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Protective Role of Arginine Against Oxidative Damage Induced by Osmotic Stress in Ajwain (*Trachyspermum ammi*) Seedlings Under Hydroponic Culture

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Abstract
Assessing the tolerance of medicinal plants is important for planting them in drought areas. Arginine is a growth regulator and its role in plants' tolerance to environmental stresses such as drought has been investigated. To evaluate the protective effects of arginine against osmotic stress induced by polyethylene glycol in ajwain (*Trachyspermum ammi*) seedlings, an experiment was conducted as a completely randomized design in a factorial arrangement with three replicates. Experimental treatments included arginine at three levels (0, 10 and 20 µM) and osmotic stress (induced by polyethylene glycol 6000) at three levels (0, 13.5% and 17% (W/V)). Results showed that arginine application through the root medium caused the reduction of H_2O_2 content, lipid peroxidation (malondialdehyde and other aldehydes) and lipoxygenases activity and increased the antioxidant enzymes activity (calatase, ascorbate peroxidase and guaiacol peroxidase), protein content and proline content under osmotic stress. Therefore, it seems that the application of arginine greatly improves the dehydration tolerance through elevated activities of antioxidant enzymes.

Keywords: Ajwain; Antioxidant enzymes; Lipid peroxidation

Abbreviations: APX— ascorbate peroxidase; Arg— arginine; CAT— catalase; GPX— guaiacol peroxidase; LOX— lipoxygenase; MDA— malondealdehyde; ROS—reactive oxygen species; TBARS— thiobarbituric acid reactive substance.

Introduction

All civilizations have always had traditions of using herbs to promote healing. Plants still remain the basis for development of modern drugs and medical plants have been used for years in daily life to treat diseases all over the world (Canter *et al.* 2005). In recent years there has been renewed interest in natural medicines that are obtained from plant parts or plant extracts. The World Health Organization has estimated that more than 80% of the world's population in developing countries depends on herbal medicine for basic health care needs. Ajwain is one of the most important plants in this regard. Ajwain (*Trachyspermum ammi*) belongs to the family of umbellifera (Apiaceae). Ajwain is used in folk medicine as a carminative, anti-spasmodic, anti-bacterial, anti-microbial, anti-nausea and anti-inflammatory. Ajwain is the only plant in the world with the highest amount of thymol. This chemical is very effective in helping the stomach release gastric juices that speed up digestion. Ajwain is also helpful to get rid of pain due to rheumatism and arthritis. Furthermore, ajwain is also considered as a spice due to terpenic

compounds isolated from its seeds and used in preparation of various dishes (Emami and Hosseini 2008).

In aromatic plants, growth is influenced by various environmental factors such as drought stress. Though metabolic responses of food crops to dry environments have been studied but such studies are lacking in medicinal and aromatic plants (Misra *et al.* 2011). Limited water supply induces several physiological, biochemical and molecular responses in plants which are characterized by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata and decrease in cell enlargement and growth (Jaleel *et al.* 2008). Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism followed by reduction of growth, dry mater and harvestable yield in a number of plant species (Jaleel *et al.* 2008). Survival under this stressful condition depends on the plant's ability to perceive the stimulus, generate and transmit the signals and initiate various physiological and chemical changes (Maksup *et al.* 2014). Drought stress, like other abiotic stresses, can also lead to oxidative stress through the increase in reactive oxygen species (ROS) such as superoxide ($O2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$), which are highly reactive and may cause cellular damage through oxidation of lipids, proteins and nucleic acids (Velikova *et al.* 2000). Lipoxygenases (LOX) are also responsible for membrane degradation because they catalyze the dioxygenation of polyunsaturated fatty acids that are toxic to the cell. LOX-generated free radicals, singlet oxygen and superoxide anion, are known to disrupt membrane selective permeability via peroxidation of membrane phospholipids, which result in membrane leakage. Malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acids, has been utilized as a biomarker of ROS (Kubis 2006). To minimize the effects of oxidative stress, plant cells have evolved a complex antioxidant system, which is composed of low-molecular mass antioxidants (glutathione, ascorbate and carotenoids) as well as ROS-scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and glutathione reductase (GR) (Shi *et al.* 2007). The regulation of these antioxidant constituents by an exogenous substance might mediate the plant tolerance to drought stress. Among different strategies which were used to cope with drought stress, priming is an easy, low cost and low risk technique and this approach has recently been used to overcome drought stress (Nasibi *et al.* 2011).

