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Exogenous polyamines improve seed germination of borage under salt stress via involvement in antioxidant defenses

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Abstract: Salinity stress is one of the most serious factors limiting crop productivity. Recently, various compounds exploited to alleviate the plant stress. Polyamines are recognized as groups of phytohormones to regulate the plant tolerance to salt stress, depending on its concentration and plant species. For this reason, an experiment was carried out in a factorial design with two factors include salinity, which was applied to the root medium as NaCl (0, 40, 80 and 120 mM), and polyamines priming (Putrescine, Spermidine and Spermine) in 40 ppm concentration for 24 h. Results revealed that salinity significantly decreased final germination percentage, energy of germination and vigor index. Salinity stress also delayed seed germination processes, in which mean germination time and T50 increased. Seedling length, seedling fresh and dry weight were also negatively affected by salt stress. Seed priming with polyamines improved all of the mentioned traits. In this respect, spermidine and spermine were superior treatments. Biochemical analysis of borage plants showed that polyamines stimulated the catalase and Superoxide dismutase activity under salt stress, which led to amelioration of stress-induced Malondialdehyde accumulation. Total protein and soluble sugar contents also are negatively affected by salinity, however, polyamines alleviated the adverse effects of salinity on proteins only. In general, these results indicated that polyamines priming (especially Spm and Spd) could be as an effective method to improve salt stress tolerance of borage seeds.

Key words: *Antioxidant enzyme; Borage; Germination behavior; Polyamine; Salinity*

1. Introduction

Borage (*Borago officinalis* L.) is a medicinal plant with high value as a vegetable crop, which is an annual member of the Boraginaceae family. The leaves of borage are reportedly used as diuretic, demulcent, emollient, expectorant, etc. (Berti et al., 2010). Knowing the importance of borage and its ever-increasing demand for pharmaceutical industry, the necessity of sowing this plant in a large amount and commercially is considered. Borage is extensively cultivated in arid and semi-arid area of the world. So, Iran has a great potential to be one of the most producers of this crop. But one of the most problematic issues which restrain its production is insufficient water supply to support seed germination and seedling growth, the most sensitive stage for water shortage. Therefore, irrigation of borage field at early stages of growth (seed germination and seedling establishment) is crucial. Surface and groundwater available for irrigation in semi-arid regions is relatively saline, causing salt and drought stress, which potentially results in harmful effects on germination and seedling establishment. Similar to other medicinal plans, borage seed germination and their seedling establishment are difficult in the field, especially when this growing stage coincide with unfavorable conditions, such as salt stress (Maher et al., 2014). Jaffel-Hamza et al. (2012) found that salinity constraint significantly reduced borage plant growth and suppressed seed yield. Willenborg et al. (2005) reported that salinity stress caused notable reduction in germination, growth and the suitable establishment of plants. So, quick germination and stand is a vital characteristic especially under this condition that can be positively influenced later stages.

A common way to improve seed germination and seedling establishment and consequently field performance of medicinal plants is seed priming. During priming, seeds are partially hydrated, so pre-germinative metabolic activities proceed, while radicle protrusion is prevented (Bradford, 1986). It not only hastens and synchronizes seedling emergence but also enhances their tolerance to biotic and abiotic stresses during the critical phase of seedling establishment (Shakirova et al., 2003). Various priming treatments have been developed to invigorate seeds (Basra et al., 2006; Farooq et al., 2007). Recently, incorporation of plants hormones in priming solution were used as the best strategy for conserving the productivity of field crops in environmental stresses.

Literature showed that under saline conditions, plants undergo changes in their environment. For instance, Munns and Tester (2008) reported that plants species often respond to salinity by increasing the concentration of compatible solutes that lead to protection of cell proteins and membranes. Many reports have indicated that salinity greatly enhanced

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accumulation of polyamines (PAs) and plants tolerance to stress is correlated with their capacity to enhance those synthesis and accumulation. Polyamines, polycationic nitrogenous compounds can associate with anionic membrane components, such as phospholipids, thereby stabilizing the bilayer surface and retarding membrane deterioration under stress conditions (Shi and Chan, 2014). Several reports showed that exogenous PAs were effective in alleviation of adverse effect of abiotic stress, including water stress, hypoxia stress and salt stress in plants (Shi et al., 2013; Shi and Chan, 2014, Mustafavi et al., 2015). Nevertheless, beneficial effects of PAs under stress could not be predicted and may be affected by several factors. Several lines of research have demonstrated that under stress, different plant species vary in their response to polyamine application. Which of the three PAs plays central roles in stress responses of plants may depend on plant species and the types of stress (Shi and Chan, 2014). To the best of our knowledge, no study has been conducted concerning changes germination behavior in salt-stressed borage plants to exogenously applied PAs. So, the present work was conducted to determine the effects of these factors on goal plants. In addition, literatures mainly showed antioxidative effects of PAs at vegetative stages and no research focused on its effect at germination and early stages of seedling establishment.

