% relative humidity, and a photon flux density of 400 µmol $m^{-2}s^{-1}$ with 16 h of light and 8 h of darkness (Hoagland and Arnon, 1938). PRO pretreatments were conducted as a foliar spray and rooting medium when seedlings treated with 0 mM PRO at seed priming were 21 days old. For the foliar spray, 0, 1, 10, and 20 mM PRO was dissolved in distilled water containing 0.1% Tween 20 and sprayed on the leaves twice every 2 days. For rooting medium treatment, 0, 1, 10, and 20 mM PRO was added to Hoagland nutrient solution and PRO pretreatments were performed for 2 days. After that, 23-day-old plants were exposed to gradual drought stress induced by dissolving polyethylene glycol (PEG_{6000}) in the nutrient solution in three equal increasing doses with an interval of 8 h until the final concentration of 20% to develop a water potential of –0.5 MPa, similar to Li et al. (2009). After that, the seedlings were subjected to drought stress for 8 h and thus they were cultured in different hydroponic systems designed as follows. Mock: Nutrient solution; PEG: nutrient solution containing 20% PEG; 1 mM PRO + PEG: nutrient solution containing 1 mM PRO and 20% PEG; 10 mM PRO + PEG: nutrient solution containing 10 mM PRO and 20% PEG; 20 mM PRO + PEG: nutrient solution containing 20 mM PRO and 20% PEG in rooting medium, seed soaking, and foliar applications. For chlorophyll fluorescence measurements, Mock, PEG, and 1 mM PRO + PEG plants in rooting medium were used. An additional hydroponic system was designed for PRO group plants treated with 1 mM proline and grown under unstressed conditions. The experimental plan was arranged by a completely randomized design with three replicates, providing a total of 6 containers with a total of 18 plants per treatment. Samples of the third leaf were harvested and the following analyses were performed.

2.2. Proline content

The method described by Bates et al. (1973) was used to determine the PRO contents of the leaves. The leaf samples were dried; dry samples (0.2 g) were homogenized in 10 mL of 3% (w/v) aqueous sulfosalicylic acid solution. Supernatants were transferred to test tubes and mixed with equal volumes of glacial acetic acid and ninhydrin reagent. Test tubes were incubated in the oven for 1 h at 100 °C. The test tubes were then placed in an ice bath and thus the reaction was stopped. The samples were rigorously mixed by using a vortex after 3 mL of toluene was added to the tubes. After 50 min, toluene phases were obtained. The absorbance was measured at 520 nm on a UV-visible spectrophotometer.

2.3. Measurement of leaf water potential

Leaf water potential (Ψ_{leaf}) was determined using a PSYPRO thermocouple psychrometer (Wescor, USA). Leaf disks (about 6 mm in diameter) were obtained from the leaves and then the disks were placed in the C-52 psychrometer chamber. Samples were equilibrated for about 60 min and then data for Ψ_{leaf} were recorded in the psychrometric mode by the instrument.

2.4. Lipid peroxidation

Lipid peroxidation was measured in the terms of malondialdehyde (MDA) content (ε = 155/(mM cm)), which is a product of lipid peroxidation, according to Heath and Packer (1968). The MDA content was expressed as nmol MDA per gram of dry weight.

2.5. Measurement of gas exchange parameters

Gas exchange parameters such as stomatal conductance (g_s), intercellular CO_2 concentration (Ci), photosynthetic rate (Pn), and transpiration (E) were measured on the third intact leaf from the top of each plant using a portable photosynthesis system (LI 6400-XT, LI-COR, USA) at a temperature of 20 ± 2 °C. PPFD, air flow rate, and relative humidity inside the sample chamber were maintained at 1000 µmol m^{-2} s^{-1}, 500 µmol s^{-1}, and 50%–60%, respectively. The portable photosynthesis system allowed for independent control of the CO_2 concentration by an integrated CO_2 mixer. After clamping the leaf, the CO_2 reference and CO_2 sample values were maintained for at least 30 min to reach a concentration of 400 µmol mol^{-1} CO_2. These measurements were completed in approximately 3 h.

2.6. Chlorophyll content

Photosynthetic pigment (chlorophyll a and chlorophyll b) contents were detected according to the method of Arnon (1949). Fresh leaf samples (0.1 g) were extracted overnight with 80% acetone at 0–4 °C. The extracts were centrifuged at 10,000 × *g* for 5 min. Supernatant was obtained and absorbance was read at 645 and 663 nm using a spectrophotometer.

2.7. Chl fluorescence measurements

Plants grown in the most effective application mode and concentration (1 mM) were used for chlorophyll fluorescence measurements. The measurements were performed with OS1-FL (at module 4), a pulse modulated fluorometer (OptiScience Corporation, USA). The leaves were dark-adapted for 20 min before Chl fluorescence was measured. The minimal fluorescence yield (F_0), maximum fluorescence yield (F_m), maximum Chl fluorescence in the light (F_m'), and steady-state chlorophyll fluorescence (F_s) were determined according to Nar et al. (2009). Photochemical quenching of Chl fluorescence (qP), nonphotochemical quenching (NPQ), maximum quantum yield of PSII photochemistry (Fv/Fm), and effective quantum yield of PSII photochemistry (Φ_{PSII}) were measured using the method described by Van Kooten and Snel (1990). The photochemical quenching and nonphotochemical quenching were calculated according to Dall'Osto et al. (2007) and Bilger and Bjorkman (1990), respectively. Fv/Fm and Φ_{PSII} were automatically calculated by a fluorometer according to the equations ($Fv/Fm = (F_m - F_0)/F_m$, $\Phi PSII = (F_m' - F_s)/F_m'$) of Genty et al. (1989). Electron transfer rate (ETR) was also determined according to Nar et al. (2009).

