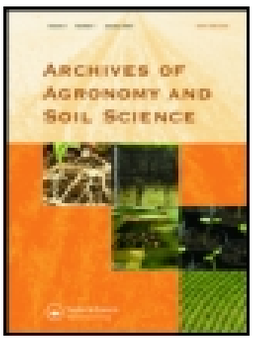

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Ameliorative effects of spermine application on physiological performance and salinity tolerance induction of susceptible and tolerant cultivars of wheat (*Triticum aestivum*)

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ABSTRACT

Polyamines are well known in environmental stress tolerance induction of plants. The present study was conducted to evaluate the interactive effects of salinity (0, 100 and 200 mM NaCl) and spermine (Spm) concentration (0, 0.5 and 1 mM solution) on physiological performance of susceptible (Sepahan) and tolerant (Neyshabour) wheat cultivars. Proline accumulation was more affected by salinity than Spm. Chlorophyll *a* and *b* content was totally improved by Spm application. Catalase and ascorbate peroxidase (APX) activity was generally increased by increasing salinity and Spm level. Highest APX activity was observed on 200 mM salinity and highest level of Spm concentration in both tolerant and susceptible cultivars. Superoxide dismutase activity was elevated with increasing salinity level and applied Spm concentration in both cultivars. Higher levels of Spm under salinity conditions showed higher activity of glutathione reductase (GR) compared with the treatment without Spm, but it reduced GR activity under normal condition. Spm application decreased sodium content in all salinity levels in both cultivar, but not with a similar trend. Higher concentration of applied Spm also enhanced potassium content. To sum up, Spm application alleviated hazardous effects of salinity stress mainly through antioxidative defense and this was more evident in tolerant cultivar.

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Antioxidants; oxidative damage; polyamines; proline

Introduction

Polyamines (PA), including spermine (Spm), spermidine (Spd) and putrescine are ubiquitous aliphatic polycations widely present in living organisms. These compounds have important roles in regulation of many fundamental cellular processes such as DNA replication, transcription, translation, cell proliferation, modulation of enzyme activities, cellular cation–anion balance and membrane stability which is mainly related to their potent-binding ability to negatively charged macromolecules in membranes (Walden et al. 1997). PA participation on tolerance induction to a wide range of environmental stress has been broadly illustrated (Wen et al. 2008). It appears that the protecting effect of PA is mostly connected with the alleviating of oxidative stress damage on plant cell structures (He et al. 2008). Mechanism of action of PA is still the matter of debate, but it is mainly due to the antioxidant property of PA. So, stress tolerance improvement is often caused by plant antioxidative defense system activity enhancement (Verma & Mishra 2005).

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Spm, which has four amino groups, is an effective scavenger than Spd, having three amino groups, suggesting the involvement of amino groups in reactive oxygen species (ROS) scavenging (Besford et al. 1993). It has been also shown that stress-tolerant plants increase endogenous PA levels to a greater extent than sensitive ones (Lee 1997).

Wheat (*Triticum aestivum* L.) is the most widely grown crop, cultivated in over 115 nations under a wide range of environmental conditions. Over the past 100 years, the yields of wheat have increased and annual global production of dry wheat in 2013 was estimated to be over 713 million ton (FAO 2013).

Salinity is an important environmental stress disrupting several physiological processes which lead to a reduced growth and productivity. Saline condition induces osmotic stress by limiting water absorption, and ionic stress is caused by high concentrations of toxic salt ions within plant cells (Kohler et al. 2009). Salinity also affects many physiological activities related to ions accumulation such as plant nutrition. Salinity often leads to increased uptake of Na^+ or decreased uptake of Ca^{2+} and K^+ in leaves which causes nutritional imbalances (Neel et al. 2002). Inhibiting Na^+ accumulation and high shoot K^+/Na^+ ratios could enhance salt tolerance in plants.