2. Materials and methods

In order to determine the effects of polyamines priming on seed germination, seedling growth and antioxidant enzymes activities of Borage plants under saline condition, a laboratory experiment was conducted in University of Maragheh, Iran. A Factorial based on complete randomized design (CRD) with three replications was used to arrange the treatments. Seeds of borage were obtained from Pakan Bazr Company of Isfahan, Iran. These seeds were divided into four sub-samples. A sub-sample was kept as control (unprimed) and the three other sub-samples were primed with 40 mg L^{-1} putrescine, Spermine and Spermidine for 24 h at 25 °C. After priming, seeds were washed with tap water and then dried for about two hours at room temperature (20-25 °C).

Seeds were placed in Petri dishes (15 seeds per Petri dish) between layers of moist Whatman at 25 °C in germinator. Drench of Petri dishes was performed according to salinity treatments. Germination was observed daily according to the Association of Official Seed Analysts (AOSA) method (AOSA, 1990). Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981) as under:

$$MGT = \frac{\sum Dn}{\sum n} \qquad (1)$$

Where n is the number of seeds, which were germinated on day D, and D is the number of days counted from the beginning of germination.

The amount of time taken to reach 50 % germination (T_{50}) was calculated according to the following formula of Farooq et al. (2005):

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{n_j - n_i} \qquad (2)$$

Where N is the final number of seeds germinated and n_i, n_j cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.

Energy of germination was recorded on the 4th day after planting. It is the percentage of germinating seeds 4 days after planting relative to the total number of seeds tested (Farooq et al., 2005).

The shoot tissues (0.5 g fresh weight) were homogenized in 2 mL of 100 mM potassium phosphate buffer, pH 7 containing 1 mM of EDTA and 1% (w/v) polyvinylpyrrolidone (PVP). The extract was then centrifuged at 4°C for 15 min at 12.000 ×g in a cooled centrifuge. This supernatant was used to measure the activities of catalase (CAT), Superoxide dismutase (SOD) and total proteins by the methods of Mishra et al., (1993), Giannopolitis and Ries (1977) and Bradford (1976), respectively.

Lipoperoxidation was monitored by the spectrophotometric determination of Malondialdehyde (MDA) using thiobarbituric acid, according to Popham and Novacky (1991). The concentration of MDA was calculated from its extinction coefficient (155 mM^{-1} cm^{-1}). Total soluble sugars were extracted and determined by the anthrone method of Riazi et al. (1985).

Analysis of variance appropriate to the experimental design was conducted, using SPSS and MSTATC software. Means of each trait were compared according to Duncan multiple range test at P=0.05. Excel software was used to draw Figs.

3. Results and discussion

Results revealed that salinity stress significantly decreased final germination percentage (FGP), energy of germination (GE) and vigor index (VI). In non-primed seeds, FGP, GE and VI decreased with increasing the salt stress. Incorporation of spermine (Spm) and spermidine (Spd) into priming media improved mentioned traits under all of the salinity level, however, under low and moderate salt stress, their positive effects were lower than severe stress condition (Table 1). Seed soaking with putrescine (Put) slightly improved these traits only under severe stress condition. Among the polyamines, the highest values for FGP, GE and VI were recorded by Spm application following with Spd. As showed in table 1, salinity delayed germination processes indicated by increasing T_{50} and Mean germination time (MGT) values. Seed priming with PAs lowered T_{50} and MGT compared with the control (Table 1), however, differences among them were not statistically significant. In fact, seed priming with PAs improved germination velocity under saline condition. Data presented in Table 1 showed that seedling growth was also negatively affected by salt stress. In control and salt stressed plants, seed priming with PAs developed the seedling length (SeL), seedling fresh weight (SeFW) and seedling dry weight (SeDW). Under control condition, seeds treated with Spd and Spm increased SeDW by 26 and 76 % respectively, but Put application had not significant effects compared with control. With increasing the stress levels, beneficial effects of Spd and Spm very slightly decreased, while those for Spm declined sharply.

