BRIEF COMMUNICATION

Effects of exogenous spermine on sweet sorghum during germination under salinity

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Abstract

Seedlings of *Sorghum bicolor* (L.) Moench were subjected to 180 mM NaCl with or without 0.25 mM spermine (SPM) for 7 d. NaCl treatment resulted in the inhibition of growth and increased the content of free proline, soluble protein and malondialdehyde (MDA). Additionally, it also enhanced the activity of catalase (CAT), peroxidase (POX) in both shoots and roots, while decreased that of glutathione reductase (GR). When exogenous spermine was added to the test solution, the growth of sweet sorghum seedlings was improved, and a smaller increase in the free proline and MDA contents was observed. The addition of spermine also partially increased the activities of POX and GR, but had no effects on soluble protein content or the activity of CAT.

Additional key words: antioxidant enzymes, oxidative stress, *Sorghum bicolor*.

Polyamines (PAs), a low molecular organic polycations, interact with some negatively charged molecules, including DNA, RNA, proteins, phospholipids and pectic polysaccharides (D'Orazi and Bagni 1987, Martin-Tanguy 2001, Groppa and Benavides 2008). Therefore they affect growth, development and senescence, as well as enzyme activities (Galston and Sawhney 1990, Shevyakova *et al*. 2006, Groppa and Benavides 2007). PAs also affect plant response to salinity (Tang and Newton 2005). Putrescine (PUT), spermidine (SPD) and spermine (SPM) are the major PAs in higher plants. It was documented that SPM plays a leading role in the development of plant stress tolerance (Kuznetsov *et al*. 2006). So far, little is known about the effects of SPM in sweet sorghum. The aim of the present study was to determine whether exogenous SPM has any protective effect against salt stress in sorghum seedlings.

Sweet sorghum [*Sorghum bicolor* (L.) Moench] cv. M-81E seeds were surface sterilized with 3 % (v/v) sodium hypochlorite solution for about 15 min and then were thoroughly rinsed several times with double distilled water. Fifty seeds were placed in Petri dishes in the dark between two sheets of filter paper at 25 °C. The Petri dishes were supplied with 3 cm^3 of one of the four test solutions: *1*) double distilled water (control); *2*) 0.25 mM SPM; *3*) 180 mM NaCl; *4*) 180 mM NaCl + 0.25 mM SPM. Germination counts were taken every 24 h. Further 2 cm^3 of solutions were added to the dishes every 2 d after sowing. The experiments were done in triplicates. The shoots and roots of seedlings were separated after 7 d and their length and fresh mass were measured. The dry mass was obtained by placing the samples in an oven at 70 °C for 2 d. Samples of fresh shoots and roots were frozen quickly in liquid nitrogen in order to assay antioxidant enzymes, lipid peroxidation, soluble protein and free proline contents.

The proline content was determined spectrophotometrically at 520 nm by following the method described by Bates *et al*. (1973). The degree of lipid peroxidation was carried out by measuring the malondialdehyde (MDA) content according to the method of Madahava Rao and Sresty (2000). Total soluble protein content was measured

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Abbreviations: CAT - catalase; GR - glutathion reductase; MDA - malondialdehyde; POX - peroxidase; ROS - reactive oxygen species; SPD - spermidine; SPM - spermine.
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using the procedure developed by Bradford (1976) using bovine serum albumin as a standard. Catalase (CAT) activity was performed by the method of Chance and Maehly (1955) and determined by monitoring the reduction of H_2O_2 at 240 nm and 25 °C. Peroxidase (POX) activity was based upon the method described by Chance and Maehly (1955) and measured by the H_2O_2-dependent oxidation of guaiacol at 470 nm. Glutathione reductase (GR) activity was determined by the procedure described by Halliwell and Foyer (1978) and measured by monitoring the reduction of NADPH at 340 nm.

The application of SPM caused no obvious effect on germination under the salinity stress (data not shown). There were no distinct improvement in the elongation of shoots and roots with the application of SPM compared with the control. However, the FM and DM of shoots were slightly reduced by SPM, while there was a small increment in the FM and DM of the roots. When 180 mM NaCl was added to the germination solution, the length, FM and DM of both shoots and roots were significantly lower when compared with the control. The treatment of SPM + NaCl caused an increment of the length of both shoots and roots (43 and 11 %, respectively) together with a large increase in the FM and root DM comparing with the M-81E seedlings exposed to NaCl alone, but had no distinct effect on the DM of the shoots (Table 1). The present study provided strong evidence that SPM improved the growth of sweet sorghum under salinity. These findings were in agreement with a previous report which found that spermine was partially responsible for restoration of growth in seedlings treated with 150 mM NaCl, and was more effective than the putrescine (Benavides *et al*. 1997).

