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Effect of Acetylsalicylic Acid (Aspirin) on Salt and Osmotic Stress Tolerance in *Solanum bulbocastanum* in Vitro: Enzymatic Antioxidants

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Abstract: In this study, we investigated the role of acetylsalicylic acid pretreatment (0, 1 and 10 µM) in inducing salt and osmotic tolerance in a wild species of potato (*Solanum bulbocastanum*). To determine whether the major influence of salinity is caused by the osmotic component or by salinity induced specific ion toxicity, we compared the effects of iso-osmstic concentrations of polyethylene glycol 6000 (15%) and NaCl (80 mM NaCl) on the physiological responses of this species explants grown in the liquid Morashige and Skoog medium. Both salt and drought reduced shoot growth parameters, photosynthetic pigments and increased lipid peroxidation, electrolyte leakage, H_2O_2 level and lipoxygenase activity and the effect of NaCl was more severe than polyethylene glycol. Salinity increased Na^+ content and decreased K^+ and K^+/Na^+ ratio. Under salt and osmotic stress, the activity of superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase, catalase and glutathione reductase enzymes was increased. Acetylsalicylic acid pretreatment (especially 1µM) alleviated the adverse effects of both stresses on all parameters measured. It is concluded that pretreatment of acetylsalicylic acid appeared to induce pre-adaptive responses to salt and water stresses leading to promote protective reactions.

Key words: Acetylsalicylic acid · Enzymatic Antioxidant Systems · In vitro · Oxidative Stress · Osmotic Stress · Salt Stress · Wild Species of Potato

INTRODUCTION

Salinity continues to be one of the world's most serious environmental problems as elevated levels of NaCl are naturally present in many agricultural fields [1]. Global scarcity of water resources and the increase salinization of soil and water are having a high impact on agricultural productivity [2]. Salinity reduces plant growth, alters ionic relation by ionic and osmotic effects and induces oxidative stress [1-3]. Crop performance may be adversely affected by salinity as a result of nutritional disorders. These disorders may derive from the effect on nutrient availability, competitive uptake, transport or partitioning within the plant [2]. Although plants in nature have evolved several adaptive mechanisms to cope with the presence of salts in their environment, the understanding of these mechanisms still remains incomplete. The osmotic effect involves limited water absorption due to salinity in the rhizosphere and the ionic effect consists of intracellular toxicity or imbalance due to excess ions [2]. Abiotic stress conditions such as salinity and drought favor the accumulation of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals and hydrogen peroxide and cause oxidative stress [1, 4]. ROS interact with a wide range of molecules causing pigment co-oxidation, lipid peroxidation, membrane destruction, protein denaturation and DNA mutation [3]. 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 resulted in membrane leakage [5]. Malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acids, has been utilized as a biomarker of ROS [6, 7]. Antioxidant defense systems in plants include both enzymatic antioxidants such as superoxid dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD) and glutathione

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reductase (GR) and non-enzymatic antioxidants such as ascorbate, glutathione and carotenoids [1, 4].

The alleviation of oxidative damage and increase resistance to environmental stresses are often correlated with an efficient antioxidative system. Such systems may be induced or enhanced by the application of chemicals such as salicylic acid (SA), or its derivates. Acetylsalicylic acid or aspirin (ASA), an artificial analogue of salicylic acid, undergoes spontaneous hydrolysis to SA in aqueous solution [8]. Salicylic acid is a common plant-produced phenolic compound. Compounds in this group can function as growth regulators [8, 9]. In addition, SA could be included in the category of phytohormones [8]. Exogenous application of SA may influence a range of diverse processes in plants, including seed germination, stomatal closure, ion uptake and transport, membrane permeability, photosynthetic and growth rate [8-11]. SA is also known as an important signal molecule for modulating plant responses to environmental stresses. It is now clear that SA provides protection against a number of biotic and abiotic stresses [9, 12-14].