Results indicated that salt stress had a significant effect ($P < 0.01$) on antioxidant enzymes activity in shoots of borage plants. In non-primed seeds, activities of CAT and superoxide dismutase (SOD) were slightly increased with increasing the NaCl from control to 120 mM (Fig. 1a, b). The present study showed that there was significant increase in SOD and CAT activity in seeds primed with Spm and Spd as compared with untreated seeds, but seeds primed with Put induced low SOD activity under all of the salinity level (Fig. 1a and b). Disregard to PAs priming, salinity lead to increase in MDA content in shoots of borage plants. In this respect, exogenous PAs treatment significantly alleviated the salt stress-induced accumulation of MDA (Fig. 1c). The results demonstrated that under non-saline condition, total protein content (TPC) increased approximately 12, 28 and 38% by exogenous application of Put, Spd and Spm, respectively (Fig. 1d). Except for spm under severe salt stress, when primed and un-primed seeds were exposed to salt stress, TPC decreased nearly by same trends. The lowest protein content was achieved for the un-primed seeds under all salinity levels that placed it in the lowest statistical group. In general, the results showed that PAs applications had positive and additive effect on soluble protein content under salinity stress. Total soluble sugars (TSS) concentration slightly changed by PAs and salt stress. Total soluble sugars of un-primed seeds increased as salinity increased up to 40 mM but further increase in salinity caused reduction in TSS (Fig. 1e). Although TSS of all pre-treated and control seeds decreased by salinity, exogenous application of PAs could not effectively ameliorate their adverse effects.

Seed germination is a physiological process modulated by phytohormones or physiological activator such as abscisic acid, or polyamines. According to the results, salinity negatively influenced borage seeds germination behavior as showed by reduction in germination percentage and velocity (Table 1). Inhibitory effect of salinity may be due to the lower water availability, or belongs to its toxic effect. Salinity prevents water uptake by seeds, resulting decreased hormones and enzymes production (Gill et al., 2002), which consequently inhibits germination and seedling's growth. Moreover, the high sensitivity of germination to salinity stress may be attributed to the reduced transition of nutrients from cotyledons to embryo axis under saline condition (Jaleel et al., 2007). Almansouri et al., (2001) in wheat and Ghoulam et al., (2001) in sugar beet reported the high levels of NaCl decreased final germination percentage. Application of PAs improved germination behavior of borage seeds (Table 1). It seems that exogenously applied PAs contributed towards enhanced buildup of PAs that resulted in increased their intracellular pools. Therefore, it can be protect cell membranes stability, and modulate the pre-germination processes. It seems that PAs by influencing the seed metabolic activities, hastened germination process. Similar findings have been proposed by Farooq et al., (2008) who reported that seed priming with polyamines improves the germination and early seedling growth in fine rice. Salinity causes both ionic imbalance and water stress, creating membrane damage (Tiburcio et al., 1994). Chattopadhayay et al., (2002) reported that PAs inhibit uptake of Na^+, loss of K^+, and leakage of amino acids and electrolytes from plant tissues. These findings support the earlier work of Farooq et al (2007) and Mustafavi et al (2015a) who reported enhanced germination velocity by polyamine priming. According to the results, Spm, which has four amino groups, was the most effective treatment than other PAs, which have two or three amino groups, suggesting the involvement of amino groups in the inactivation of reactive oxygen species (ROS) (Besford et al., 1993).

Our results showed that polyamine priming ameliorated the adverse effect of salt stress on borage seedling growth. Highest seedling length, seedling dry and fresh weight were recorded from spermine solution (Table 1). Seedling growth development in primed seeds might be affected by improved cell division inside the apical meristem, which caused in an increase in plant growth. Farooq et al. (2011) demonstrated that priming with polyamines not only developed the germination rate and time significantly but also improved the seedling vigor significantly as showed by higher seedling length and fresh weight compared by control. In general, exogenous application of PAs not only showed improved germination percentages and shortened mean germination times but also displayed significantly enhanced seed vigor as indicated by longer seedling length, seedling fresh and dry weights compared with control.