There are some reports indicating the alleviative role of PA in salinity stress. For instance, the response of susceptible and tolerant varieties of rice to exogenously application of Spm and Spd under salinity stress was evaluated, and it was shown that these PA are able to mitigate harmful effects of salinity through various mechanisms including maintaining proper K^+/Na^+ balance or triggering the level of osmolytes and activity of antioxidant enzymes (Roychoudhury et al. 2011). Rahdari and Hoseini (2013) found that Spd and putrescine reduced the detrimental effects of salinity stress in wheat seedlings by decreasing the rate of chlorophyll and protein destruction in NaCl-stressed plants. Shu et al. (2012) also announced that exogenous application of Spd improved the photosynthetic capacity of salt-stressed cucumber seedlings. In another study, when the effects of Spd on salt-stress-induced oxidative damage in two Kentucky bluegrass cultivars was examined, it was demonstrated that exogenous Spd might improve turfgrass quality and promote the salinity tolerance through reducing oxidative damages and increasing enzyme activity both at protein and transcriptional levels (Puyang et al. 2015).

Regards to the importance of PA in ROS scavenging and alleviating the salinity stress effects and in order to get better understanding of their mechanism of action on plant cells, this study was conducted to find the effect of exogenous Spm on physiological properties and antioxidant activity of wheat cultivars in the presence of four levels of salinity.

Materials and methods

Plant materials and growth conditions

The experiment was conducted under laboratory and glasshouse condition at Department of Agronomy and Plant Breeding, Ferdowsi University of Mashhad, Iran during 2011. The glasshouse environmental conditions were having a light/dark period of 16/8 h with 26–31/15–18°C day and night temperature, 48/62% relative humidity and with the illumination of about 1650 lx. To determine the interaction effects of Spm and salinity on germination and seedling growth, seeds were primed with different Spm concentrations including 0, 0.5 and 1 mM solutions. Seeds were soaked in each concentration for 10 min and then transferred to the petri dishes. Salinity solutions (control, 100 and 200 mM NaCl treatments) were also added to petri dishes. Germination percentage was recorded every day. After 10 days, shoot and root length of seedlings were recorded and seedling dry weight was also determined. In glasshouse experiment, 1 kg plastic pots were used and filled with soil–sand–litter mixture (1:1:1). Neyshabour and Sepahan wheat cultivars were selected as the tolerant and susceptible cultivars, respectively. Seeds were surface sterilized with 1% sodium hypochlorite (NaClO) for 5 min and extensively washed with distilled water. Salt-stress treatments were applied 12 days after emergence when seedlings were established and the first

leaf was completed and the second leaf appeared (Z11 stage according to the Zadoks classification). Spm treatments were applied through foliar application soon after the salinity treatments application. The treatments included salinity at three levels (control, 100 and 200 mM NaCl treatments) and Spm concentrations at three levels (0, 0.5 and 1 mM solution). Plants were harvested 41 days after planting (Z15 according to the Zadoks classification). Plant dry weight was measured after drying at 70°C for 48 h. The youngest fully expanded leaf was selected and used for biochemical analysis.

Biochemical analysis

Proline measurement

Leaf proline content was determined based on the method of Bates et al. (1973). One 100 mg sample of leaf tissue was homogenized with 10 ml of 3% aqueous sulfosalicylic acid and centrifuged at 10,000×*g* for 10 min; 2 ml of supernatant were mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin for 1 h at 100°C. The developed color was extracted in 4 ml toluene and measured calorimetrically at 520 nm. Proline content was expressed as μmol g^{-1}·fresh weight (FW).

Chlorophyll determination

Samples (100 mg leaves) were homogenized in chilled 80% (v/v) acetone and centrifuged at 10,000×*g* for 10 min at 4°C. The absorbance of the acetone extracts was measured at 663 and 645 nm. Chlorophyll *a* and *b* contents were calculated as described by Lichtenthaler (1987) using the following formula:

$$\text{Chlorophyll } a\ (\text{mg})\ \text{content on 1g of leaf} = \left\{\frac{12}{7}(\text{A}_{663}) - \frac{2}{69}(\text{A}_{645})\right\} \times \frac{\text{V}}{\text{W}} \times 1000$$

$$\text{Chlorophyll } b\ (\text{mg})\ \text{content on 1 g of leaf} = \left\{\frac{22}{9}(A_{645}) - \frac{2}{68}(A_{663})\right\} \times \frac{V}{W} \times 1000$$

Enzyme assays

Leaf tissues (100 mg FW) were placed into liquid nitrogen and then homogenized with a prechilled mortar and pestle under ice-cold conditions in 4 ml 50 mM potassium phosphate buffer, pH 7.0, with the addition of 1 mM ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 25,200×*g* at 4°C for 20 min. The supernatant was stored at −20°C and used for the assay of enzyme activity.

