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BRIEF COMMUNICATION

Acetylsalicylic acid ameliorates negative effects of NaCl or osmotic stress in *Solanum stoloniferum in vitro*

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Abstract

The role of acetylsalicylic acid (0, 1 and 10 μM) pre-treatment in amelioration of salt and osmotic stress in a wild species of potato (*Solanum stoloniferum*) was investigated. We compared the effects of iso-osmotic concentrations of polyethylene glycol 6000 (15 %) and NaCl (80 mM) on the physiological responses of this species in explants grown in the liquid Murashige and Skoog medium. Both salt and drought reduced shoot growth parameters, photosynthetic pigment contents and increased lipid peroxidation, electrolyte leakage, H_2O_2 content and lipoxygenase activity. The effect of NaCl was more severe than that of polyethylene glycol. Salinity also increased Na^+ content and decreased K^+ content and K^+/Na^+ ratio. Under both stresses, the activities of superoxide dismutase, guaiacol peroxidase, ascorbate peroxidase, catalase and glutathione reductase enzymes were increased. Acetylsalicylic acid pre-treatment alleviated the adverse effects of both stresses on all parameters measured.

Additional key words: antioxidative enzymes, carotenoids, chlorophyll, oxidative stress, polyethylene glycol.

Salinity is a common environmental problem, and affects almost all plants functions including plant growth due to ionic and osmotic effects (Parida and Das 2005, Molassiotis *et al*. 2006, Silva *et al*. 2008). Salinity induces the accumulation of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals and hydrogen peroxide, and causes oxidative stress. Antioxidant defence systems in plants include both enzymatic antioxidants such as superoxid dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD) and glutathione reductase (GR), and non-enzymatic antioxidants such as ascorbate, glutathione and carotenoids (Parida and Das 2005).

Exogenous application of salicylic acid (SA) may influence a range of diverse processes in plants, including seed germination, growth rate, stomatal closure, photosynthesis, ion uptake and transport, membrane permeability, *etc*. (Raskin 1992, Horvath *et al*. 2007a,b). SA is also known as an important signal molecule modulating plant responses to environmental stresses. Acetylsalicylic acid (ASA), an artificial analogue of salicylic acid, undergoes spontaneous hydrolysis to SA in aqueous solution (Raskin 1992). Present study investigated the effects of iso-osmotic concentrations of NaCl and polyethylene glycol (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 stoloniferum*). Research was performed *in vitro* to allow better control of the stress conditions and environmental parameters.

Potato (*Solanum stoloniferum* Schlechtd.) seeds were received from the United State Department of Agriculture Research Service, Inter-Regional Potato Introduction Station, Stargeon Bay, WI, USA and held at temperature 4 °C until required. Seeds were disinfected 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

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Abbreviations: APX - ascorbate peroxidase; ASA - acetylsalicylic acid; Car - carotenoids; CAT - catalase, Chl - chlorophyll, GPOD - guiacol peroxidase, PEG - polyethylene glycol; ROS - reactive oxygen species, SOD - superoxide dismutase.
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Murashige and Skoog (1962; MS) salts and vitamins and 3 % sucrose (pH 5.7) in *Phytacone* vessels (*Sigma*, St. Louis, USA). The cultures were incubated at temperature of 25 ± 2 °C and 16-h photoperiod at photon flux density 40 μmol m^{-2} s^{-1} (cool white fluorescent tubes). NaCl (80 mM), PEG 6000 (15 %) and ASA (1 or 10 μM) were added to the medium before being autoclaved. The water potentials of media, determined by a vapor pressure osmometer (*Vapro-5520*, *Wescor*, Logan, UT, USA), were -0.57 MPa for MS medium with iso-osmotic solutions of PEG and NaCl, and -0.17 MPa for control MS medium. For ASA pre-treatments, six single nodes of this species were cultured in *Phytacone* vessels containing liquid MS medium or liquid MS medium + ASA. After one week, the explants were aseptically transferred to liquid MS medium, with or without 15 % PEG or 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 shoots were frozen in liquid nitrogen and stored at -80 °C for subsequent analysis.