One of the factors that complicates the study of salt stress is the fact that salt is both an ionic and an osmotic component. The use of both an osmotic agent and salt could help to discern specific ion toxicity effects from those induced by the osmotic component. Osmotic stress effects in plant cell and tissue culture studies can be investigated by using either ionic and penetrating (e. g., NaCl and KCl), non-ionic and penetrating (e. g., mannitol and sorbitol) or non-ionic and non-penetrating (e. g., polyethylene glycol (PEG)) stress agents [15]. Among these osmotic agents, PEG is the most widely used osmoticum to study the water status of plants. It is an inert, non-ionic and non-toxic chemical of high molecular weight. PEG of high molecular weight (more than 4000) induces water stress in plants by decreasing the water potential of the nutrient solution without being taken up and without evidence of toxicity [15]. On the other hand, NaCl is an ionic and penetrating stress agent as it is well known to produce specific ionic toxicities in plant culture studies. In addition, it can be easily taken up by the cultured cells and can cause ionic as well as osmotic effects [15].

The experimental environment for salinity and drought studies should be consistent, highly controlled and monitored since fluctuating environmental conditions confound the exact plant response to salt and water stresses. And also the expression of salt and water stresses may be amplified or obscured by response to other environmental parameters. In vitro cultures can provide an effective alternative to avoid soil or environmental complexities when studying plant response to imposed stress factors and can offer a means to focus exclusively on physiological and biochemical processes which contribute to salinity and drought tolerance [15, 16].

To find genes responsible for tolerance in diseases and pests, wild species of potato are used. With respect to the various habitats of these wild species of potato and their wide range to tolerance against the various climatic conditions, studying the amount of tolerance in these species against abiotic stresses such as salinity and drought and their responses to stresses can be so effective. Present study, investigated the effects of iso-osmotic concentrations of salt (NaCl) and drought (PEG) on growth and physiological responses and also the possible role of ASA in mitigating the adverse effect of those stresses in a wild species of potato (*Solanum bulbocastanum*) which is highly tolerant of blight disease and has been used to produce a tolerant cultivar by somatic hybridization.

MATERIALS AND METHODS

Plant Materials and Treatments: True potato seeds from *Solanum bulbocastanum* (275188) (accession in brackets) were received from the United State Department of Agriculture Research Service, Inter-Regional potato Introduction Station, at Stargeon Bay, WI., USA and held at fridge temperature (4 °C) until required. Seeds were disinfested for 20 min in 30% household bleach and 70% ethanol for 1 min, rinsed 3 times with sterile distilled water, soaked for 24 h in 2g dm^{-3} filter-sterilized gibberellic acid (GA_3) solution to break dormancy, again rinsed with sterile distilled water and aseptically transferred to 0.8 % agar plates for in vitro germination.

The plants were maintained by subculture of nodal cuttings on sterile liquid medium consisting of Morashige and Skoog [17] (MS) salts and vitamins and 3% sucrose without agar (pH 5.7) in phytacone vessels (Sigma, USA). The cultures were incubated at 25±2 °C with 16/8 h D/N at 40 μmol m^{-2} s^{-1} photon flux density (cool white fluorescent light).

NaCl (80 mM), PEG (15%) and ASA (1and 10 μM) were added to the medium before being autoclaved. The water potentials of media, determined by a vapor pressure osmometer (VAPRO-5520, Wescure Inc.,Logan, Utah, USA), were -0.4 MPa for 15% PEG (6000) and 80 mM NaCl (iso-osmotic) and -0.17 MPa for control (MS medium). After adding of the iso-osmotic agents to MS medium, the final water potential of media was -0.57 MPa. For ASA pretreatments, six single nodes of this species were

cultured in phytacone vessels including: liquid MS medium and/or liquid MS medium + ASA. After one week, the explants were aseptically transferred to liquid MS medium, with or without iso-osmotic agents (15% PEG and 80 mM NaCl) with three replicates per treatment. The culture conditions were the same as above. After 4 weeks, morphological parameters were recorded for each treatment and the plant materials were frozen in liquid nitrogen and were stored at -80 °C for subsequent analysis.

Growth Parameters: Shoot length (cm) of plants from each treatment was measured. For dry weight determination samples were oven dried at 70°C for 48 h and then weighed.