Catalase (CAT, EC 1.11.1.6) activity was determined by following the consumption of H_2O_2 at 240 nm for 1 min (Aebi 1984). The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2 and 50 μl of enzyme extract in a 3 ml volume. The enzyme activity was calculated using the extinction coefficient (39.4 mM^{-1} cm^{-1}) and expressed as units (1 μmol of H_2O_2 decomposed per min) per mg protein.

The reactive solution for ascorbate peroxidase (APX, EC 1.11.1.11) activity determination included 50 mM sodium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM H_2O_2 and 10 μl of enzyme extracts. The decrease in absorbance at 290 nm was read. Activity was calculated using the extinction coefficient (2.8 mM^{-1} cm^{-1}). One unit of APX was defined as the amount of degrading 1 μmol of ascorbate min^{-1} mg^{-1}·protein under the assay conditions (Nakano & Asada 1981).

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined by measuring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) (Giannopolitis & Ries 1977). The color was developed by adding the following reagents: 2.4 ml of 50 mM potassium phosphate buffer solution (pH 7.8), 0.2 ml of 195 mM methionine, 0.1 ml of 0.3 mM EDTA, 50 μl enzyme extract, 0.2 ml of 1.125 mM NBT and 0.2 ml of 60 μM riboflavin. Reaction mixtures were illuminated for 15 min at light intensity of 5000 lx. The absorbance of solution was measured at 560 nm. One unit of SOD

was defined as the amount of enzyme causing half-maximal inhibition of the NBT reduction under the assay condition.

Glutathione reductase (GR) activity was determined according to Jablonski and Anderson (1978). The reaction mixture consisted of 10 mM GSSG, 1 mM EDTA, and 200 mM phosphate buffer. The supernatant was preincubated at 25°C for 5 min. The reaction was initiated by an addition of 1 mM nicotinamide adenine dinucleotide phosphate (NADPH), and the rate of oxidation of NADPH was monitored at 340 nm. The enzyme activity is expressed as μmol·NADPH·min^{-1} mg^{-1} protein.

Ion measurements

Leaf samples were ashed and dissolved in HNO_3 and stored at 80°C for 1 h. The amounts of Na^+ and K^+ were determined using flame photometer (UK-Jenway, Serial No. 2199).

Statistical analysis

The experiment was conducted as a 3 × 3 factorial based on a completely randomized design with three replications and three seedlings on each pot. All data were analyzed using analysis of variance and the least significant difference (LSD) was calculated at $p = 0.05$ for the means comparisons.

Results

Germination properties

Results of the germination test revealed that Spm application could improve germination ability of both susceptible and tolerant cultivars under salinity stress, although the differences between the Spm concentrations were mostly not significant (Figure 1). Applied Spm levels on Sepahan cv. at 200 mM salinity not only did not improve the germination percentage, but also it was decreased lower than control treatment. Spm application did not show any significant effect on germination rate, seedling shoot and root length and seedling dry weight (data are not given).

Whole plant and leaf dry matter

Plants' biomass production was significantly affected by salinity stress and Spm application, but the response of tolerant and susceptible cultivars were different (Tables 1 and 2). Total dry matter