Shoot length was measured. For dry mass determination samples were oven dried at 70 °C for 48 h. The amount of photosynthetic pigments (chlorophyll *a*, *b*, and carotenoids) was determined according to Lichtenthaler (1987). K^+ and Na^+ contents were determined using an atomic absorption spectrophotometer (model *610*, *Shimadzu*, Kyoto, Japan) after wet digestion of the ash with nitric acid. The lipid peroxidation was determined according to malondialdehyde (MDA) content (Heath and Packer 1969). The electrolyte leakage and H_2O_2 content were determined as described by Ben Hamed *et al*. (2007) and Velikova *et al*. (2000), respectively. Frozen shoot samples (0.5 g) were homogenized in 2.5 cm^{-3} of 50 mM phosphate buffer (pH 7.2) containing 1 mM ethylendiamine tetraacetic acid (EDTA), 1 mM phenylmethanesulfonyl fluoride (PMSF) and 1 % polyvinylpyrrolidone (PVP). The homogenate solution was centrifuged at 14 000 *g* for 15 min at 4 °C and the supernatant was used for the assay of enzyme activities and protein content according to Bradford (1976). The activity of lipoxygenase (LOX) (EC 1.13.11.12), superoxide dismutase (SOD) (EC 1.15.1.1), guaiacol peroxidase (GPOD) (EC 1.11.1.7), ascorbate peroxidase (APX) (EC 1.11.1.1), catalase (CAT) (EC 1.11.1.6), and glutathione reductase (GR) (EC 1.6.4.2) were measured according to the method of Minguez-Mosquera *et al*. (1993), Giannopolitis and Ries (1977), Plewa *et al*. (1991), Nakano and Asada (1987), Dhindsa *et al*. (1981), and Foyer and Halliwell (1976), respectively. The experimental design was a completely randomized design with 3 replicates of 9 treatments and repeated twice. The data were subjected to an analysis of variance (two ways) and the means were separated using Duncan Multiple Range test (DMRT; $P \leq 0.05$).

Salinity and PEG-induced drought adversely affected growth and decreased contents of chlorophylls (Chl) and carotenoids (Car; Table 1). The decrease in Chl content in stressed plants could be attributed to the increased activity of the Chl-degrading enzyme, chlorophyllase, or to inhibition in Chl biosynthesis (El-Tayeb 2005). The decrease in Car under stress condition is due to the

Table1. The effects of ASA pretreatments (1 and 10 μM), NaCl (80 mM) and PEG (15 %) treatments on the growth and physiological parameters in *S. stoloniferum*. Means (n = 6) followed by different letters in a row are significantly different ($P \leq 0.05$) according to DMRT.