Photosynthetic Pigments: The amount of photosynthetic pigments, (chlorophyll a, b, total and carotenoids), was determined according to the method of Lichtenthaler [18]. Shoot samples (0.25 g) were homogenized in acetone (80 %). Extract was centrifuged at 3000×g and absorbance was recorded at wavelengths of 646.8 and 663.2 nm for chlorophyll assay and 470 nm for carotenoids assay by a UV-Vis spectrophotometer (Cary50, Germany). Chla, Chlb, ChlT and carotenoids were calculated using the following formulas (Content of photosynthetic pigments was expressed as μg g^{-1} f w.):

Chla= (12.25 $A_{663.2}$ – 2.79 $A_{646.8}$)
Chlb= (21.21 $A_{646.8}$ -5.1 $A_{663.2}$)
ChlT= Chla + Chlb
Car= (1000 A_{470} – 1.8 Chla – 85.02 Chlb)/ 198

Mineral Content: K^+ and Na^+ contents were determined using an atomic absorption spectrophotometer (Shimadzu, model 610, Japan) after wet digestion of the ash with nitric acid and expressed as mg g^{-1} d w.

Lipid Peroxidation: The level of lipid peroxidation in plant tissues was measured by determination of malondialdehyde (MDA) [19] and other aldehydes [20] which are known to be the breakdown products of lipid peroxidation. Shoot samples (0.1 g) were homogenized in 10 ml of 0.1% Trichloroacetic acid (TCA), then centrifuged at 10000×g for 15 min. one ml of supernatant was then vortexed with 4 ml of 20% TCA containing 0.5% 2-thiobarbituric acid (TBA) and the solution was heated for 30 min at 90 °C. Samples were cooled on ice for 5 min and recentrifuged for 10 min at 10000×g.The non-specific absorbance of supernatant at 600 nm was subtracted from the maximum absorbance at 532 nm for MDA measurement and at 455 nm for other aldehyds. For the MDA and other aldehyds calculation, an extinction coefficient (ε) of $1.55\times10^5 M^{-1}$ cm^{-1} was used at 532 nm for MDA and an extinction coefficient of $0.457\times10^5 M^{-1}$ cm^{-1} was used at 455 nm which is the average of ε obtained from five other aldehydes (propanal, butanal, hexanal, heptanal and propanal dimethylacetal). Results were expressed as nano mol g^{-1} f w.

Electrolyte Leakage: The electrolyte leakage was determined as described by Ben Hamed *et al*. [21]. Shoot samples (0.2 g) were placed in test tubes containing 10 ml of double distilled water. The tubes were incubated in a water bath at 32 °C for 2 h and the initial electrical conductivity of the medium (EC_1) was measured by an EC meter (Metrhom, Swiss). The samples were autoclaved at 121 °C for 20 min to release all the electrolytes, cooled at 25 °C and then the final electrical conductivity (EC_2) of each was measured. The electrolyte leakage (EL) was calculated by using the formula: EL= (EC_1/ EC_2) ×100

H_2O_2 content: H_2O_2 content was determined using the method given by Velikova *et al*. [22]. Shoot samples were extracted with 5 ml of 0.1% TCA and centrifuged at 12000×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 1M potassium iodide and the absorbance was determined at 390 nm. The amount of H_2O_2 was calculated using the extinction coefficient 0.28μ M^{-1} cm^{-1} and expressed as μM g^{-1} f w.

Enzyme Extractions and Assays: Frozen shoot samples (0.5 g) was homogenized in 2.5 ml of 50mM phosphate buffer (pH 7.2) containing 1 mM ethylendiamine tetraacetic acid (EDTA), 1mM phenylmethanesulfonyl fluoride (PMSF) and 1% polyvinyl pyrrolidone (PVP). The homogenate solution was centrifuged at 14000×g for 15 min at 4 °C and the clear supernatant was used directly for the assay of enzyme activity and estimation of protein. Activity of enzyme was determined at 25°C with a spectrophotometer. The supernatant was used for measurement of total soluble protein according to Bradford [23] and expressed in mg g^{-1} f w. Bovine serum albumin was used as standard (data not shown).

Lipoxygenase Activity (LOX) (EC 1.13.11.12): Lipoxygenase activity was estimated according to the method of Minguez-Mosquera *et al*. [24]. For measurement of LOX activity, the substrate solution was prepared by adding 0.035 ml linoleic acid to 5 ml distilled

water containing 0.05 ml 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, 0.1M phosphate buffer (pH 6.5) was added to make a total volume of 100 ml. LOX activity was determined spectrophotometrically by adding 0.05 ml of the enzyme extract to 2.95 ml substrate. Solution absorbance was recorded at 234nm and the LOX activity was calculated using an extinction coefficient of 25000 M^{-1} cm^{-1} and expressed as unit per mg of protein. 1 unit is defined as an amount of enzyme that utilizes 1 µM linoleic acid in one min.

