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Exogenous application of ascorbic acid stimulates growth and photosynthesis of wheat (*Triticum aestivum* L.) under drought

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Abstract

Drought causes considerable reduction in plant growth. A hydroponic experiment was conducted to appraise the potential role of exogenously applied ascorbic acid in alleviating the effect of drought on wheat. Two contrasting wheat genotypes, a drought tolerant cultivar Chakwal-86 and a drought sensitive strain 6544-6 were used in the study. Drought was induced by dissolving 20% Polyethylene glycol (PEG_{8000}) in the nutrient solution producing -0.6MPa osmotic stress. Drought caused a significant decrease in chlorophyll pigments and net photosynthesis resulting in growth reduction of both wheat genotypes. However, this decrease was more severe in the genotype 6544-6 compared to Chakwal-86. Ascorbic acid (AsA) was applied through rooting medium, as a foliar spray and seed soaking treatment. Ascorbic acid treated seedlings of both genotypes maintained higher chlorophyll contents, net photosynthesis and growth compared to the non-treated plants. Of the three different modes of ascorbic acid application, rooting medium was more effective in alleviating the adversities of drought in wheat.

Key words: Wheat, drought, ascorbic acid, net photosynthesis, growth, chlorophyll

Introduction

Limited availability of water is a serious constraint to agricultural production of major crops (Shao *et al*., 2009) because water is vital factor in plant development. Reduced supply of water is known to hamper important physiological and biochemical mechanisms leading to reduction in plant growth. Substantial yield losses have been observed in different crops due to reduced supply of water even for a short period of time (Pinheiro *et al*., 2005). Drought stress causes reduced stomatal conductance resulting in decreased net photosynthetic rate. Chlorophyll degradation due to drought stress also inhibits photosynthetic rate in wheat (Moaveni, 2011). Drought stress deteriorates membranes which adversely affects a number of metabolic reactions occurring within the cell (Ashraf, 2009). Membrane stability is adversely affected due to oxidative damage caused by higher cellular concentrations of reactive oxygen species (ROS) like hydrogen peroxide, superoxide, singlet oxygen (Navarri-Izzo *et al.*, 1994) produced as a result of drought stress. Reactive oxygen species due to their reactive nature negatively interact with DNA, proteins, lipids and pigments causing huge cellular damage (Ashraf, 2009).

A number of enzymatic and non-enzymatic antioxidants are produced in plants in response to abiotic stresses which save plant from oxidative damage caused by ROS (Ashraf, 2009). Major enzymatic antioxidants reported are superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) whereas, ascorbic acid (vitamin C) and tocopherols are the main non-enzymatic antioxidants exploited by plants under stressful conditions to ameliorate the adverse effects imposed by ROS (Mittler, 2002). A few studies report that exogenously applied ascorbic acid ameliorates adverse effects of drought (Singh *et al*., 2001; Amin *et al*., 2009; Dolatabadian *et al.*, 2009a; Dolatabadian *et al.*, 2010; Khalil *et al.*, 2010). However, they have applied ascorbic acid either as a foliar application or in the rooting medium under soil conditions. In the present study, ascorbic acid was applied by three different modes; pres-sowing seed treatment, rooting medium and foliar spray to wheat plants under drought stress. Objective of the study was to investigate the comparative effect of ascorbic acid in three different modes.

Materials and Methods

Two wheat genotypes, a drought tolerant cultivar, Chakwal-86 and a drought sensitive genotype 6544-6 were used in the study. Ten seeds of each genotype were sown on wet filter paper in Petri plates kept in a growth chamber. The temperature and humidity was not controlled and varied between 15-20^{0}C and 30-60%, respectively. The light intensity was maintained at 500 µmol m^{-1} s^{-1} for 10 hours daily using fluorescent tubes. A week after germination, seedlings of both genotypes were transplanted