L-arginine (Arg) is one of the most functionally diverse amino acids in living cells. In addition to serving as a constituent of proteins, arginine is a precursor for biosynthesis of polyamines (PAs), Agmatine and proline as well as the cell signaling molecules, glutamine and nitric oxide (NO) (Chen *et al.* 2004). It has been suggested that both endogenous and exogenous arginine have roles in plant stress responses such as drought and salinity (Nasibi *et al.* 2011). However, there are few researches on the effect of exogenous arginine as a precursor of these compounds for the possible antioxidative responses of plants against drought stress.

There is no report on the effect of Arg under drought stress in the Ajwain plant. Therefore, the

objective of the present experiment was to investigate the effects of arginine pretreatment on alleviation of oxidative damages induced by osmotic stress. Comparing these responses can be useful in understanding the physiological and biochemical mechanisms of this compound in plants which have to cope with the drought stress.

Materials and Methods

Plant material

Ajwain (*Trachyspermum ammi*) seeds were grown in plastic pots containing sand and compost. The seedlings were irrigated with water daily and half-strength Hoagland's solution once a week. After five weeks of growth, the uniform seedlings were transferred to bottles containing Hoagland's solution aerated with air pump and were treated with 0 (as a control), 10 and 20 µM arginine (arginine was added to the nutrient solution). After 24 h, plants were subjected to the osmotic stress. Polyethylene glycol (PEG_{6000}) compound has been used to simulate osmotic stress effects in vitro for plants to maintain uniform water potential throughout the experimental period. For this purpose, seedlings were placed in the aerated bottle containing distilled water served as a control and polyethylene glycol of 13.5% and 17% (W/V) strengths to achieve osmotic stress levels of -0.3 and -0.5 MPa. These concentrations of arginine and PEG were optimized in the preliminary experiment. After 72 h, the plant shoots were gathered and immediately frozen in liquid nitrogen and stored at -80 °C for the subsequent analyses.

Hydrogen peroxide content: H_2O_2 content was determined using the method given by Velikova *et al.* (2000). Shoot samples were extracted with 5 ml of 0.1% trichloroacetic acid (TCA) and centrifuged at 12,000 × g for 15 min. Then 0.5 ml of supernatant was mixed with 0.5 ml of 10 mM phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide and the absorbance was determined at 390 nm. The amount of H_2O_2 was calculated using the extinction coefficient (ε) of 0.28 $mM.cm^{-1}$ and expressed as µmol/g DW.

Thiobarbituric acid reactive substance (TBARS): One hundred mg of the leaf tissue of plants was homogenized in 10 ml of 0.1% TCA (W/V), and then centrifuged at 10,000×g for 15 min. One ml of supernatant was then swirled with 4 ml of 20% TCA (W/V) containing 0.5% 2-thiobarbituric acid (TBA) (W/V), and the solution was heated for 30 min at 90°C. Samples were cooled on ice for 5 min and then re-centrifuged for 10 min at 10,000×g. For malondealdehyde (MDA) measurement, the non-specific absorbance of supernatant at 600 nm was subtracted from the maximum absorbance at 532 nm and an extinction coefficient (ε) of 1.55×10^5 $M^{-1}cm^{-1}$ was used for determination of MDA concentration (Heath and Packer 1969). For other aldehydes measurement, absorbance of 600 nm was subtracted from maximum absorbance of 455 nm and the extinction coefficient of 0.457×10^5 M^{-1} cm^{-1} was used for the calculations (Meirs *et al.* 1992).

Enzyme extraction and activity determination: 500 mg of leaves were homogenized in 50 mM potassium phosphate buffer (pH 7.0) containing 1% soluble polyvinyl pyrrolidone (PVP), 1 mM ethylene diamine tetra acetic acid (EDTA) and 1 mM phenylmethylsulfonyl fluoride (PMSF) with the addition of 10 mM ascorbic acid in the case of the APX assay. All of the procedures were done at

4 °C. The homogenate was centrifuged at 20,000×g for 20 min, and the supernatant used for the assay of the activity of enzymes and protein content.