Superoxide Dismutase (SOD) (EC 1.15.1.1): Superoxide dismutase activity was measured by monitoring the inhibition of nitroblue tetrazolium (NBT) reduction at 560 nm [25]. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM Na-EDTA, 75 µM riboflavin, 13 mM methionine and 0.05 ml the enzyme extract. Reaction was carried out in test tubes at 25 °C under fluorescent lamp (40 W) with irradiance of 75 µmol m^{-2} s^{-1}. The reaction was allowed to run for 8 min and stopped by switching the light off. Blank and control were run in the same manner but without irradiation and enzyme, respectively. Under the experimental condition, the initial rate of reaction, as measured by the difference in increase of absorbance at 560 nm in the presence and absence of the extract, was proportional to the amount of enzyme. The unit of SOD activity was defined as the amount of enzyme that inhibits the NBT photoreduction by 50 %. SOD activity values are given in units per mg of protein.

Guaiacol Peroxidase (GPOD) (EC 1.11.1.7): Peroxidase activity was assayed by the method of Plewa *et al*. [26] using the guaicol test. The tetraguaiacol formed in reaction has a maximum absorption in 470 nm. Thus, the reaction can be readily followed photometrically. The enzyme was assayed in a solution containing 50mM phosphate buffer (pH 7.0), 0.3% H_2O_2 and 1% guaiacol. The reaction was started by adding 0.02 ml of the enzyme extract to the reaction mixture at 25°C. Guaiacol oxidation (tetraguiacol formation) was monitored by reading the absorbance at 470 nm at the moment of the enzyme extract addition and 1 min later. The difference in absorbance (ΔA_{470}) was divided by the tetraguaiacol extinction coefficient (25.5 mM^{-1} cm^{-1}) and the enzyme activity expressed as unit per mg protein. The enzyme unit was calculated for the formation of 1mM tetraguiacol for one min.

Ascorbate Peroxidase (APX) (EC 1.11.1.1): Ascorbate peroxidase activity was measured using method of Nakano and Asada [27]. Reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.15 mM H_2O_2, 0.1 mM EDTA and 0.05 ml enzyme extract. Decrease in absorbance at 290 nm was considered as oxidation of ascorbic acid and was followed 2 min after starting the reaction. One unit of APX oxidized 1mM ascorbic acid in one min.

Catalase (CAT) (EC 1.11.1.6): Catalase activity was assayed using method of Dhindsa *et al*. [28]. The enzyme activity was estimated by monitoring the decrease in absorbance of H_2O_2 at 240 nm. The assay solution contained 50 mM potassium phosphate buffer (pH 7.0) and 15 mM H_2O_2. The reaction was started by adding 0.1 ml of the enzyme extract to the reaction mixture. The change in absorbance was followed 1 min after starting of the reaction. Unit of activity was taken as the amount of enzyme which decomposes 1 mM of H_2O_2 in one min.

Glutathione Reductase (GR) (EC 1.6.4.2): Glutathione reductase activity was determined according to Foyer and Halliwell [29]. The oxidized Glutathione (GSSG)-dependent oxidation of NADPH was followed at 340 nm in a 1 ml reaction mixture containing 100 mM sodium phosphate buffer (pH 7.8), 0.5 mM GSSG, 0.1 mM NADPH and 0.05 ml extract. GR activity was calculated using an extinction coefficient of 6.2 mM^{-1} cm^{-1} and expressed as unit per mg of protein. One unit of GR activity is defined as the amount of enzyme that oxidizes 1 nM of NADPH in one min.

Statistical Analysis: The experimental design was a completely randomized design with 3 replicates of 9 treatments and repeated twice. The treatments consisted of three levels of stress (0, 80 mM NaCl and 15% PEG-6000) and three levels of ASA (0, 1 and 10 µM). The data were subjected to an analysis of variance (two ways) and the means were separated using Duncan Multiple Range test ($p = 0.05$).