2.8. Statistical analysis

All experiments were repeated three times with six biological replicates. All results were presented as means ± standard deviation. Statistical analysis was done with the Duncan multiple comparison test (one-way ANOVA) using SPSS 15.0 for Windows (SPSS Inc., USA) to evaluate if the means were significantly different. The significance level among all treatments was appraised at 5% ($P < 0.05$).

3. Results

3.1. Proline content

Short-term drought stress caused a considerable increase in the PRO content of maize plants in all application modes. Plants with PRO applied at 1 mM concentration under stress, in comparison with PEG plants, exhibited higher endogenous PRO content in rooting medium and foliar spray modes. However, 10 mM PRO application as a rooting medium showed nonsignificant effects on internal PRO content in comparison with PRO-untreated plants. Conversely, plants with 20 mM PRO application in seed soaking mode under stress (20 mM PRO + PEG) had a statistically decreased endogenous proline content as compared to PEG plants. PRO applied in foliar mode increased endogenous PRO content more than the rooting medium and seed soaking modes. The highest PRO content in foliar spray mode was observed at the 20 mM concentration (Figure 1).

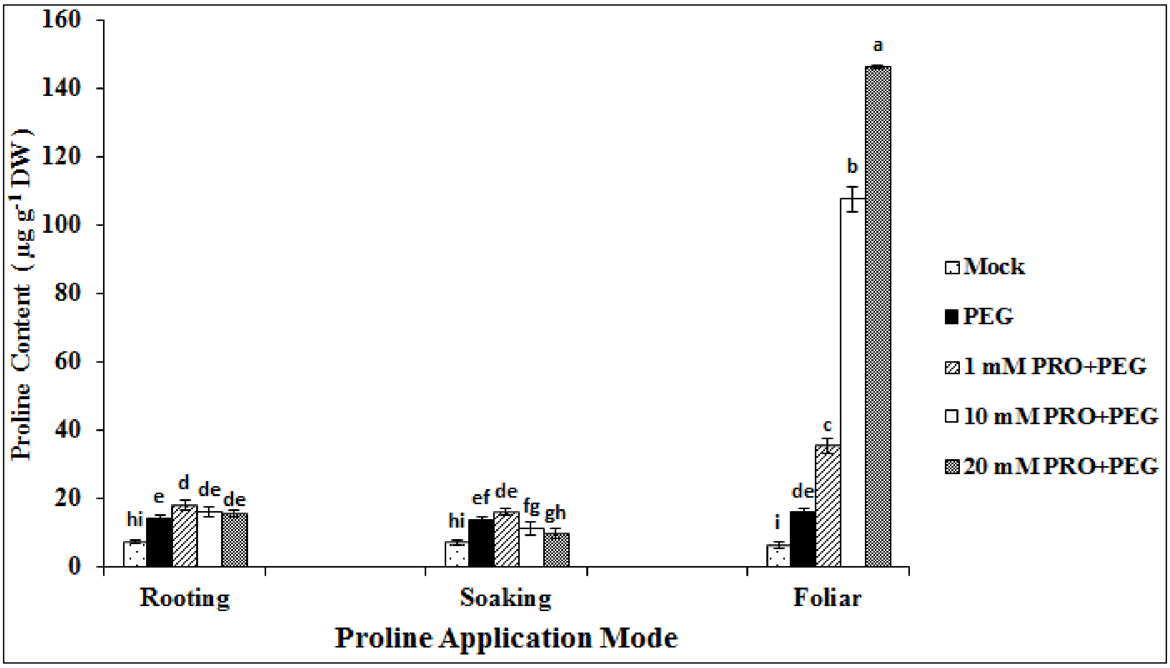

Figure 1. Effect of PRO pretreatment in different modes on endogenous proline content in the leaves of maize seedlings under drought stress conditions. Vertical bars represent standard deviations of the means of three replicates. Different letters denote significant differences among all treatments at $P < 0.05$.

3.2. Water status

A marked decrease in leaf water potential was recorded when the seedlings were subjected to PEG-induced drought stress in all application modes. PRO applications in three different concentrations (1, 10, and 20 mM) and application modes alleviated the negative effects of drought on leaf water status. However, rooting medium treatments exhibited a relatively higher leaf water potential as compared to foliar and seed priming application modes. Moreover, the highest leaf water potential under short-term drought stress was observed in the leaves treated with 1 mM PRO in rooting medium (Figure 2).

3.3. Membrane damage

Lipid peroxidation in the membranes was determined according to MDA content. It was found that the MDA content of PEG plants increased in all application modes as compared to Mock plants. Moreover, there was no difference between the different application modes for PEG plants. PRO applications under short-term drought caused an alleviative effect on the membrane damage as compared to untreated plants in all application modes. The ameliorating effect of PRO application under stress was the highest in the leaves treated with 1 mM PRO in rooting medium. PRO application (10 mM) in foliar application mode decreased MDA content much more than PRO applications in soaking mode. However, 20 mM foliar PRO application caused higher lipid peroxidation as compared to untreated plants under short-term drought stress (Figure 3).