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into plastic tubs containing Hoagland's nutrient solution (Epstein, 1972). The tubs were placed in a covered wirehouse to protect against rainfall. Aeration was maintained in the hydroponic system with the help of an electric pump. The environmental conditions during the course of experiment were 20.50-13.75ºC average day and night temperature and 41.71-76.28% average relative humidity. A week after transplantation when the seedlings were 2 weeks old, PEG_{8000} was dissolved in the hydroponic culture in three equal doses with an interval of one day until the final concentration of 20% to develop -0.6 MPa drought stress. Optimized concentrations of ascorbic acid evaluated from the preliminary trials (data not mentioned) were exogenously applied as a foliar spray, seed priming and rooting medium treatments soon after dissolving PEG in the nutrient solution. Twenty four plastic tubs were used. There were twenty plants (10 plants of each genotype) in a tub. There were 3 tubs for each treatment. The experimental plan was a completely randomized design with three replications. The treatment breakup of the 24 tubs of hydroponic system was as below:

- Hoagland's nutrient solution
- Hoagland's nutrient solution + PEG
- Hoagland's nutrient solution + 0.5*mM* AsA in rooting medium
- Hoagland's nutrient solution + PEG and 0.5*mM* AsA in rooting medium
- Hoagland's nutrient solution + 1*mM* AsA foliar spray
- Hoagland's nutrient solution + PEG and 1*mM* AsA foliar spray
- Hoagland's nutrient solution + 1*mM* AsA applied as seed priming
- Hoagland's nutrient solution + PEG and 1*mM* of AsA as seed priming

For rooting medium treatment, 0.5*mM* AsA was added to nutrient solution of the plants. Seeds were soaked for 10 h in a solution of 1*mM* AsA for seed priming. Foliar spray was prepared by dissolving 1*mM* AsA in distilled water with the addition of 0.1% tween-20 as a surfactant to increase the penetration of ascorbic acid solution into the leaves. Foliar spray was applied twice, once on 2 weeks old seedlings and then on 3 weeks old seedlings. The data for gas exchange parameters were recorded on 3rd leaf of each plant using an open system portable infra red gas analyzer (Analytical Development Company, Hoddeson, England) after five weeks of transplantation. The plants were then harvested and fresh biomass was recorded with the help of an electronic balance. Harvested fresh leaves were used for the quantification of photosynthetic pigments (Chlorophyll "a" and "b") following the procedure described by Arnon (1949). The data were subjected to analysis of variance using COSTAT software. LSD was calculated to see the differences among the means (Steel *et al.*, 1997).

Results and Discussion

A marked reduction in fresh biomass was recorded when plants of both wheat genotypes were exposed to PEG-induced drought stress. However, cultivar Chakwal-86 exhibited relatively higher shoot and root fresh biomass compared to the genotype 6544-6 (Figure 1; Table 1). Reduction in plant biomass due to water stress has been widely reported (Kamara *et al*., 2003; Kusaka *et al*., 2005; Amin *et al*. 2009). In the present investigation, ascorbic acid applied via different methods improved growth of the treated plants compared to non-treated ones which indicated that AsA helped plants to mitigate adverse effects of drought stress. The results of the present study on the role of ascorbic acid in circumventing the adverse effects of drought on plant fresh biomass are in line with some earlier reports. For example, Shalata and Neuman (2001) reported the ameliorative effect of ascorbic acid on fresh biomass of tomato seedlings when exposed to PEG or NaCl-induced osmotic stress. They found 0.5 *mM* as the most effective concentration of ascorbic acid in the rooting medium. The present study also revealed that 0.5 *mM* AsA concentration was the most effective in rooting medium. Of different modes of AsA application in the present study, rooting medium application was more effective compared to that of other modes. Athar *et al*. (2009) also applied ascorbic acid through different modes to counteract the adverse effects of salinity on the growth of wheat plants and found that rooting medium was the most effective mode of ascorbic acid application.