RESULTS

Both NaCl and PEG adversely affected growth parameters and photosynthetic pigments and the effect of iso-osmotic salt was more severe than osmoticum (Table 1). The relative reductions in shoot height, shoot dry weight, total chlorophyll and carotenoids were 68%, 80%, 92% and 90% for salt and 58%, 73%, 36% and 28% for PEG treatment, respectively (Table 1). Significant

Table 1: The effects of ASA pretreatments (0, 1 and 10 µM) and stresses (0, 80mM NaCl and PEG (15%)) on the growth parameters and photosynthetic pigments in the shoots of *S. bulbocastanum*

ASA	Stress	shoot length (cm)	dry weight (g)	Chla (µg g^{-1} fw)	Chlb (µg g^{-1} fw)	chlT (µg g^{-1} fw)	Carotenoids (µg g^{-1} fw)
0	0	23.16±0.16b	0.095±0.0032b	59.70±0.96b	20.81±0.85a	80.52±1.76b	19.64±0.62b
1	0	24.66±0.44a	0.100±0.0023a	62.63±0.17a	22.72±0.12a	85.35±0.24a	22.62±0.21a
10	0	24.33±0.60ab	0.089±0.0031b	60.10±0.92b	21.22±0.70a	81.32±1.55b	21.80±0.15a
0	NaCl	7.33±0.33f	0.018±0.0003f	5.27±0.31f	1.12±0.12f	6.39±0.41f	1.99±0.16f
1	NaCl	13.33±0.44c	0.048±0.0003c	10.63±0.66e	4.56±0.37e	15.20±1.01e	4.07±0.32e
10	NaCl	11.66±0.88d	0.035±0.0018d	10.56±0.58e	4.50±0.45e	15.06±1.01e	3.98±0.32e
0	PEG	9.66±0.33e	0.026±0.0030e	38.20±0.61d	13.11±0.53d	51.32±1.12d	14.13±0.45d
1	PEG	13.50±0.29c	0.046±0.0005c	49.34±0.19c	15.93±0.09c	65.28±0.11c	17.31±0.41c
10	PEG	11.83±0.44d	0.036±0.0014d	48.85±0.46c	15.56±0.03c	64.42±0.43c	17.08±0.54c

*Means ± SE if followed by different letters in a column, are significantly different (p = 0.05) according to Duncan test

Table 2: The effects of ASA pretreatments (0, 1 and 10 µM) and stresses (0, 80mM NaCl and PEG (15%)) on the k^+, Na^+ and K^+/Na^+ ratio, lipid peroxidation (MDA and other aldehydes), electrolyte leakage and H_2O_2 content in the shoots of *S. bulbocastanum*

ASA	Stress	K^+ (mg g^{-1} dw)	Na^+ (mg g^{-1} dw)	K^+/Na^+	MDA (nM g^{-1} fw)	other aldehydes (nM g^{-1} fw)	electrolyte leakage (%)	H_2O_2 (µM g^{-1} fw)
0	0	41.25±0.98c	2.49±0.089d	16.19±0.18c	7.73±0.12d	0.084±0.0094d	4.73±0.14g	1.59±0.88g
1	0	45.03±0.54ab	2.57±0.028d	17.55±0.13a	6.80±0.15e	0.082±0.0060d	4.24±0.39g	0.93±0.03h
10	0	41.72±0.39c	2.63±0.033d	16.08±0.23c	7.76±0.14d	0.098±0.0015d	5.06±0.09g	0.89±0.47h
0	NaCl	22.46±0.38f	21.04±0.740a	1.07±0.05e	11.32±0.29a	0.230±0.0120a	50.00±1.15a	13.62±0.19a
1	NaCl	32.39±0.29d	17.60±0.300c	1.84±0.03d	9.26±0.12c	0.170±0.0028bc	35.73±1.07c	9.17±0.12c
10	NaCl	29.55±0.43e	18.76±0.145b	1.57±0.03d	10.16±0.09b	0.187±0.0014b	40.76±0.72b	11.53±0.08b
0	PEG	44.17±0.36b	2.71±0.049d	16.29±0.15c	9.90±0.06b	0.186±0.0026b	31.40±1.26d	7.99±0.12d
1	PEG	46.34±0.56a	2.75±0.028d	16.85±0.02b	9.19±0.04c	0.167±0.0015c	19.26±0.77f	6.45±0.056f
10	PEG	44.14±0.71b	2.62±0.043d	16.81±0.03b	9.47±0.03c	0.175±0.0017bc	25.66±0.89e	7.31±0.076e