3.4. Gas exchange attributes

All seedlings showed a significant reduction in net photosynthetic rate, transpiration rate, stomatal conductance, and substomatal CO_2 concentration under short-term drought stress in three application modes. Marked increases of Pn were observed in the leaves treated with 1, 10, and 20 mM PRO in all application modes as compared to untreated leaves under stress. The increase in Pn was highest in 1 mM PRO + PEG seedlings of rooting medium (Figure 4A). Similarly, applications of PRO at 1 and 10 mM concentrations generally increased the values of E, g_s, and Ci in three application modes (Figures 4B–4D), whereas 20 mM PRO applications in seed soaking and foliar spray modes caused only slight, nonsignificant changes. The increase in E was highest at the 1 mM PRO concentration of rooting medium (Figure 4B). Values of g_s and Ci were high in 1 mM PRO + PEG and 10 mM PRO + PEG plants in rooting medium as compared to the other PRO application modes under stress (Figures 4C and 4D). Moreover, all gas exchange parameters of seed priming were higher than those of foliar application (Figures 4A–4D).

3.5. Chlorophyll content

Chlorophyll a and chlorophyll b contents of maize leaves were significantly reduced when the seedlings were

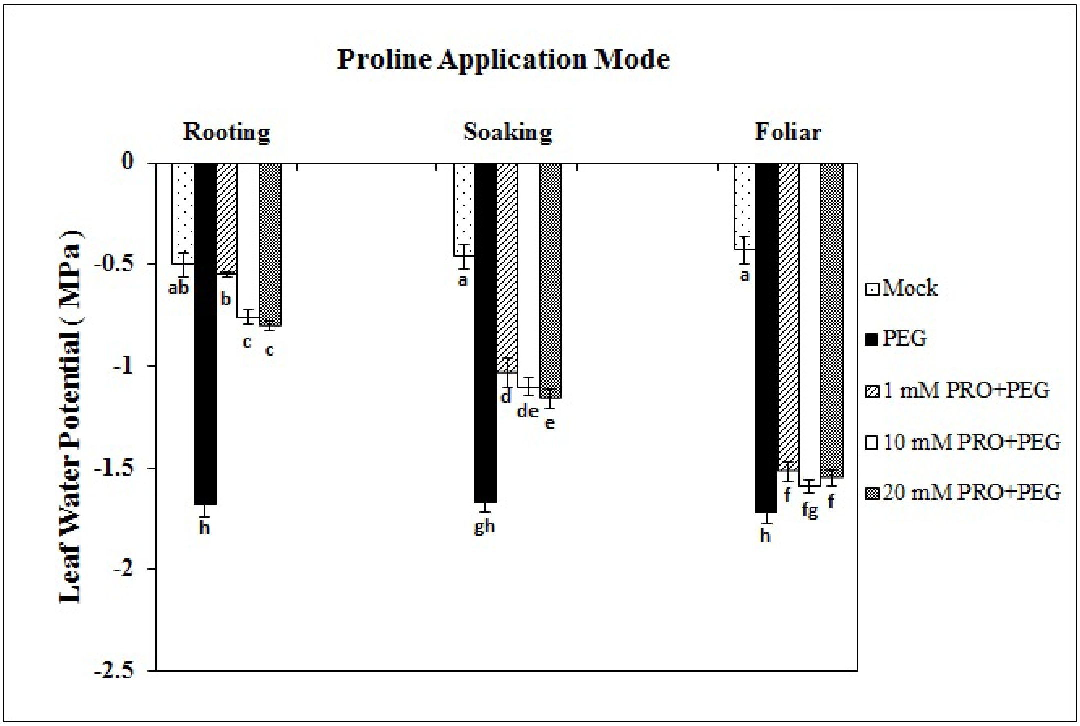

Figure 2. Effect of PRO pretreatment in different modes on leaf water potential (Y_{leaf}) in the leaves of maize seedlings under drought stress conditions. Vertical bars represent standard deviations of the means of three replicates. Different letters denote significant differences among all treatments at P < 0.05.

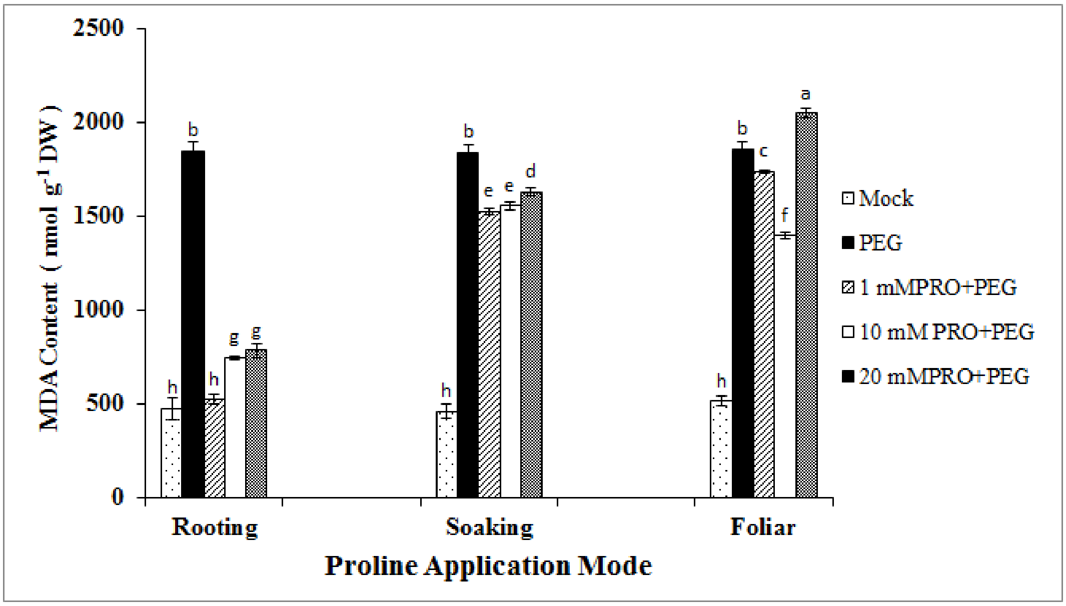

Figure 3. Effect of PRO pretreatment in different modes on membrane damage (MDA content) in the leaves of maize seedlings under drought stress. Vertical bars represent standard deviations of the means of three replicates. Different letters denote significant differences among all treatments at P < 0.05.