Lipoxygenase (LOX) activity (EC 1.13.11.12): LOX was estimated according to the method of Doderer *et al.* (1992). For measurement of LOX activity, the substrate solution was prepared by adding 35 µl linoleic acid to 5 ml distilled water containing 50 µl Tween 20. The solution was kept at pH 9.0 by adding 0.2 M NaOH until all the linoleic acid was dissolved and the pH remained stable. After adjusting the pH to 6.5 by adding 0.2 M HCl, 0.1 M phosphate buffer (pH 6.5) was added to make a total volume of 100 ml. LOX activity was determined by the spectrophotometer via adding 50 µl of enzyme to 2.95 ml substrate. Solution absorbance was recorded at 234 nm and the activity was expressed as a change in absorbance per minute per mg protein in the leaves.

Catalase (CAT) activity (EC 1.11.1.6): CAT activity was determined by the spectrophotometer via following the decrease of absorbance of H_2O_2 within 30 s at 240 nm (Dhindsa *et al.* 1981). The 3 ml reaction solution consisted of 50 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2, and 100 ml of enzyme extract. Addition of H_2O_2 started the reaction and the decrease in absorbance was recorded after 30 s.

Guaiacol peroxidase (GPX) activity (EC1.11.1.7): GPX activity was measured using guaiacol as a substrate. Reaction mixture (3 ml) contained 25 µl of enzyme extract, 2.77 ml of 50 mM phosphate buffer (pH 7.0), 0.1 ml of 1% H_2O_2 (V/V), and 0.1 ml of 4% guaiacol (V/V). The increase in absorbance at 470 nm due to the guaiacol oxidation was recorded for 3 min. One unit of enzyme activity was defined as the amount that causes a change of 0.01 in the absorbance per minute (Zhang *et al.* 2005).

Ascorbate peroxidase (APX) activity (EC 1.11.1.11): Ascorbate peroxidase was determined by the spectrophotometer according to the oxidation of ascorbate (ASA). The reaction solution contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM H_2O_2 and 150 µl enzyme extract. H_2O_2-dependent oxidation of ASA was followed by measuring the decrease in absorbance within 1min at 290 (extinction coefficient (ε) of 2.8 $mM^{-1}cm^{-1}$) (Nakano and Asada 1981).

Total soluble proteins: Protein content was determined according to the method of Bradford (1976) using Bovine serum albumin as the standard.

Proline determination: The proline content was determined according to Bates *et al.* (1973).

Statistical analysis: The experiment was conducted in a factorial arrangement based on completely randomized design with nine treatments and three replications. Statistical analyses were carried out by one-way analysis of variance using LSD test to evaluate whether the means were significantly different, taking $p \leq 0.05$ as significance level. Computations and statistical analysis were done using SAS and MSTATC.

Results

H_2O_2 content: The results of analysis of variance showed that osmotic stress caused a significant ($p \leq 0.01$) effect on H_2O_2 content (Table 1). The highest level of drought stress (-0.5MPa) caused an increasing of 68.1% in the H_2O_2 content as

compared to the control (Table 2). Arg pretreatment markedly alleviated the effects of osmotic stress (Table 2). The concentration of 20 µM Arg was significantly different from 10 µM Arg and non-primed seedlings under normal condition and -0.5 MPa osmotic stress (Table 2). At the osmotic potential of -0.3 MPa, there was statistically significant difference between both concentrations of Arg and control (Table 2).

Table 1. Mean squares for hydrogen peroxide content (H_2O_2), malondealdehyde (MDA), other aldehydes, Lipoxygenase (LOX) activity, Catalase (CAT) activity, Guaiacol peroxidase (GPX) activity, Ascorbate peroxidase (APX) activity, protein content, and Proline content of *Trachyspermum ammi*.

Source of variation	df	Mean squares								
		H_2O_2 content	MDA	Other aldehydes	LOX activity	CAT activity	GPX activity	APX activity	Protein content	Proline content
Arginine	2	1.79^{**}	0.054^{**}	0.373^{**}	0.026^{**}	0.289^{ns}	4.75^{**}	0.308^{**}	2.845^{**}	10.92^{*}
Osmotic stress	2	35.08^{**}	0.285^{**}	3.55^{**}	0.604^{**}	196.5^{**}	343.58^{**}	7.943^{**}	36.702^{**}	1052.25^{**}
Arginine × Osmotic stress	4	0.25^{**}	0.011^{**}	0.089^{**}	0.0025^{*}	0.062^{ns}	1.45^{**}	0.1002^{**}	0.944^{**}	2.69^{ns}
Error	18	0.039	0.001	0.013	0.0006	0.124	0.007	0.0136	0.141	2.70018
CV%		5.05	6.51	13.02	4.75	5.5	1.05	8.77	4.03	9.96

*, ** and ns denote significant differences at 0.05 and 0.01 % probability levels, and not significant, respectively.