*Means ± SE if followed by different letters in a column, are significantly different (p = 0.05) according to Duncan test

Table 3: The effects of ASA pretreatments (0, 1 and 10 µM) and stresses (0, 80mM NaCl and PEG (15%)) on the activity of LOX, SOD, GPOD, APX, CAT and GR enzymes in the shoots of *S. bulbocastanum*

ASA	Stress	LOX (U mg^{-1} protein)	SOD (U mg^{-1} protein)	GPOD (U mg^{-1} protein)	APX (U mg^{-1} protein)	CAT (U mg^{-1} protein)	GR (U mg^{-1} protein)
0	0	0.133±0.003e	0.12±0.0115e	1.95±0.11d	0.45±0.011g	0.90±0.057f	1.19±0.054f
1	0	0.093±0.0037e	0.24±0.0057d	3.10±0.06e	0.59±0.005f	1.58±0.062e	1.58±0.072e
10	0	0.096±0.0033e	0.24±0.0231d	2.88±0.06cd	0.51±0.008fg	1.51±0.049e	1.50±0.057e
0	NaCl	1.620±0.08a	0.44±0.0252b	5.30±0.54b	1.12±0.028d	4.46±0.240d	3.22±0.088b
1	NaCl	1.200±0.057b	0.57±0.0211a	10.75±0.43a	2.23±0.053a	8.92±0.260a	3.90±0.029a
10	NaCl	1.260±0.033b	0.54±0.0311a	10.90±0.66a	1.91±0.044b	7.03±0.145b	3.82±0.044a
0	PEG	0.880±0.015c	0.37±0.0115c	2.77±0.04cd	0.89±0.023e	6.26±0.310c	2.06±0.068d
1	PEG	0.720±0.0028d	0.53±0.0083a	3.09±0.35c	1.40±0.049c	8.66±0.210a	2.42±0.017c
10	PEG	0.760±0.0057d	0.56±0.012a	3.19±0.03c	1.18±0.050d	7.36±0.145b	2.36±0.029c

*Means ± SE if followed by different letters in a column, are significantly different (p = 0.05) according to Duncan test

increase in shoot Na^+ contents was observed only under salt stress conditions (8 fold vs. control) (Table 2). Shoot K^+ contents was significantly decreased under salt stress and the reverse was true under PEG stress (Table 2). Both stresses caused significant increase in the level of MDA, other aldehydes, electrolyte leakage, H_2O_2 and LOX activity and the effect of salt was more pronounced (Table 2 and 3). The increases under NaCl and PEG were respectively, 46% and 28% for MDA, 173% and 120% for other aldehydes, 900% and 560% for electrolyte leakage, 750% and 400% for H_2O_2 and 1100% and 560% for LOX (Table 2 and 3). The activity of SOD, GPOD, APX, CAT and GR enzymes was elevated under both types of stresses and the effect of salt was more pronounced

(Table 3). The increases under NaCl and PEG were respectively, 260% and 208% for SOD, 170% and 42% for GPOD, 149% and 98% for APX and 170% and 73% for GR (Table 3).

Both levels of ASA significantly improved most parameters measured and the effect of lower level (1 µM) was more pronounced. Compared with the corresponding control, 1 µM ASA increased shoot length by 82% and 40%, shoot dry weight by 58% and 76%, total chlorophyll by 138% and 27%, carotenoides by 255% and 23% under salt and drought stress, respectively (Table 1). This treatment also, reduced Na^+ uptake by 16% and increased shoot K^+ by 44% and K^+/Na^+ by 72% under salt stress (Table 2). ASA also reduced MDA by 18% and 7%, other aldehydes by 26% and 10%, electrolyte leakage by 29% and 39%, H_2O_2 by 33% and 19% and LOX by 26% and 18% under NaCl and PEG, respectively (Table 2 and 3). The activity of antioxidant enzymes was also affected by 1 µM ASA under stress and non stress conditions (Table 3). Corresponding to the relative control, the increases under salt and drought stresses were respectively, 30% and 44.3% for SOD, 102.8% and 11.5% for GPOD, 99% and 57.3% for APX, 100% and 38.3% for CAT and 21% and 17.4% for GR (Table 3).