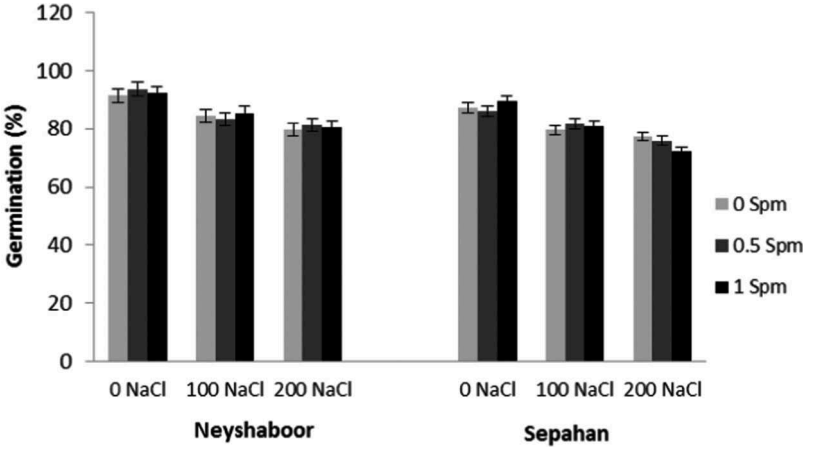

Figure 1. Germination percentage of cultivars Neyshabour (tolerant) and Sepahan (susceptible) under different levels of salinity and Spm.

Table 1. Effects of salinity levels and Spm application on dry matter production, relative water content, proline and chlorophyll content of salinity tolerant wheat cultivar (Neyshabour).

Treatments	Total dry matter ($g \cdot plant^{-1}$)	Leaf dry matter ($g \cdot plant^{-1}$)	Relative water content (%)	Proline ($\mu mol\ g^{-1} \cdot FW$)	Chlorophyll *a* content ($\mu g\ g^{-1}$)	Chlorophyll *b* content ($\mu g\ g^{-1}$)	Chlorophyll *a*/*b* ratio
0NaCl ± 0Spm	14.28 ± 0.80	10.43 ± 0.59	75.40 ± 1.38	1.94 ± 0.20	751 ± 26.51	571 ± 27.50	1.31 ± 0.02
0NaCl ± 0.5Spm	16.39 ± 0.92	11.96 ± 0.67	77.11 ± 1.41	1.61 ± 0.16	822 ± 29.01	585 ± 28.01	1.40 ± 0.02
0NaCl ± 1Spm	14.45 ± 0.81	10.55 ± 0.59	76.74 ± 1.40	1.84 ± 0.19	812 ± 29.01	598 ± 28.50	1.36 ± 0.02
100NaCl ± 0Spm	11.95 ± 0.66	8.72 ± 0.49	68.81 ± 1.26	4.15 ± 0.42	580 ± 20.50	392 ± 18.50	1.48 ± 0.02
100NaCl ± 0.5Spm	12.97 ± 0.72	9.47 ± 0.53	70.11 ± 1.28	4.84 ± 0.49	562 ± 20.01	378 ± 18.50	1.49 ± 0.02
100NaCl ± $1Spm_3$	13.78 ± 0.77	10.06 ± 0.57	70.2 ± 1.28	5.25 ± 0.53	623 ± 22.01	415 ± 20.00	1.5 ± 0.02
200NaCl ± 0Spm	8.79 ± 0.49	6.41 ± 0.36	64.19 ± 1.17	12.9 ± 1.29	412 ± 14.50	242 ± 11.50	1.70 ± 0.03
200NaCl ± 0.5Spm	8.31 ± 0.47	6.07 ± 0.34	65.25 ± 1.19	12.57 ± 1.26	422 ± 15.01	264 ± 12.50	1.6 ± 0.02
200NaCl ± 1Spm	9.34 ± 0.52	6.82 ± 0.38	65.10 ± 1.19	15.46 ± 1.55	454 ± 16.01	273 ± 13.00	1.66 ± 0.03
LSD (5%)	1.48	1.07	4.14	2.24	68.9	49.98	0.04

Data represent the mean ± SE of three replicates. Treatments were applied in mM concentrations.

Table 2. Dry matter production, relative water, proline and chlorophyll content of susceptible wheat cultivar (Sepahan) plants under different levels of salinity and Spm.