Malondealdehyde, other aldehydes and lipoxygenase activity: MDA and other aldehydes were measured as an indicator of lipid peroxidation. The effect of both levels of osmotic stress on MDA and other aldehydes was significant (Table 1). The data showed that osmotic stress induced increasing of MDA and other aldehydes (Table 2). Water deficit at the level of -0.5 MPa increased MDA and other aldehydes by approximately 56.5 and 82.7% compared to the control, respectively (Table 2). Arg applied through root medium was more effective at both levels of osmotic stress (Table 2). Lipoxygenase is an oxidative enzyme that contributes to oxidation of polyunsaturated fatty acids. Drought had a significant effect on LOX activity (Table 1). The activity of this enzyme increased under water deficit when compared with the control (Table 2). The highest amount of this enzyme belonged to -0.5 MPa (0.825) and application of Arg reduced the activity of LOX in the drought stressed plants (Table 2). The lowest activity of this enzyme was achieved in the seedlings pretreatment with 10 µM Arg, which was statistically similar with another concentration of Arg under -0.3 MPa osmotic potential (Table 2). Pretreatment of ajwain plants with both concentrations of Arg had significant effect on reduction of LOX activity compared to the control (Table 2).

Table 2. The effect of arginine (Arg) pretreatment on hydrogen peroxide (H_2O_2) content, malondealdehyde (MDA), other aldehydes, lipoxygenase (LOX) activity, catalase (CAT) activity, guaiacol peroxidase (GPX) activity, ascorbate peroxidase (APX) activity, protein content, and proline content of *Trachyspermum ammi* under different levels of osmotic stress.

Arg × Drought	H_2O_2 content (µmol/g DW)	MDA (µmol/gDW)	Other aldehydes (µmol/gDW)	LOX activity (U/mg protein)	CAT activity (U/mg protein)	GPX activity (U/mg protein)	APX activity (U/mg protein)	Protein content (µmol/g DW)	Proline content (µmol/gDW)
0 Arg									
Control	2.15f	0.35054e	0.3093d	0.265f	1.64c	1.564f	0.299d	11.353a	2.617cd
-0.3 MPa	4.33d	0.65269b	1.3041b	0.646c	7.055b	7.609e	1.283c	8.383bc	2.483d
-0.5 MPa	6.74a	0.80753a	1.785a	0.825a	11.107a	12.314c	1.779b	6.253d	2.048e
10 µmol Arg									
Control	2.07f	0.35806e	0.252d	0.235fg	1.626c	1.505f	0.213d	11.616a	3.131ab
-0.3 MPa	3.69e	0.47742c	0.665c	0.55d	6.495b	9.1108d	1.814b	8.91b	2.639cd
-0.5 MPa	5.7b	0.62796b	1.497b	0.705b	10.768a	14.428b	2.208a	8.0746c	2.922bc
20 µmol Arg									
Control	1.69g	0.30538e	0.249d	0.212g	1.462c	1.5078f	0.355d	11.657a	3.44a
-0.3 MPa	3.58e	0.41720d	0.732c	0.504e	6.538b	9.1824d	1.691b	8.96b	2.882bc
-0.5 MPa	5.33c	0.64624b	1.297b	0.714b	10.85a	14.7584a	2.346a	8.527bc	3.4179a

Means followed by the same letter(s) in each column are not significantly different at the 5% probability level