The total soluble protein of M-81E seedlings showed no distinct difference between the control and seedlings treated with SPM in either the shoots or the roots (Fig. 1*A*). When NaCl was applied, the protein content was substantially higher than that found in the control group. There was no difference between the group treated with SPM + NaCl and NaCl alone. In addition, shoots had higher total soluble protein content than roots.

It is well known that proline is a nitrogen source available for the recovery from stress and restoration of growth (Trotel *et al*. 1996). In addition, proline also acts as an osmolyte, which reduce the osmotic potential of the cell and the uptake of toxic ions (Woodward and Bennett 2005). The content of free proline increased significantly in both in shoots and roots of sweet sorghum seedlings treated with 180 mM NaCl when comparing with the control groups. However, the less increase in the proline content after SPM + NaCl treatment in both shoots and roots might be due to alleviation of the NaCl-stress (Fig. 1*B*). This was consistent with the results of Jiménez-Bremont *et al*. (2006), who found less accumulation of the proline in bean tissues under saline condition after application of polyamines. In addition, the content of free proline was greater in the shoots than in roots, which is also in accordance with the result of Jiménez-Bremont *et al*. (2006).

Tang and Newton (2005) reported that polyamine plays a vital role in reducing the oxidative stress in Virginia pine. Additionally, Hsu and Kao (2007) showed that spermine protected rice against Cd toxicity by preventing the increase of MDA and enhancing the activities of antioxidant enzymes. Similarly, we observed the increase in MDA content in seedlings treated by NaCl alone, but much less in those treated by SPM + NaCl (Fig. 1*D*). Moreover, Roberts *et al*. (1985) demonstrated that spermine had the beneficial effects of reducing membrane fluidity and protecting rigidity. This fact strengthens the possibility that the appropriate concentrations of added spermine could induce a stabilization of the plasmalemma (Hsu and Kao 2007).

Reactive oxygen species (ROS) are responsible for salinity-induced damage to macromolecules and cellular structures (Özdemir *et al*. 2004). Therefore, the roles of antioxidative enzymes, including CAT, POX and GR assume great importance. As it was shown in our study, NaCl resulted in an increase in the activity of CAT (Fig. 1*C*) and POX (Fig. 1*E*) but a decrease in GR activity (Fig. 1*F*). The results are in accordance with those of Mandhania *et al*. (2006) who reported the enhancement in the POX and CAT activities in wheat under salinity. Similarly, POX and CAT activities were increased in potato seedlings (Rahnama and Ebrahimzadeh 2005) and barley seedlings (Fedina *et al.* 2009). The lowering of GR activity in sweet sorghum seedlings under salt stress was consistent with the result of Özdemir *et al*. (2004). When exogenous SPM was added to the test solution, the activities of POX and GR increased, but no effect on the CAT activity was observed. Whereas, the salicylic acid treatments significantly inhibited CAT activity and increased POX activity under salinity (Muthu *et al*. 2009).

Table1. The effects of SPM on growth of sweet sorghum seedlings under 180 mM NaCl stress. Values are means ± SE based on 15 replicates for length and four replicates for fresh and dry masses.

Parameter		Control	SPM	NaCl	NaCl + SPM
Length [cm]	shoot	7.86 ± 0.04 a	7.86 ± 0.04 a	2.11 ± 0.02 c	3.02 ± 0.01 b
	root	12.35 ± 0.10 a	11.94 ± 0.05 b	4.59 ± 0.10 d	5.09 ± 0.02 c
FM [mg]	shoot	80.09 ± 0.45 a	74.46 ± 0.31 b	21.13 ± 0.29 d	24.71 ± 0.29 c
	root	4.94 ± 0.08 a	7.29 ± 0.18 b	1.36 ± 0.40 d	7.74 ± 0.27 c
DM [mg]	shoot	7.48 ± 0.12 a	6.71 ± 0.08 b	2.84 ± 0.23 c	5.06 ± 0.62 c
	root	3.06 ± 0.34 a	3.32 ± 0.13 a	1.22 ± 0.02 c	2.82 ± 0.11 b