The chloroplast pigments, chlorophylls "a" and "b" play an important role in photochemical reactions (Taiz and Zieger, 2006). The present study showed that drought stress significantly reduced the leaf chlorophyll "a" and "b" content of drought sensitive as well as drought tolerant genotype. This is in line with what has been earlier reported in sunflower (Zhang and Kirkham, 1996; Manivannan *et al.*, 2007), rice (Pattanagul, 2011), barley (Havaux, 1998), maize Dolatabadian *et al*. 2009a), okra (Amin *et al*. 2009) and wheat (Moaveni, 2011). The decrease of chlorophyll content under water limited conditions is reported to take place because of its photo-oxidation and degradation under drought (Anjum *et al.*, 2011). Under drought stress, degradation of chlrorophyll takes place due to the increased activity of chlorophyllase enzyme (Mihailovic *et al.*, 1997). Non-stomatal decrease in photosynthesis due to drought stress in the present study may have been as a result of chlorophyll degradation (Guo and Li, 1996; Sairam *et al.*, 1998; Anjum *et al*., 2011).

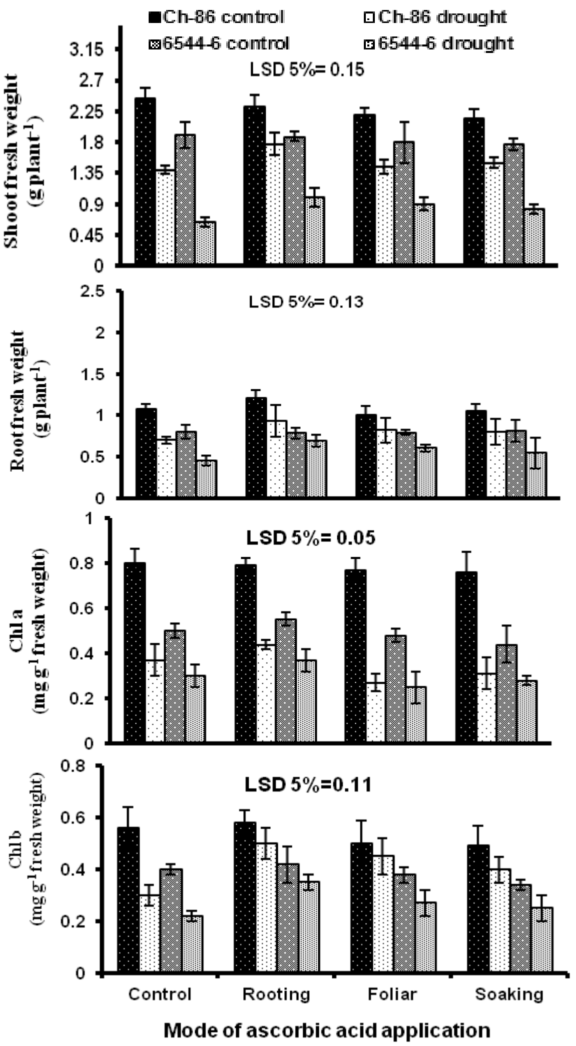

Figure 1: Comparison of shoot fresh biomass, root fresh biomass, chlorophyll a (Chl a) and chlrorophyll b (Chl b) contents of drought stressed and non-stressed 6 week old plants of two wheat genotypes, Chakwal-86 (Ch-86) and 6544-6 with ascorbic acid application through different modes (mean ± S.E.)

The cultivar Chakwal-86 maintained higher chlorophyll "a" and "b" content compared to that of genotype 6544-6 under drought stress. Earlier studies have also reported that the chlorophyll content of drought resistant genotypes of barley, wheat and maize were higher compared to the sensitive genotypes in drought stress conditions (Pastori and Trippi, 1992; Rong-Hua *et al.*, 2006). The present study revealed that exogenous application of ascorbic acid via rooting medium helped plants maintaining the chlorophyll pigments and hence mitigated the adverse effects of drought stress. These findings are in line with some earlier reports on *Cassia* (Singh *et al.*, 2001), okra (Amin *et al.*, 2009), wheat (Azzedine *et al.*, 2011), and maize (Dolatabadian *et al.*, 2009a). Reactive oxygen species produced under stress conditions have been reported to cause pigment degradation (Sairam and Saxena, 2000; Anjum *et al.*, 2011). However, ascorbic acid being an antioxidant actively scavenges these ROS, thereby reducing the chlorophyll degradation under stress (Ashraf, 2009).