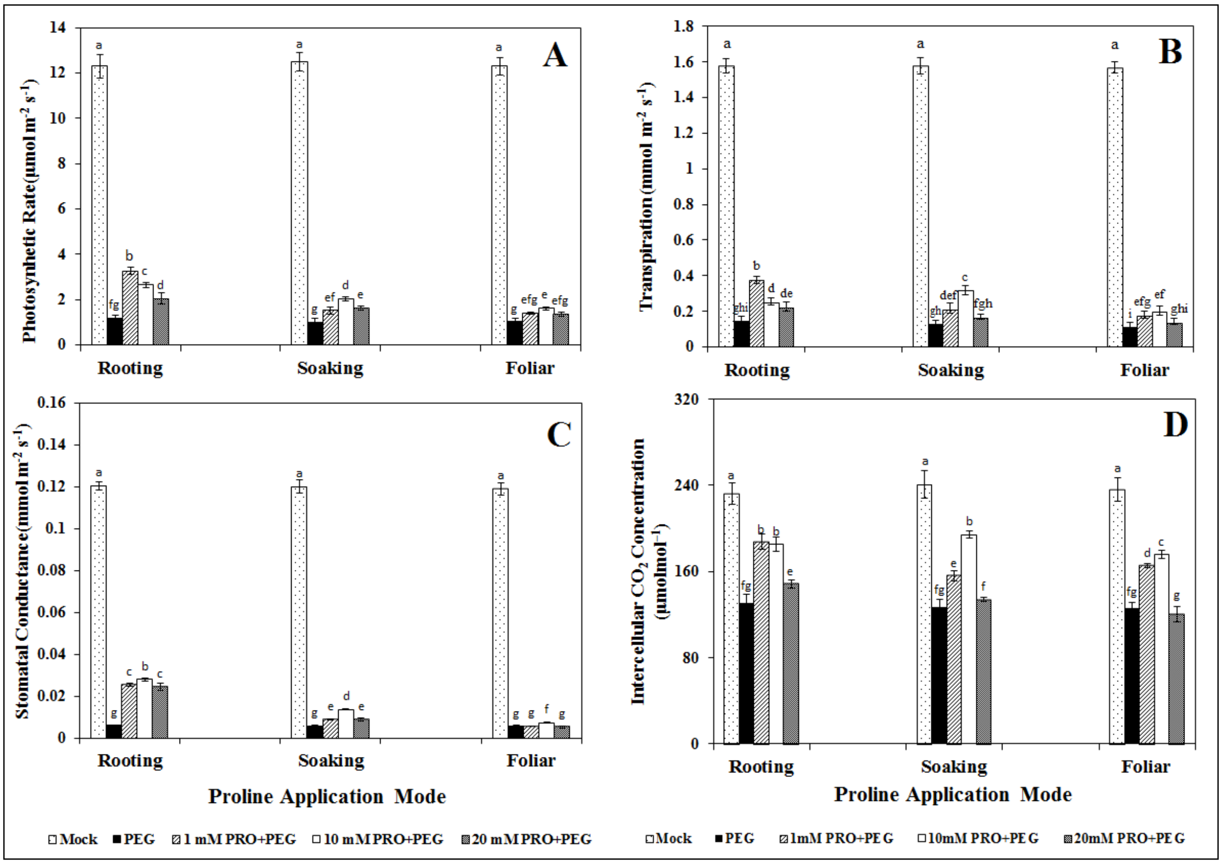

Figure 4. Effect of PRO pretreatment in different modes on net photosynthetic rate (A), transpiration rate (B), stomatal conductance (C), and substomatal CO_2 concentration (D) in the leaves of maize seedlings under drought stress. Vertical bars represent standard deviations of the means of three replicates. Different letters denote significant differences among all treatments at $P < 0.05$.

exposed to PEG-induced drought stress. Alleviative effects on chlorophyll losses appeared in PRO-applied plants under stress as compared to untreated plants. Similar responses were observed in all application modes. The most alleviative effect was observed in plants treated with 1 mM PRO in rooting medium. PRO applications in seed soaking mode under stress increased the Chl a and Chl b contents more than those of foliar spray mode (Figures 5A and 5B).

3.6. Chlorophyll fluorescence parameters

Changes in the Fv/Fm, Φ_{PSII}, ETR, and qP values in the most effective proline application mode were determined, and it was observed that Fv/Fm, Φ_{PSII}, ETR, and qP values decreased under stress conditions as compared to unstressed plants but the NPQ value increased (Figures 6A, 6B, and 7A–7D). Interestingly, the Fv/Fm value decreased slightly in PRO group plants as compared to Mock plants, but it increased in PRO + PEG plants as compared to PEG plants (Figure 6A). Similarly, Φ_{PSII}, ETR, and qP increased in PRO + PEG plants in comparison with PEG plants (Figures 6B, 7A, and 7B). The nonphotochemical quenching values decreased significantly in PRO-applied plants in comparison with PRO-untreated plants under stressed and unstressed conditions (Figure 7C).