Antioxidant enzyme activities: Change in specific activity of antioxidant enzymes is the consequence of oxidative stress. The effect of osmotic stress on CAT, GPX and APX in ajwain plant leaves, either with or without Arg pretreatment was assayed. As shown in Table 1, the effect of osmotic potential on CAT, GPX and APX activities was significant. Results showed that the activity of CAT, GPX and APX was higher in the stressed plants (especially in -0.5MPa) than control groups, which may be related to the reflection of oxidative burst under drought stress. Pretreatment of plants with 10 and 20 µM Arg increased the activity of antioxidant enzymes in those plants which were subjected to drought stress and this may be related to the key role of these enzymes in ROS detoxification under this condition. At the level of -0.5 MPa, CAT activity was increased by approximately 85.2% compared to the control condition (Table 2). Both concentrations of Arg were not significantly different from the non-primed seedlings under normal and stress conditions (Table 2). No significant differences were observed in the GPX activity between both concentrations of Arg and non-pretreated plants under normal condition (Table 2). On the basis of these results, increasing drought rose the GPX level remarkably (Table 2). The difference in the activity of this enzyme was statistically significant between control and Arg (Table 2). The data indicated that drought treatments at the potentials of -0.3 and -0.5MPa caused an increase of 76.7 and 83.2% in the APX activity, respectively compared with the control (Table 2). Pretreatment of ajwain plants with Arg had significant effect on the activity of this enzyme under drought condition (Table 2).

Protein content: Water deficit had a significant effect on the protein content of *Trachyspermum ammi* (Table 1). Osmotic stress at the level of -0.5 MPa reduced protein content by approximately 45% compared to the control (Table 2). Pretreatment of plants with Arg was more effective under severe drought stress (Table 2).

Proline content: The effect of different concentrations of PEG on proline content was shown in Table 1. The response of proline content to the interaction of Arg concentrations and drought levels were different, and the highest concentration of this trait belonged to the control (Table 2).

Discussion

Environmental stresses trigger a wide variety of plant responses, ranging from altered gene expression and cellular metabolism to changes in the growth rate and plant productivity. Among the environmental stresses, drought stress is one of the most adverse factors to plant growth and productivity. The numerous physiological responses of plants to water deficit generally vary with the severity as well as with the duration of water stress (Jaleel *et al.* 2008; Maksup *et al.* 2014). Compounds that are able to reduce the damaging effects of various stresses are prominent in both theoretical and practical points of view (Nasibi *et al.* 2011). In the present research, Arg was used as an important signal molecule for modulating plant responses to osmotic stress and participating in the regulation of physiological processes and to study the effect of this amino acid on some physiological characters under this stress.

Increased drought stress results in enhanced accumulation of reactive oxygen species (ROS) which are harmful to the plant growth due to their detrimental effects on the subcellular components and metabolism of the plant, leading to the oxidative destruction of cells and autoxidative chain reactions on unsaturated fatty acids (Gunes *et al.* 2007; Maksup *et al.* 2014). Active oxygen species cause deterioration of membrane lipids, leading to increased MDA and leakage of solutes from membranes (Gunes *et al.* 2007). Also, activity of LOX as an oxidative enzyme which contributes to the lipid peroxidation, has been reported to increase at drought conditions. MDA and other aldehydes are often used as a measure of free radical damage to cell membranes under stress conditions (Halliwel and Gutteridge 1984). At the present study, MDA and other aldehydes products of lipid peroxidation and an indicator of membrane damage were significantly increased on plants which were not pretreated with Arg under the osmotic stress (Table 2). In the case of control samples, the level of MDA and other aldehydes was nearly the same in all concentrations of Arg. Upon PEG imposition, MDA and other aldehydes' level were increased on non-Arg treated plants (Table 2). A protective effect of Arg on membrane injury has been reported under drought stress (Nasibi *et al.* 2011). It has been indicated that the role of Arg in the prevention of lipid peroxidation is related to its ability to react with lipid alcoxyl ($LO^{\cdot}$) and lipid peroxyl ($LOO^{\cdot}$) radicals and stopping the chain of peroxidation (Nasibi *et al.* 2011), which is in good agreement with our results for the decrease of TBARS content (Table 2). Several studies showed that MDA content and other aldehydes in susceptible plants were higher than in resistant plants (Halliwel and Gutteridge 1984; Juan *et al.* 2005). Maintaining the integrity of cellular membranes under stress conditions is considered an the integral part of salinity and drought tolerance mechanisms (Meirs *et al.* 1992).