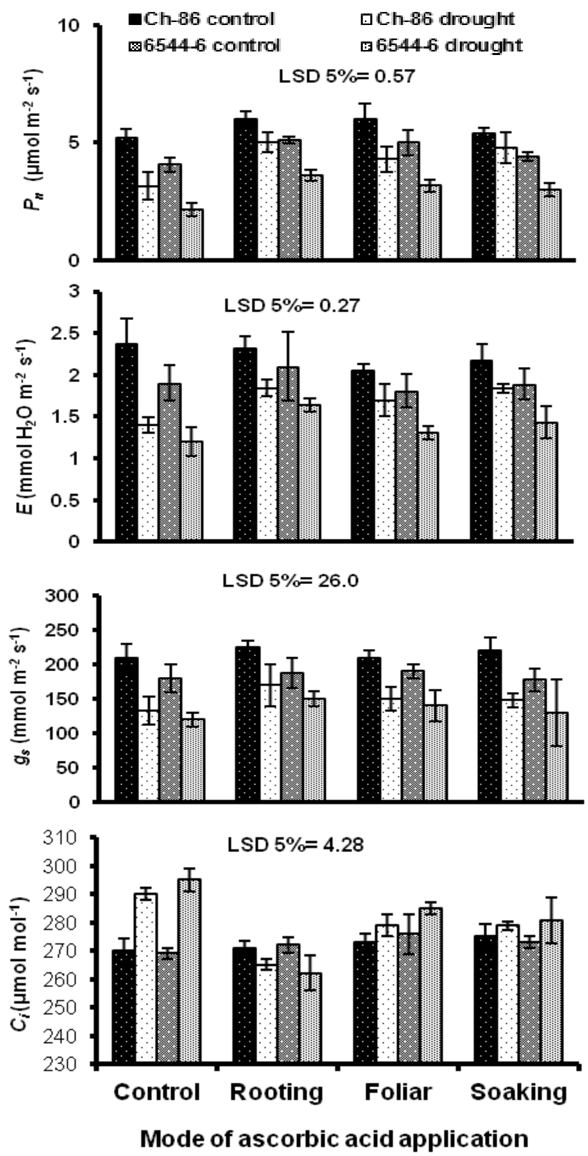

Figure 2: Comparison of net photosynthetic rate (P_n), transpiration rate (E), stomatal conductance (g_s), sub-stomatal CO_2 concentration (C_i) of drought stressed and non-stressed 6 week old plants of two wheat genotypes, Chakwal-86 (Ch-86) and 6544-6 with ascorbic acid application in different modes (mean ± S.E.)

Drought stress is also known to cause a significant reduction in the gas excahge attributes; net photosynthesis, transpiration rate and stomatal conductance of plants (El-hafid *et al*., 1998; Shah and Paulsen, 2003; Flexas *et al*., 2004; Ali and Ashraf, 2011). In the present investigation, drought stress caused a marked reduction in net photosynthesis, transpiration rate and stomatal conductance in both wheat genotypes. However, the drought tolerant cv. Chakwal-86 was superior to the drought sensitive genotype 6544-6 with respect to these gas exchange attributes (Figure 2; Table 2). Drought-induced reduction in photosynthesis and transpiration rate has been reported earlier in a number of crops including wheat (El Hafid *et al*., 1998), maize (Ali and Ashraf, 2011), and sorghum (Kreig and Hutmacher, 1983). In the present study, exogenous application of ascorbic acid mitigated the adverse effects of drought on photosynthesis in both wheat genotypes by increasing stomatal conductance. This could have also been due to the fact that ascorbic acid as an antioxidant has the ability to mitigate the negative effects of stress on plants by neutralizing harmful oxidants which have been reported to damage plant membranes such as the thylakoid membranes of chloroplasts (Miguel *et al*, 2006; Dolatabadian *et al*., 2009b). In the present investigation, ascorbic acid applied through the rooting medium was found to be more effective compared to the other modes of application in alleviating the adverse effects of drought on different gas exchange attributes of both wheat cultivars.