Treatments	Total dry matter ($g\ plant^{-1}$)	Leaf dry matter ($g\ plant^{-1}$)	Relative water content (%)	Proline ($\mu mol\ g^{-1} \cdot FW$)	Chlorophyll *a* content ($\mu g\ g^{-1}$)	Chlorophyll *b* content ($\mu g\ g^{-1}$)	Chlorophyll *a*/*b* ratio
0NaCl ± 0Spm	13.51 ± 0.76	9.66 ± 0.54	73.8 ± 1.90	1.43 ± 0.07	782 ± 27.50	518 ± 18.08	1.51 ± 0.01
0NaCl ± 0.5Spm	15.2 ± 0.86	10.87 ± 0.61	72.44 ± 1.86	1.32 ± 0.06	769 ± 26.50	504 ± 18.08	1.52 ± 0.01
0NaCl ± 1Spm	15.41 ± 0.87	11.02 ± 0.62	75.69 ± 1.95	1.51 ± 0.07	793 ± 27.50	533 ± 19.08	1.48 ± 0.01
100NaCl ± 0Spm	10.13 ± 0.57	7.24 ± 0.41	65.52 ± 1.68	2.93 ± 0.14	493 ± 17.50	310 ± 11.06	1.59 ± 0.01
100NaCl ± 0.5Spm	11.20 ± 0.63	8.02 ± 0.45	66.77 ± 1.71	3.34 ± 0.16	512 ± 18.00	324 ± 11.06	1.58 ± 0.01
100NaCl ± $1Spm_3$	11.07 ± 0.62	7.92 ± 0.45	65.86 ± 1.69	3.27 ± 0.16	550 ± 19.50	304 ± 10.54	1.81 ± 0.01
200NaCl ± 0Spm	7.26 ± 0.41	5.19 ± 0.29	60.7 ± 1.56	8.43 ± 0.41	356 ± 12.50	198 ± 7.02	1.79 ± 0.01
200NaCl ± 0.5Spm	7.94 ± 0.45	5.67 ± 0.32	61.59 ± 1.58	9.12 ± 0.45	336 ± 12.00	215 ± 7.55	1.56 ± 0.01
200NaCl ± 1Spm	6.97 ± 0.39	4.98 ± 0.28	60.39 ± 1.55	9.89 ± 0.48	149 ± 5.00	118 ± 7.55	1.26 ± 0.01
LSD (5%)	0.99	0.82	2.52	1.51	52.36	40.16	0.02

Data represent the mean ± SE of three replicates. Treatments were applied in mM concentrations.

(TDM) was reduced with increasing salinity level and the lowest amount of TDM was obtained under 200 mM salinity for both cultivars ($p \leq 0.01$). But the interaction of Spm and salinity was more sensible in Neyshabour (tolerant) cultivar (Table 1). The trend of leaf dry matter was also similar to whole plant trend (Tables 1 and 2).

Relative water content and proline content

Spm application showed different effects on relative water content (RWC) of wheat plants under saline and normal conditions. RWC did not have a similar or specific trend for both cultivars, but when plants were exposed to salinity stress, Spm enhanced RWC in almost all treatments of both cultivars. Higher concentration of Spm reduced the amount of RWC (Tables 1 and 2).

Proline accumulation was more affected by salinity than Spm. In Neyshabour cultivar and under non-saline condition, applied Spm decreased proline content to a lower level than control treatment in the highest concentration of Spm (Table 1). Salinity-stressed plants showed higher accumulation of proline with increasing the level of salinity and Spm.

Chlorophyll content

Under normal condition, chlorophyll *a* and *b* contents were generally improved by Spm application, but it was not significant among Spm concentrations in both cultivars (Tables 1 and 2, $p \leq 0.05$). Plants exposed to salinity caused a substantial decline in chlorophyll *a* and *b* contents.

Spm application showed an alleviative effect and raised chlorophyll content, especially in higher concentration. There was a decreasing trend in chlorophyll *a* content of Sepahan cultivar in 200 mM salinity and highest Spm concentration. An approximately similar trend was observed for chlorophyll *b* content. The ratio of chlorophyll *a*/*b* did not reveal considerable difference between the treatments and only the differences of ratios at 200 mM salinity were significant for both varieties (Tables 1 and 2, $p \leq 0.05$).

Antioxidative system response

Catalase

An increased amount of CAT activity was observed as the salinity levels were raised (Tables 3). The positive interaction of salinity and applied concentrations of Spm was significant and it was more considerable in higher levels of salinity and Spm ($p \leq 0.01$).