4. Discussion

When plants are exposed to biotic and abiotic stresses, increased PRO accumulation contributes to the stress tolerance of the plants (Ashraf and Foolad, 2007). In this context, studies have been conducted on the ameliorating effect of PRO application against stress damages. PRO is applied to the plants to enhance abiotic stress tolerance in different application modes, such as application to rooting medium, foliar spray, and seed soaking application. In the current study we aimed to detect the most effective application mode of exogenous PRO under short-term drought stress in plants. Therefore, we determined the effects of PRO application in maize on leaf water potential, membrane damage, gas exchange parameters, chlorophyll contents, and PRO level under short-term drought stress in three different application modes: rooting medium, foliar spray, and seed soaking. Our results showed that

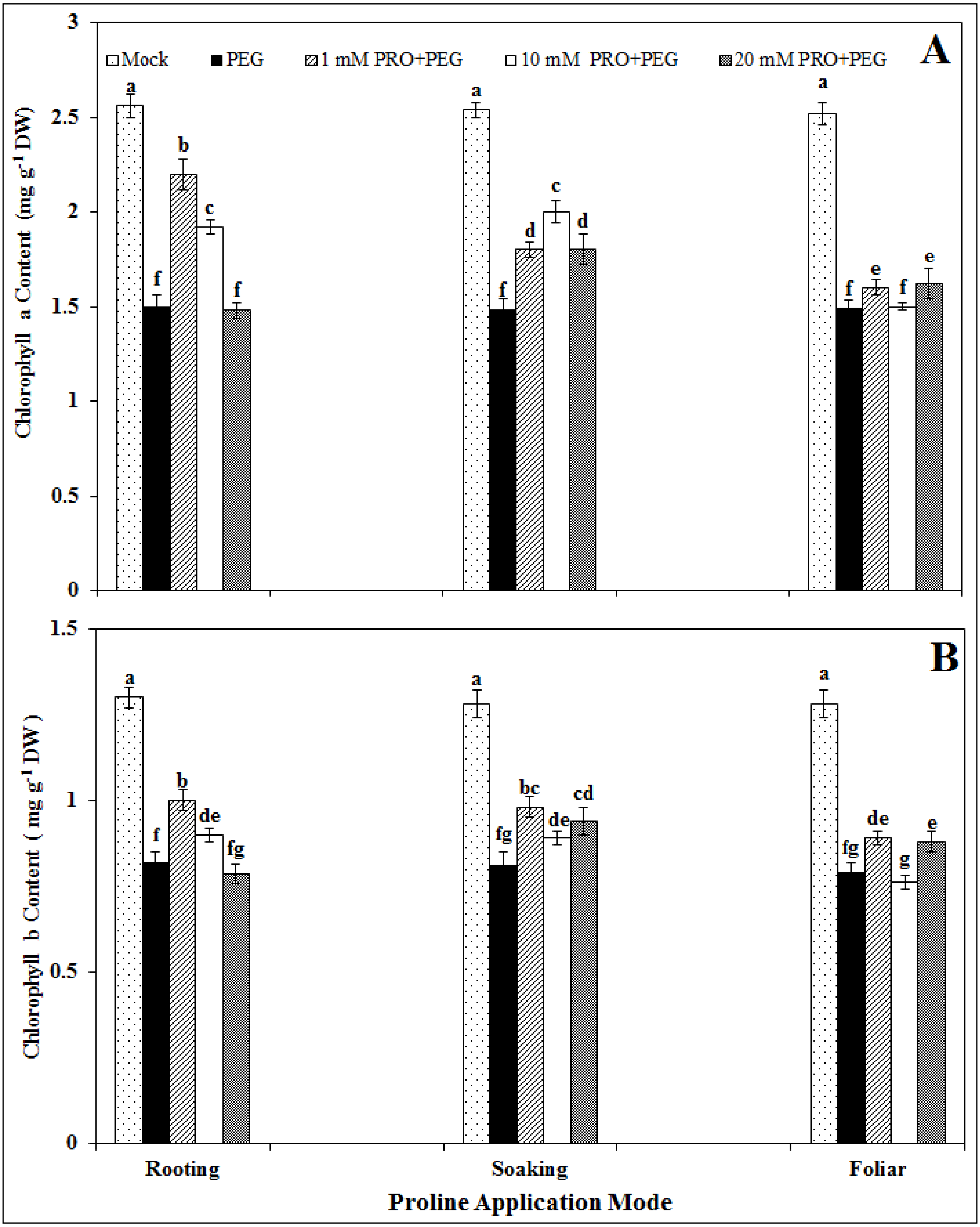

Figure 5. Effect of PRO pretreatment in different modes on chlorophyll a (A) and chlorophyll b (B) contents in the leaves of maize seedlings under drought stress. Vertical bars represent standard deviations of the means of three replicates. Different letters denote significant differences among all treatments at $P < 0.05$.

endogenous PRO content increased under short-term drought stress induced by PEG_{6000}. Increase in PRO content under stress in many plant species was correlated with stress tolerance and its concentration was shown to be significantly higher in stress-tolerant genotypes than in stress-sensitive ones (Ashraf and Foolad, 2007). We