Parameters	Control	ASA (1 μM)	ASA (10 μM)	NaCl	NaCl+ ASA (1 μM)	NaCl+ ASA (10 μM)	PEG	PEG+ ASA (1 μM)	PEG+ ASA (10 μM)
Shoot length [cm]	20.50b	22.00a	21.33a	10.60f	13.66e	12.83e	13.50e	15.66d	14.66d
Shoot dry mass [mg $plant^{-1}$]	83.70b	95.00a	91.70a	27.30g	56.70d	38.70f	46.00e	63.70c	53.70d
Chlorophyll *a* [mg g^{-1} (d.m.)]	64.51c	66.80a	65.54b	12.19h	16.47f	14.52g	16.50f	20.03d	18.48e
Chlorophyll *b* [mg g^{-1} (d.m.)]	40.50b	46.03a	46.24a	5.54d	7.66cd	6.64cd	6.91cd	8.03c	7.70cd
Carotenoids [mg g^{-1} (d.m.)]	30.47c	33.17a	32.47b	6.63h	8.77f	7.50g	8.72f	10.60d	9.80e
Malondialdehyde [nmol g^{-1} (d.m.)]	42.80c	44.10bc	44.66b	25.17f	37.07d	34.10e	47.07a	46.43a	47.03a
K^+ [mg g^{-1} (d.m.)]	3.08d	3.20d	3.10d	46.31a	40.40c	43.50b	3.26d	3.26d	3.43d
Na^+ [mg g^{-1} (d.m.)]	13.86a	13.80a	14.41a	0.54b	0.91b	0.78b	14.44a	14.23a	13.71a
K^+/Na^+ ratio	6.50f	5.85g	5.70g	14.75a	11.37c	13.27b	10.70c	8.40e	9.57d
Electrolyte leakage [%]	3.95f	3.93f	4.00f	18.00a	14.80c	16.50b	15.20c	11.86e	13.43d
H_2O_2 [μmol g^{-1} (d.m.)]	1.79g	0.94h	0.96h	5.70a	4.21c	4.83b	4.10c	3.06f	3.28d
LOX activity [U mg^{-1} (prot.)]	0.20g	0.18g	0.20g	0.80a	0.59d	0.69c	0.52d	0.33f	0.38e
SOD activity [U mg^{-1} (prot.)]	0.03f	0.07e	0.06e	5.88c	8.63a	7.32b	4.03d	5.44c	5.20c
GPOD activity [U mg^{-1} (prot.)]	2.22f	3.53ef	3.16f	13.37b	17.90a	16.56a	4.89de	7.10c	6.10cd
APX activity [U mg^{-1} (prot.)]	0.54e	0.92d	0.86d	1.40c	1.91b	1.78b	2.01b	3.03a	2.81a
CAT activity [U mg^{-1} (prot.)]	0.91e	2.13d	1.83de	5.81c	9.93a	8.33b	5.93c	9.51a	8.23b
GR activity [U mg^{-1} (prot.)]	3.02g	3.73e	3.40f	5.49b	6.56a	6.50a	4.34d	5.21bc	4.93c

degradation of β-carotene and formation of xanthophylls, which are apparently involved in protection against photoinhibition (Sultana *et al*. 1999). At the nutrient level, the inclusion of PEG in the MS medium did not cause severe nutrient imbalance. On the contrary, salinized plants displayed shoot Na^+ accumulation as well as decrease in K^+ and K^+/Na^+ ratio (Table 1). This imbalance has been reported widely and described as a mechanism of competition between cations (Aqueel Ahmad *et al*. 2007, Molassiotis *et al*. 2006). 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. Similarly, addition of PEG to the growth medium of rice was accompanied by the accumulation of K^+ which facilitated the osmotic adjustment (Aqueel Ahmad *et al*. 2007).

Under present experimental conditions, NaCl induced stronger oxidative stress than the iso-osmotic PEG as it is evident from the magnitude of lipid peroxidation, electrolyte leakage, H_2O_2 content and also the increased activity of LOX. Our findings illustrated that both PEG and NaCl stresses could up-regulate the antioxidant enzymes in this species. In our research, SOD, CAT, APX, GPOD and GR activities increased during osmotic and salt stresses (Table 1). The enhancement of antioxidant enzymes activity under salt and drought stress has been reported in many plant species (Sing and Usha 2003, Agarwal and Pandey 2004, Molassiotis *et al*. 2006, Eraslan *et al*. 2008).

SA, when applied at low concentration, causes transient oxidative stress in plants, which acts as a hardening process, increasing antioxidant capacity of plants (Horvath *et al*. 2007b, Wang *et al*. 2009). It appears that SA induces redox signal (H_2O_2 as a secondary messenger), which is linked to inhibition of CAT, APX or plasma membrane NADPH oxidase (Horvath *et al*. 2007b). 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 conditions. 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 and LOX activity. Similar results were also reported by Mutlu *et al*. (2009) and He and Zhu (2008) in wheat and *Lycopersicon esculentum*, respectively. Maintaining the integrity of cellular membranes under stress conditions is considered an integral part of salinity and drought tolerance mechanisms (Agarwal *et al*. 2005, Korkmaz *et al*. 2007). The beneficial effect of ASA was also reflected on membrane stability, contents of chlorophylls, carotenoids and ions, and ultimately growth parameters (Table 1).

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