DISCUSSION

Salinity and drought adversely affected growth, photosynthetic pigments and carotenoids. The decrease in chlorophyll concentration in stressed plants could be attributed to the increased activity of the chlorophyll-degrading enzyme, chlorophyllase, or inhibition in chlorophyll biosynthesis [30, 31]. The decrease in carotenoids under stress condition is due to the degradation of ß-carotene and formation of zeaxanthins, which are apparently involved in protection against photoinhibition [31].

At the nutrient level, the inclusion of osmoticum in the MS medium did not seem to cause severe nutrient imbalance. On the contrary, salinized plants displayed shoot Na^+ accumulation as well as decrease in K^+ and K^+/Na^+ ratio. This imbalance has been reported widely and described as a mechanism of competition between cations [1, 3, 15]. Although shoot K^+ content of this species was decreased due to NaCl stress in the growth medium, the reverse was true in PEG induced osmotic stress. Similar findings were reported in rice plants [15] Adding PEG to the growth medium is accompanied by the accumulation of cations (such as K^+) which facilitate the plant to withstand the harmful effects of water stress through osmotic adjustment [15].

The increase in oxidative stress under salt and water stress has also been reported in several plant species [1, 4, 5, 32-35]. Under present experimental conditions, NaCl induced stronger oxidative stress than the iso-osmotic PEG as it is evident from the magnitude of lipid peroxidation (MDA and other aldehydes), electrolyte leakage, H_2O_2 and also the increased activity of LOX.

Our findings illustrated that both PEG and NaCl stresses could up-regulate the antioxidant mechanisms in this species. Indeed, we observed that SOD activity increased under osmotic and saline conditions in this species. Although SOD functions as the first line of defense against ROS, its end-product is the toxic H_2O_2. Therefore, an efficient H_2O_2 scavenging system is required to enable rapid removal of H_2O_2 in the plant cells. A number of enzymes regulate H_2O_2 intracellular levels, but CAT, APX, GPOD and GR are considered the most important [1, 4]. In our research, SOD, CAT, APX, GPOD and GR activities increased during osmotic and salt stresses. The enhancement of antioxidant enzymes activity under salt and drought stress has been reported in many plant species [3, 4, 6, 9, 34, 36-39].

SA when applied at low concentration causes transient oxidative stress in plants, which acts as a hardening process, increasing antioxidant capacity of plants [9]. It is appears that SA induces redox signal (H_2O_2 as a secondary messenger) and leading to increase in antioxidant activity is linked to inhibition of CAT or APX or plasma membrane linked NADPH oxidase [9]. However, to induce antioxidant activity, low concentration of H_2O_2 is required.

In this study, ASA was effective in improving plant performance under stress and non-stress conditions. The improving effect of ASA under non-stress conditions has been reported in many plant species [9-12, 30, 40]. In wheat seedlings, SA promoted plant growth via increase in IAA and cytokinins under no stress conditions [41]. Although we did not measure the hormonal changes under ASA treatments, but we could attribute the better performance growth to more K^+ and improved K^+/Na^+ ratio in the shoot and enhanced activities of all antioxidant enzymes and photosynthetic pigments.

ASA very profoundly decreased the severity of NaCl and PEG stresses on all parameters measured by inducing the activity of SOD, CAT, APX, GPOD and GR in plants, which led to reductions in H_2O_2 content, lipid peroxidation (MDA and other aldehydes contents) and LOX activity. Maintaining the integrity of cellular membranes under stress conditions is considered an integral part of salinity and drought tolerance mechanisms [35, 42, 43]. The beneficial effect of ASA was also reflected on membrane

stability, chlorophylls and carotenides contents and ultimately growth parameters. Conclusion

The present study revealed that *S. bulbocastanum* was more salt intolerant than drought which indicated that apart from salt the induced drought stress, the toxic ions (Na^+) had contribution. Although ASA did not completely eliminate the deleterious effects of the stresses, by an enhancement in activity of antioxidant systems and reduction of oxidative stress, it did improve plant tolerance to salinity and drought stresses. Signaling compounds such as ASA that are able to reduce the effect of stresses on plants and thus increase productivity could be of great importance to restoration of natural ecosystems as well as agricultural, horticultural and forestry production systems around the world.

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