Ascorbate peroxidase

Activity of APX was also enhanced due to the interactions of salinity and Spm. Highest APX activity was observed on 200 mM salinity and 1 mM Spm level in both cultivars (Tables 3, $p \leq 0.05$). It should be taken into account that the activity of APX in tolerant cultivar (Neyshabour) was generally higher than susceptible cultivar (Sepahan).

Superoxide dismutase

Increased levels of salinity and Spm were followed by an enhanced activity of SOD (Tables 3). The highest concentration of Spm caused the highest amount of SOD activity between salinity-stressed plants, but under non-saline condition, this concentration reduced the activity of SOD.

Glutathione reductase

Activity of GR was raised with increasing salinity level, but stressed plants responded differently to various levels of applied Spm in both cultivars (Tables 3). Similar to SOD, higher levels of Spm under salinity conditions showed higher activity of GR compared with the treatment without Spm, but it reduced GR activity under normal condition. The difference between GR activity of tolerant and susceptible cultivars was also substantial.

Sodium and potassium content

Leaf sodium content of wheat plants under salinity stress and normal condition did not show any particular trend in different concentrations of Spm (Tables 3). In tolerant cultivar (Neyshabour) and

Table 3. Antioxidants activity (CAT, APX, SOD and GR) and sodium and potassium content of salinity tolerant wheat cultivar (Neyshabour) under different levels of salinity and Spm.

Treatments	CAT activity ($\mu mol\ min^{-1}\ \mu g^{-1}$ leaf protein)	APX activity ($\mu mol\ min^{-1}\ \mu g^{-1}$ leaf protein)	SOD activity ($Units\ mg^{-1}$ protein)	GR activity ($\mu mol\ min^{-1}\ mg^{-1}$ leaf protein)	Na^+ content ($\mu g\ g^{-1}$)	K^+ content ($\mu g\ g^{-1}$)
0NaCl + 0Spm	5.55 ± 0.27	0.65 ± 0.04	40.39 ± 2.63	8.77 ± 0.48	40 ± 2.0	530 ± 15.01
0NaCl + 0.5Spm	6.20 ± 0.30	0.81 ± 0.04	42.84 ± 2.79	6.90 ± 0.38	47 ± 3	517 ± 14.53
0NaCl + 1Spm	5.32 ± 0.26	0.77 ± 0.04	40.38 ± 2.63	8.43 ± 0.46	43.33 ± 2.5	525 ± 14.53
100NaCl + 0Spm	8.23 ± 0.40	0.83 ± 0.05	38.19 ± 2.49	9.33 ± 0.51	212.3 ± 12.5	304 ± 8.50
100NaCl + 0.5Spm	9.80 ± 0.47	0.9 ± 0.05	41.17 ± 2.68	9.92 ± 0.54	218.3 ± 13.5	319 ± 9.02
100NaCl + $1Spm_3$	9.19 ± 0.44	0.93 ± 0.05	43.28 ± 2.82	10.87 ± 0.60	202.3 ± 12.5	289 ± 8.02
200NaCl + 0Spm	10.16 ± 0.49	1.11 ± 0.06	55.50 ± 3.62	13.41 ± 0.73	422.7 ± 25	167 ± 4.51
200NaCl + 0.5Spm	9.90 ± 0.48	1.02 ± 0.06	53.83 ± 3.51	15.41 ± 0.84	404.7 ± 24	180 ± 5.51
200NaCl + 1Spm	10.89 ± 0.52	1.14 ± 0.06	57.30 ± 3.74	15.21 ± 0.83	416.7 ± 25	191 ± 5.51
LSD (5%)	1.04	0.12	8.1	1.9	55.3	29.80

Data represent the mean ± S.E. of three replicates. Treatments were applied in mM concentrations.

Table 4. Antioxidants activity (CAT, ASP, SOD and GR) and sodium and potassium content of susceptible wheat cultivar (Sepahan) under different levels of salinity and spermine.