Another effect of Arg on lipid peroxidation is related to the lipoxygenase activity. Lipoxygenase

is an oxidative enzyme which may contribute to the lipid peroxidation. It is a non-heme enzyme that contains a single iron atom which is thought to oscillate between ferrous (inactive) and ferric (active) forms during each cycle of catalysis. Arg was thought to inhibit enzyme activity by reducing the iron of the active site from an active Fe^{3+} to an inactive Fe^{2+} form and trapping the iron in the reduced inactive form (Zeid 2009). Our results showed that the activity of this enzyme increased at the drought-stress conditions and when we used Arg pretreatment, the activity of this enzyme decreased (Table 2).

Under normal conditions, the total amount of ROS formed in the plants is determined by the balance between the multiple ROS producing pathways and the ability of the enzymatic and non-enzymatic mechanism to deal with them. Under stress conditions, ROS formation is higher than the ability of plants to remove it and this could result in oxidative damages (Laspina *et al.* 2005). In ajwain plants under osmotic stress, CAT, GPX and APX activities were elevated over the controls, therefore, we can assume that the plant antioxidant machinery was effectively struggling against the stressful condition (Table 2). Relatively higher activities of ROS-scavenging enzymes have been reported in the tolerant genotypes when compared to the susceptible ones, suggesting that the antioxidant system plays an important role in plant tolerance against environmental stresses (Shi *et al.* 2007). In addition, our results showed that under osmotic stress, H_2O_2 content increased (Table 2). When Arg was applied before drought stress, the activity of CAT, GPX and APX increased (Table 2). In the Arg pretreatment plants, H_2O_2 content also declined, which may relate to the antioxidant enzyme activity. In many studies, it was found that the function of Arg alleviation of oxidative stress was attributed to the induction of various ROS-scavenging enzyme activities (Kubis 2006; Zeid 2009; Nasibi *et al.* 2011). Decrease in H_2O_2 content in the drought-stressed plants may be related to the CAT, GPX and APX activity. The ranges of CAT activity for scavenging of H_2O_2 is limited to peroxisome so, APX and GPX were more effective to prevent the accumulation of excess H_2O_2 in cells via ascorbate–glutathione cycle and guaiacol respectively (Foyer and Halliwell 1976). Enhanced expression of APX and GPX have been reported in cytosol as well as in cellular organelles under stressful conditions and it appears to constitute a basic mechanism of deployment for antioxidative defense in plants (Madhusudhan *et al.* 2003).

Data presented in Table 2 demonstrated that protein content decreased under the osmotic stress. Reduction of protein content was the prevalent phenomenon in drought stress, because water deficiency has a major effect on the nitrogen metabolism. There is a considerable decline in protein synthesis in the drought-stressed plants, due to the reduced number of polysomal complexes in the tissues with lower water content. In this study, the generation of ROS caused the oxidation of amino acids and could burst the protein structure; therefore, oxidative stress was the most important reason for the reduction of proteins in ajwain plants under the osmotic stress. However, Arg pretreatment increased protein contents under the stress condition (Table 2). Arg has caused the increase of protein content in tomato (Nasibi *et al.* 2011), sunflower (Nejadalimoradi *et al.* 2014) and

bean (Zeid 2009). Increasing the activation of nitrate reductase and nitrate contents caused the increase of protein content on Arg treated plants (Chen *et al.* 2004; Laspina *et al.* 2005). It seems that the character of Arg may well contribute to the protective reactions of plants, acceleration of reparative processes and the effect on protein content (Nasibi *et al.* 2011).

Metabolic acclimation via the synthesis, accumulation and transitory increase of compatible solutes are regarded as basic strategies to counteract osmotic stress symptoms by either protecting the vital enzymes and cellular macromolecular structures or detoxifying free radicals. Osmotic stress has increased the endogenous content of proline (Nayyar 2003), sugars and other osmolytes (Ahmadi and Baker 2001), pointing to its involvement in influencing the process of osmoregulation. Our experimental results revealed that exogenous Arg application greatly improves the dehydration tolerance through the increase in proline content (Table 2). However, the accumulation of proline as the osmoregulator observed at the highest concentration of Arg (20 µM) which may be related to the osmo-protectant function of arginine against free radicals and membrane damages.

Conclusion

In conclusion, PEG-induced osmotic stress could cause oxidative damage in ajwain leaves through excessive generation of ROS and exogenous Arg greatly improves the dehydration tolerance through elevated activities of antioxidant systems under the osmotic stress. Based on our results, the application of exogenous Arg can be a method to decrease water stress damages to plants. However, the application dose of Arg needs further investigation with regard to different plant species and growth stages.

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