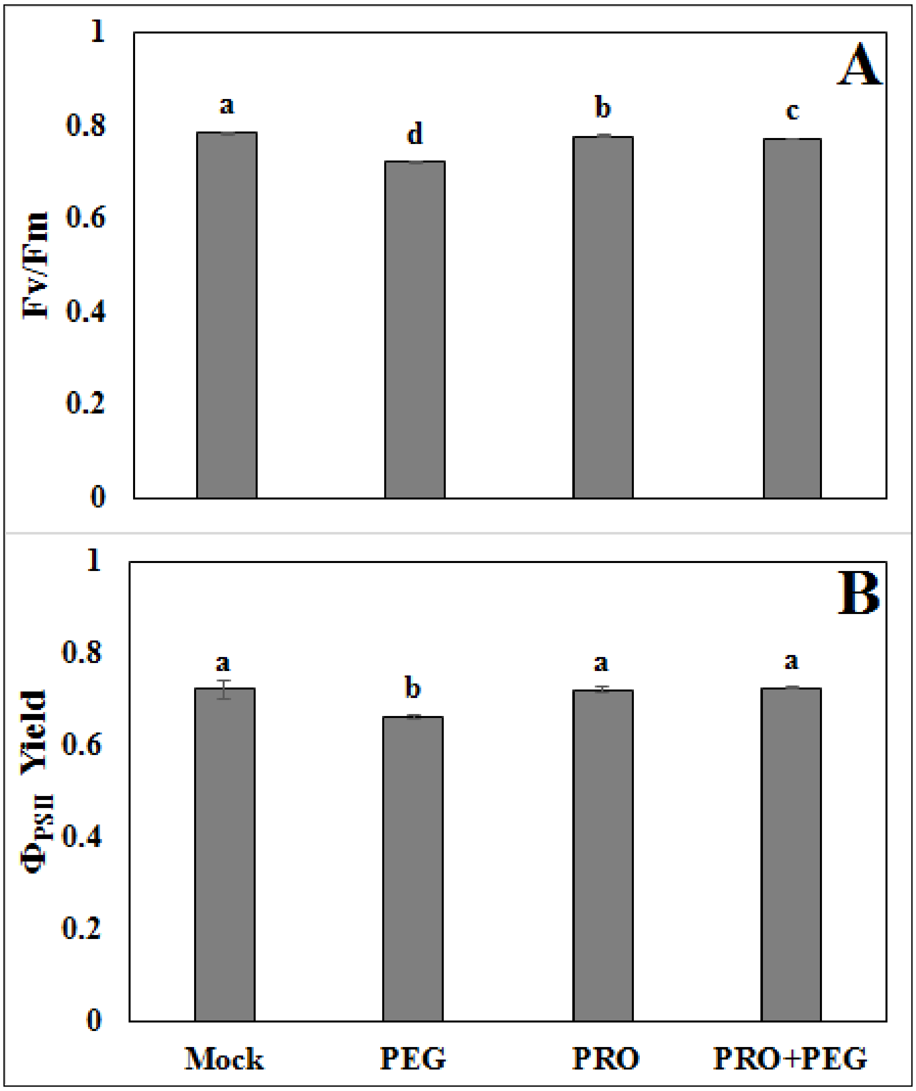

Figure 6. Effect of proline application in suitable mode on Fv/Fm (A) and Φ_{PSII} (B) under drought stress. Vertical bars represent standard deviations of the means of three replicates. Different letters denote significant differences among all treatments at P < 0.05.

determined that exogenous PRO at the 1 mM concentration increased the stress-induced PRO accumulation slightly in rooting medium and foliar spray modes. Posmyk and Janas (2007) recorded that exogenous PRO application enhanced leaf PRO content and thus alleviated the injuries of abiotic stress. Similar findings were reported by Moustakas et al. (2011) in PRO-applied *Arabidopsis thaliana* leaves under drought. PRO application increased the endogenous PRO content in foliar application mode too much as compared to the other modes, because of the fact that PRO solutions were sprayed on the leaves twice every 2 days. On the other hand, PEG + 20 mM PRO application in the seed soaking mode resulted in lower endogenous PRO concentration as compared to PEG alone. These findings indicate that stress-induced PRO accumulation can be reduced by exogenous PRO application.

We found that 1, 10, and 20 mM PRO ameliorated the undesirable effects of drought stress on leaf water potential in all application modes. Moreover, 1 mM PRO application in rooting medium exhibited a relatively higher leaf water potential than other application modes under stress. When compared to rooting medium and seed soaking application modes, PRO applications in the rooting medium, where plants had a higher endogenous PRO content, increased the leaf water potential more than those of seed soaking mode. In accordance with our study, it was reported that stress tolerance increased when PRO was supplied exogenously at low concentrations (Hayat