In the present study, ascorbic acid increased transpiration rate of wheat under drought stress compared to non-treated plants. Similar results were reported in okra plants under drought using ascorbic acid as a foliar spray (Amin *et al*., 2009). It has been reported that maintenance of water status is regulated by stomatal conductance and rate of transpiration (Ashraf, 2009). In the present study, there was a significant decline in stomatal conductance of drought tolerant as well as drought sensitive genotype under drought. Application of ascorbic acid improved the stomatal conductance of plants compared to non-treated plants.

Table 1: Mean squares from analysis of variance of data for shoot fresh weight, root fresh weight, chlorophyll a (Chl a) and chlrorophyll b (Chl b) contents of drought stressed and non-stressed 6 week old plants of two wheat genotypes (Chakwal-86 and 6544-6) with ascorbic acid application in different modes

Source	df	Shoot fresh wt	Root fresh wt	Chl a	Chl b
Main effects					
Genotype	1	3.44***	0.55***	0.62***	0.12*
Drought	1	7.83***	0.97***	0.82***	0.007ns
AsA	3	0.06ns	0.06ns	0.04***	0.01ns
Interaction					
Genotype x drought	1	0.20*	0.03ns	0.17***	0.04ns
Genotype x AsA	1	0.25***	0.04ns	0.04***	0.02ns
Drought x AsA	3	0.73***	0.26***	0.07***	0.09**
Genotype x drought x AsA	3	0.17**	0.03ns	0.02**	0.00655ns

Table 2: Mean squares from analysis of variance of data for net photosynthetic rate (P_n), transpiration rate (E), stomatal conductance (g_s), sub-stomatal CO_2 concentration (C_i) of drought stressed and non-stressed 6 week old plants of two wheat genotypes (Chakwal-86 and 6544-6) with ascorbic acid application in different modes

Source	df	P_n	E	g_s	C_i
Main effects					
Genotype	1	14.73*	0.47*	0.0007ns	43.12ns
Drought	1	23.98***	2.46***	0.067***	134.29**
AsA	3	4.72***	0.50**	0.0004ns	282.11***
Interaction					
Genotype x drought	1	0.12ns	0.34ns	0.0003ns	47.16ns
Genotype x AsA	1	0.36ns	0.18ns	0.0008ns	78.47ns
Drought x AsA	3	1.98*	0.26ns	0.0001ns	243.36***
Genotype x drought x AsA	3	0.01ns	0.17ns	0.0009ns	139.26**

*, **, *** = significant at 0.05, 0.01 and 0.001 levels, respectively, ns = non-significant

Although the ascorbic acid application was effective through all three modes, the effect of ascorbic acid through the rooting medium was relatively higher. This may be expected as in rooting medium, a continuous supply of ascorbic acid is available to the plant compared to the other two modes.

Conclusion

Overall, ascorbic acid application through rooting medium was comparatively more effective in overcoming the adverse effects of drought stress in wheat compared to that of foliar spray or seed priming treatment in terms of photosynthetic rate and plant growth. Ascorbic acid treated plants showed higher net photosynthetic rate, transpiration and stomatal conductance compared to non-treated ones. Moreover, relatively less stress-induced degradation of chlorophyll pigments took place in plants supplied with ascorbic acid. Thus, it may be concluded that exogenously applied ascorbic acid is effective in ameliorating the adverse effects of drought stress.

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