Treatments	CAT activity ($\mu mol\ min^{-1}\ \mu g^{-1}$ leaf protein)	ASP activity ($\mu mol\ min^{-1}\ \mu g^{-1}$ leaf protein)	SOD activity (Units mg^{-1} protein)	GR activity ($\mu mol\ min^{-1}\ mg^{-1}$ leaf protein)	Na^+ content ($\mu g\ g^{-1}$)	K^+ content ($\mu g\ g^{-1}$)
0NaCl + 0Spm	4.13 ± 0.16	0.43 ± 0.03	54.30 ± 3.61	5.32 ± 0.26	35.33 ± 1.53	255 ± 20.07
0NaCl + 0.5Spm	4.02 ± 0.16	0.51 ± 0.04	60.07 ± 3.99	5.89 ± 0.29	44.33 ± 2.52	244 ± 19.08
0NaCl + 1Spm	4.24 ± 0.17	0.37 ± 0.03	59.18 ± 3.94	5.44 ± 0.27	47.33 ± 2.52	260 ± 18.08
100NaCl + 0Spm	5.91 ± 0.23	0.55 ± 0.04	60.19 ± 4	6.43 ± 0.31	265.6 ± 12.58	144 ± 11.53
100NaCl + 0.5Spm	5.42 ± 0.22	0.61 ± 0.05	58.11 ± 3.86	6.30 ± 0.31	284.6 ± 13.58	154 ± 10.54
100NaCl + $1Spm_3$	6.91 ± 0.28	0.70 ± 0.05	64.28 ± 4.28	9.07 ± 0.44	254.6 ± 12.58	122 ± 11.53
200NaCl + 0Spm	7.93 ± 0.31	0.83 ± 0.06	72.05 ± 4.79	10.11 ± 0.49	474.6 ± 23.12	144 ± 6.03
200NaCl + 0.5Spm	8.26 ± 0.33	0.7 ± 0.05	70.22 ± 4.67	9.22 ± 0.45	491 ± 23.64	154 ± 7.02
200NaCl + 1Spm	8.47 ± 0.33	0.84 ± 0.07	74.03 ± 4.72	10.89 ± 0.53	472 ± 22.65	122 ± 5.51
LSD (5%)	0.66	0.16	9.82	1.09	48.95	66.38

Data represent the mean ± S.E. of three replicates. Treatments were applied in mM concentrations.

at 100 mM salinity, lowest content of sodium was achieved in 0.5 mM Spm level, but in 200 mM salinity, 1 mM Spm concentration leaded to the lowest level of sodium content (Table 3). In susceptible cultivar (Sepahan), this trend was not similar and lowest level of sodium content was observed in highest level of Spm in both levels of salinity (Table 4). Although the decreasing trend of sodium was not accompanied with increasing trend of potassium content in all treatments, the general trend showed higher content of potassium in the treatments with reduced amount of sodium content. It seems that Spm effects on sodium and potassium content under saline conditions depended on applied concentration of Spm and also the level of salinity.

Discussion

Exogenous application of PA is known as an appropriate and efficient approach for enhancing salinity tolerance of crops which improves crop productivity under salinity stress (Chattopadhayay et al. 2002). It was shown that exogenous application of Spm could improve the germination properties and seedling dry weight of sunflower under salinity stress (Mutlu & Bozcuk 2000). Regards to the results of the germination test, it seems that at this level of salinity and for this cultivar Spm application was not efficient enough to improve the germination rate and other parameters related to germination properties.

Exogenous PA application has been used efficiently for enhancing salinity tolerance of different species (Verma & Mishra 2005). Growth and dry-matter accumulation of evaluated cultivars were enhanced due to the Spm application. These results might indicate that Spm application was an enhancing factor in dry-matter accumulation of wheat under saline and non-saline conditions, but this role was more pronounced under the latter condition.

Proline accumulation is one of the most frequently reported modifications induced by salt stress in plants, and it is often considered to be involved in stress-resistance mechanisms. Proline serves as a membrane protectant and accumulates in the cytoplasm at a higher concentration under stress conditions without interrupting the cellular structure and metabolism due to its zwitterion characteristics (Goyal & Asthir 2010). It was well established that proline could alleviate salt-stress-induced damages through several physiological mechanism (Sivakumar et al. 2000). The role of Spm in proline accumulation enhancement was more obvious under saline conditions. It is also necessary to note that tolerant cultivar showed higher concentration of proline and so better coping ability.