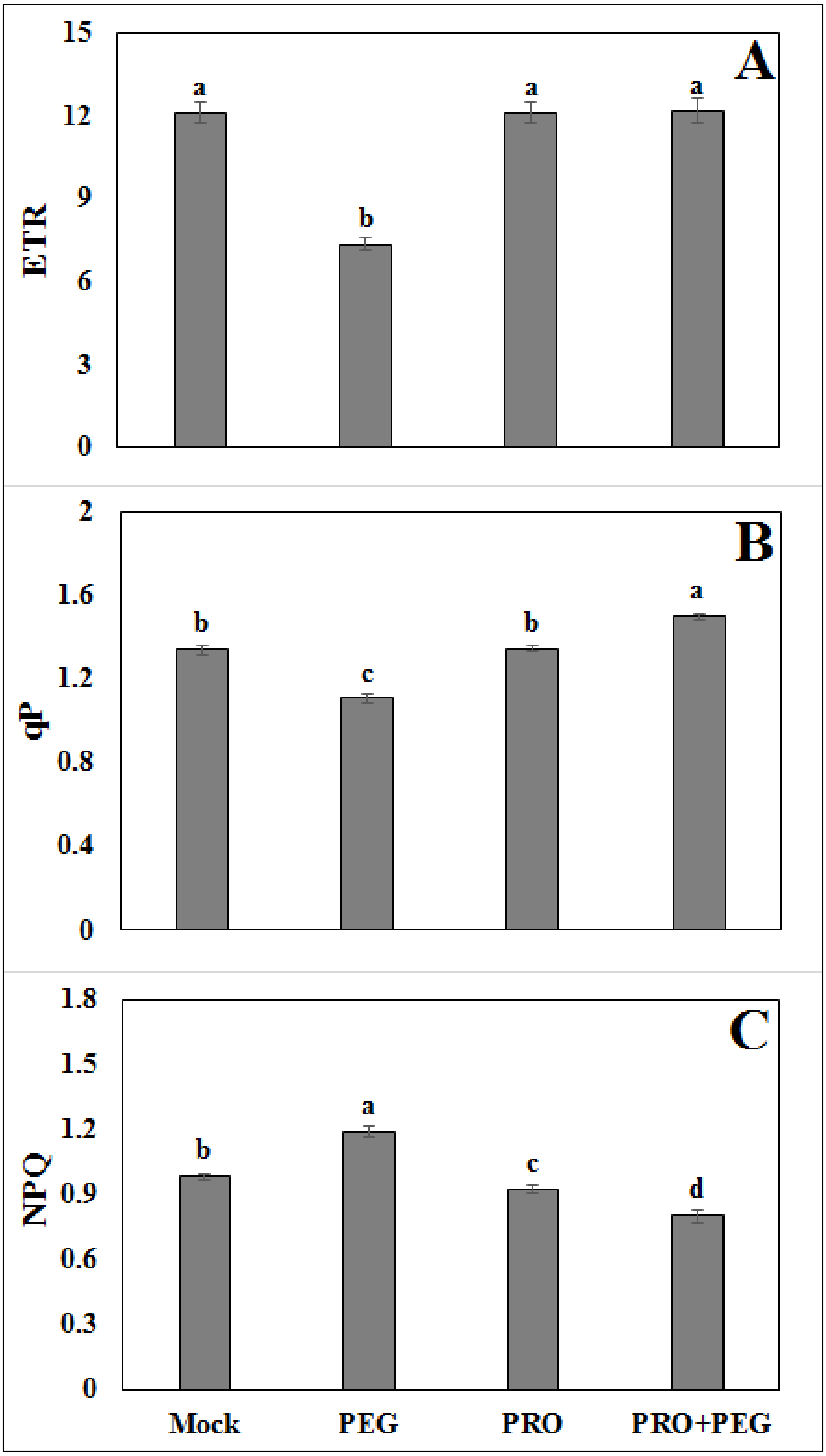

Figure 7. Effect of proline application in suitable mode on ETR (A), qP (B), and NPQ (C) under drought stress. Vertical bars represent standard deviations of the means of three replicates. Different letters denote significant differences among all treatments at $P < 0.05$.

et al., 2012). Despite the high endogenous PRO level, the ameliorative effect of PRO on water status in foliar application mode was low as compared to seed soaking applications. It was reported that PRO played a protective role against abiotic stress, but the increased level of PRO did not provide enough osmotic adjustment in some plants (Hamilton and Heckathorn, 2001). For this reason, similar to the findings of Sun and Hong (2010), we concluded that providing more PRO sources through exogenously adding PRO could enhance PRO accumulation in maize, but excessive PRO content may induce membrane damage and suppress the growth of the seedlings.

Compatible with the findings of leaf water potential, MDA content increased in plants exposed to stress in three application modes. However, PRO applications (1, 10, and 20 mM) under stress caused alleviative effects on the membrane damage as compared to untreated plants in all application modes, except 20 mM foliar PRO application. The highest alleviative effect was observed in plants treated with 1 mM PRO in rooting medium. Interestingly, 20 mM foliar PRO application caused high lipid peroxidation as compared to PRO-untreated plants under short-term drought stress. This finding suggests that high PRO accumulation on the leaf surface could cause increased MDA content in foliar PRO-applied plants. Likewise, Ashraf and Foolad (2007) reported that the effectiveness of PRO applied as a foliar spray depended on the concentration, time of application, type of species, and plant developmental stage. Shahid et al. (2014) recorded that the effect of exogenous PRO on stress damages was dependent on the concentration, and foliar-applied PRO at a low concentration reduced the oxidation of lipid membranes in *Pisum sativum* under salinity-stressed conditions. In our study, to the best of our knowledge, it has been shown for the first time that exogenous PRO application in rooting medium and seed soaking modes alleviated the hazardous effects of drought stress on membranes in maize plants.