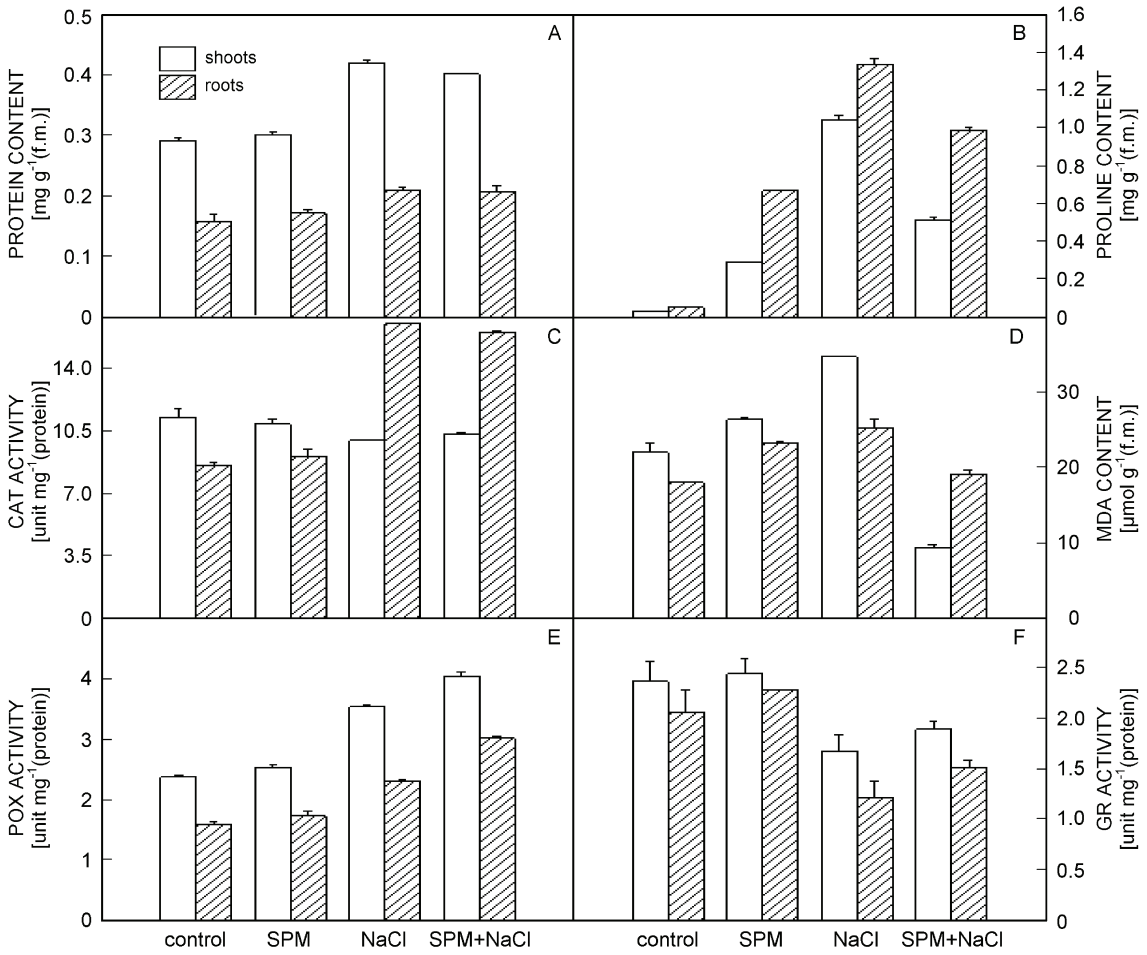

Fig.1. The effects of exogenous SPM on sweet sorghum seedlings protein content (*A*), proline content (*B*), CAT activity (*C*), MDA content (*D*) POX activity (*E*) and GR activity (*F*) under salinity stress.

The increased activity of the POX and GR under the SPM treatment indicated that SPM alleviated oxidative stress. Exogenous SPM might reduce NaCl-induced oxidative damage by restraining the Na^+ uptake from the medium.

In conclusion, exogenous SPM partially protected sweet sorghum during the germination stage under salinity stress.

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