Chlorophyll degradation is one of the most common consequences of salinity stress in plants. The role of PA in ameliorating the harmful effects of salinity on chlorophyll content was studied and reported by some researchers (Demetriou et al. 2007; Roychoudhury et al. 2011). It was also reported that exogenous Spd prevented chlorophyll loss induced by salinity stress conditions

(Anjum 2010). Results of this experiment regards to chlorophyll content are also supported in the previous reports, although the trend was not completely similar.

Environmental stresses such as salinity cause serious damages to cellular structures through the production and accumulation of ROS. Under normal conditions, ROS are mostly controlled by enzymatic and non-enzymatic antioxidants. However, an overproduction of ROS under stress conditions could impose serious cellular damages to cellular structures (Mittler 2002; Zhou et al. 2008). It has also been documented that the levels of antioxidant enzymes are higher in tolerant than in sensitive species under various environmental stresses (Bor et al. 2003; Turkan et al. 2005). Based on the results of this experiment, activity of enzymatic antioxidants was amplified due to salinity. Interactions of salinity and Spm application were also significant and enhanced antioxidants activity which amplified the resistance of wheat cultivars under saline conditions. Tolerant cultivar also revealed higher activity of enzymatic antioxidants compared with susceptible cultivar which could improve the ability of plant to cope with stress conditions.

It was clearly shown that enhanced PA biosynthesis can alleviate hazardous effects of salinity by scavenging free radicals, stabilizing membrane and cellular structures and most importantly, cation–anion balance maintenance (Tonon et al. 2004). In a very recent study, exogenous Spd was applied on two salt-tolerant and salt-sensitive rice cultivars under salinity stress and it was observed that seed priming with Spd improved salt-induced reductions in growth. Salt-stress-induced pronounced increases in Na^+/K^+ ratios which were significantly reduced in the seedlings grown from Spd-primed seeds. Priming rice seeds with Spd also resulted in seedlings which possessed greater antioxidant capacity than the control seedlings (Chunthabureea et al. 2015). In another study, when ginseng seedlings were exposed to saline conditions, application of Spd (0.01, 0.1 and 1 mM) to the salinized-nutrient solution showed increased plant growth by preventing chlorophyll degradation and increasing antioxidant enzymes activity such as CAT, APX and GPX (Parvin et al. 2014). Amri et al. (2011) also confirmed the protective effect of foliar applied Spd and putrescine on salt-stressed pomegranate grown under greenhouse conditions. Different aspects of the role of PAs in the abiotic stress of plants were comprehensively discussed by Gupta et al. (2013) and they declared that complicated role of PAs under saline conditions, especially their role as signaling molecule.

Enhanced sodium accumulation in plants grown under saline conditions was clearly documented (Tuna et al. 2008). Furthermore, the antagonistic effect between sodium and potassium is a common process under saline conditions (Pirlak & Esitken 2004). Discussion about the effects of Spm application on salinity tolerance of plants is controversial. For instance, Verma and Mishra (2005) found that salinity-caused reduction in seedling growth of *Brassica juncea* was alleviated by exogenous putricine. In contrast, in rice shoots, Krishnamurthy and Bhagwat (1989) observed that salinity caused excessive accumulation of putricine with little change in Spd and Spm content in salinity-sensitive cultivars, whereas in salinity-tolerant cultivars, the same stress induced a remarkable increase in Spd and Spm content and decreased putricine. Chattopadhayay et al. (2002) found that salinity-induced injury of rice plants was greatly mitigated by Spd or Spm which was added to the salinized nutrient solution. On the other hand, it has been reported that putricine, Spd and Spm did not alleviate salinity-induced growth suppression in rice (Ndayiragije & Lutts 2006).

Conclusion

In general, although seed priming with Spm showed a slight effect on germination process on both cultivars, it can be concluded that Spm application was an effective approach in salinity tolerance induction of wheat cultivars mostly through the activation of enzymatic antioxidants and increasing osmolytes production.

Disclosure statement

No potential conflict of interest was reported by the authors.

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