Drought stress progressively decreases the net photosynthetic rate due to reduced stomatal conductance. Drought stress also induces reduction in the substomatal CO_2 concentration and transpiration rate. Moreover, the reduced content of leaf chlorophyll in drought-stressed plants can cause a decrease in the net photosynthetic rate (Reddy et al., 2004). We found a significant reduction in gas exchange parameters under short-term drought stress as compared to unstressed plants (Mock). Likewise, Anjum et al. (2011) recorded that drought stress caused considerable declines in net photosynthesis, transpiration rate, stomatal conductance, and intracellular CO_2 concentration in maize. However, determining the effect of exogenous PRO on drought-induced photosynthetic responses can contribute to understanding the tolerance mechanism of plants. In the current study, we found that exogenous PRO at 1 and 10 mM concentrations alleviated the negative effects of stress on g_s in all modes, especially in the rooting medium mode, although a reduction in g_s value was induced by drought stress. In accordance with the increase in g_s, PRO applications caused increases in Pn. Indeed, the rate of photosynthesis was highly linked with the rate of stomatal conductance, which was a marked indication of increased photosynthetic activity by regulation of stomatal conductance (Shahid et al., 2014). Furthermore, we found that PRO applied at 1 and 10 mM concentrations increased the values of E and Ci as compared to PRO-untreated leaves in three application modes. The increase in E was highest at the 1 mM PRO concentration of rooting medium. Similarly, the value of Ci was high in 1 mM PRO + PEG and 10 mM PRO + PEG plants in rooting medium as compared to the other PRO application modes under stress. Ali et al. (2007) reported that foliar PRO at a concentration of 30 mM induced an improvement in the growth of water-stressed maize plants, and exogenous PRO was associated with gas exchange attributes, net photosynthesis, transpiration rate, substomatal CO_2, and stomatal conductance. However, in contrast to their study, we found that the effective PRO concentration was low. In our experiment, PRO was applied to maize plants before they were exposed to stress. Therefore, we could say that the effective PRO concentration applied before exposure to stress to reduce stress damage might be lower than the concentrations after exposure to stress. Moreover, we determined that 20 mM PRO applications in seed soaking and foliar spray modes caused only slight, nonsignificant changes as compared to PEG plants. Ashraf and Foolad (2007) and Hayat et al. (2012) recorded that high PRO concentrations were inhibitory to growth, while PRO supplied exogenously at optimal concentrations provided beneficial effects to the plants. Thus, PRO applied to the rooting medium at 1 mM was most effective in ameliorating the damages of drought stress. Moreover, the rooting medium mode of PRO application was more effective compared to other modes.

The decrease in the photosynthetic rate under drought stress can also be attributable to the reduction in chlorophyll content, which is one of the major chloroplast components for photosynthesis (Athar and Ashraf, 2005; Anjum et al., 2011). Various abiotic stress factors can alter stomatal functioning and chlorophyll synthesis, which results in reduced photosynthesis (Hayat et al., 2012). The results of our study show that drought stress induced a sharp decline in chlorophyll a and chlorophyll b content in maize. Large declines in photosynthetic pigment contents such as chlorophyll a, chlorophyll b, and total chlorophyll content under drought stress have been reported in plants (Anjum et al., 2011). However, in the current study, the reductions of pigment contents were ameliorated by PRO pretreatment under short-term drought. Ali et al. (2007) recorded that PRO application as a foliar spray improved chlorophyll contents in water-stressed maize plants. In addition, in the current research, we revealed that the most alleviative effect was observed in plants treated with 1 mM PRO in the rooting medium. Furthermore, foliar application mode had the lowest alleviative effect on photosynthetic damages because of excessive PRO content.

Chlorophyll fluorescence parameters are also assumed to closely reflect the functioning of the photosynthetic apparatus (Fracheboud and Leipner, 2003). Thus, we measured the changes in Fv/Fm, Φ_{PSII}, ETR, and qP values to verify the ameliorating effect of exogenous PRO on photosynthetic damages in the most effective PRO application mode and at the effective application concentration. We found that values of Φ_{PSII}, ETR, and qP increased while NPQ decreased significantly in PRO-applied plants under stress as compared to PRO-untreated plants. Similarly, Moustakas et al. (2011) reported that exogenous foliar application of PRO by spraying maintained the PSII function in *Arabidopsis thaliana* subjected to drought. In this experiment, a slight decrease in the Fv/Fm value in PRO plants under unstressed conditions in comparison with Mock plants demonstrated that exogenous PRO may decrease the maximum quantum yield of PSII photochemistry under unstressed conditions, depending on the concentration. Indeed, a proper concentration of PRO was involved in the osmotic potential of some plants under stress.

In conclusion, the presented data have shown that although the PRO pretreatment through all application modes increased the photosynthetic performance of the plants, its application via rooting medium at a concentration of 1 mM was relatively more effective for increasing photosynthetic performance and reducing water loss. This may be attributed to the continuous uptake of PRO in the rooting medium as compared to other application modes. PRO application in an effective mode can induce the photosystem II photochemical efficiency under short-term drought in maize. PRO-treated plants under stress showed a higher net photosynthetic rate, transpiration, and stomatal conductance as compared to untreated plants. Moreover, the decrease in the content of chlorophyll pigments and the increase in membrane damage was relatively lower in PRO-pretreated plants. Thus, exogenously applied PRO is effective in overcoming the adverse effects of drought stress. Furthermore, it may be concluded that PRO application in maize seedlings through the rooting medium was relatively more effective in alleviating the hazardous effects of water stress on the photosynthetic rate and plant water status as compared to those of foliar spray or seed priming treatments. The increase in photosystem II photochemical efficiency in PRO-applied plants at 1 mM under drought verifies that the concentration and the application mode are suitable for decreasing photosynthetic damages.

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