One of detrimental effects of salinity is loss of integrity of biological membranes due to oxidative damage; accordingly, maintenance of balance between the generation and quenching of reactive oxygen species (ROS) in plants is crucial to survive salt stress. Earlier reports show that polyamines can improve stress tolerance, and these roles are mainly related to alleviation of oxidative damage (Davies, 2004). For this reason, we determined two most important antioxidative enzymes (CAT and SOD) activity. Catalase (CAT) has an important role in the antioxidant system, as it operates in the scavenging of hydrogen peroxide (H_2O_2) into oxygen and water (Asada, 2006). This enzyme is considered very sensitive to conditions of abiotic stress as serves as a marker of stress. Salinity caused a change in activities of antioxidant enzymes, such as SOD and CAT, which may lead to adjustment to stress condition by reducing damage of reactive oxygen species (ROS). Results revealed that antioxidant enzymes (CAT and SOD) activity increased by addition of PAs (especially Spd and Spm) into priming media (Fig. 1a, b). Therefore, it seems that germination and seedling growth improvement in primed seeds subjected to salinity may be attributed to the role of PAs in oxidative damage ameliorating. Velikova et al. (2000) indicated that PAs are involved in plant reactions to stress as protectants, and that the more effect of Spm in comparison with Spd and Put could be accounted for by its longer chain and more positive charges, thus providing more effective neutralizing and membrane-stabilizing effects. Malondialdehyde (MDA) is one of the final products of the peroxidation of unsaturated fatty acids in phospholipids and is responsible for cell membrane damage (Sharma et al., 2011). Results showed that MDA content increased with increasing the salinity intensifying (Fig. 1c). The higher MDA concentration under salinity stress means more seedling damage suffering from salt stress. The present study showed lower MDA concentration from soaking seeds with Spd and Spm compared to control. All of these indicating that seed priming with Spd and Spm alleviated the salinity injury of the borage seedlings. This indicates that Spd and Spm are able to influence the antioxidant defense system to moderate the oxidative stress intensity and its injuries induced by salt stress during seed germination. Previous results suggest that polyamines are able to elevate the activities of antioxidant enzymes and thereby control free radical production in plants during exposure to salinity (Tang and Newton, 2005; Duan et al., 2008), led to plasma membrane stability and reduction in electrolytes leakage.

The results demonstrated that salinity significantly altered the levels of total protein. The trends of total protein content were decreased with increasing in salinity levels (Fig. 1d). Free radicals produced under salt stress conditions may damage the proteins and decrease its content (Noctor and Foyer, 1998). Several researchers have reported a decrease in the amount of protein and an increase in nitrate, ammonium, and free amino-acids under salt stress (Yonis et al., 1993). The results showed that PAs application had positive and additive effect on protein content under control and saline conditions (Fig. 1d). Results revealed that total soluble sugars concentration was not significantly affected by salinity stress and PAs application. Total soluble sugars (TSS) concentration slightly decreased by salt stress, however, PA pre-treatments did not alleviate the adverse effects of salt stress on total soluble sugars concentration (Fig. 1d). The reduction of chlorophyll content and the inhibition of carbon metabolism and photosynthetic activity (Chattopadhayay et al., 2002) led to the observed reduction of total soluble sugars concentration in salt-stressed plants. The enhancement effect of PAs may be attributed to increased stability of thylakoids membrane and amine group supplying for protein synthesis. These are in agreement with previous findings of many authers (Alsokari, 2011).

Table 1: Effect of priming with polyamines on the germination potential of borage officinalis under salinity

priming	NaCl (mM)	FGP (%)	GE (%)	VI	MGT (days)	T_{50} (days)	SeL (cm)	SeFW (g)	SeDW (g)
control	0	80.6 c	65 c	12.293 d	5.61 bcde	3.56 bcd	15.23 de	0.968 cd	0.485e
	40	60.6 e	50.36 fg	8.112 fg	6.63 ab	4.58 b	13.367 fg	0.918 de	0.456e
	80	51.38 f	43.6 h	4.074 i	7.65 a	5.6 a	7.933 j	0.696 g	0.231h
	120	35 h	36.29 i	1.293 j	7.57 a	5.648 a	3.767 k	0.473 i	0.101i
Put	0	80.59 c	57 e	12.35 d	5 de	3 def	15.3 de	0.994 bcd	0.475e
	40	61.35 e	54.3 ef	7.746 fg	5 de	3.32 cde	12.633 gh	0.958 cd	0.421ef
	80	52.6 f	45.78 gh	5.564 h	6 bcd	4.6 b	10.567 i	0.818 f	0.348g
	120	41 g	35.51 i	3.411 i	5.7 bcde	4 bc	8.333 j	0.602 h	0.218h
Spm	0	96 a	84 a	17.275 a	5 de	2.133 f	18 a	1.393 a	0.857a
	40	84.64 c	75 b	13.881 c	5.3 cde	3.3 cde	16.4 bc	1.047 bc	0.659b
	80	71 d	62.7 cd	10.129 e	5.6 bcde	4 bc	14.267 ef	0.96 cd	0.486e
	120	67.667 d	56 e	8.42 f	6.33 bc	4.343 b	12.467 gh	0.844 ef	0.378f
Spd	0	89.667 b	77.34 b	15.186 b	4.657 e	2.667 ef	16.933 b	1.08 b	0.612bc
	40	82.34 c	65 c	12.851 d	5.64 bcde	3.61 bcd	15.6 cd	0.996 bcd	0.545c
	80	71.63 d	57.6 de	10.49 e	5.6 bcde	3.627 bcd	14.633 de	0.947 d	0.501d
	120	60.26 e	45 gh	7.33 g	6.61 ab	4.6 b	12.167 h	0.82 f	0.365f
Means with the same letters in each column are not significantly different at 5% of probability level.									

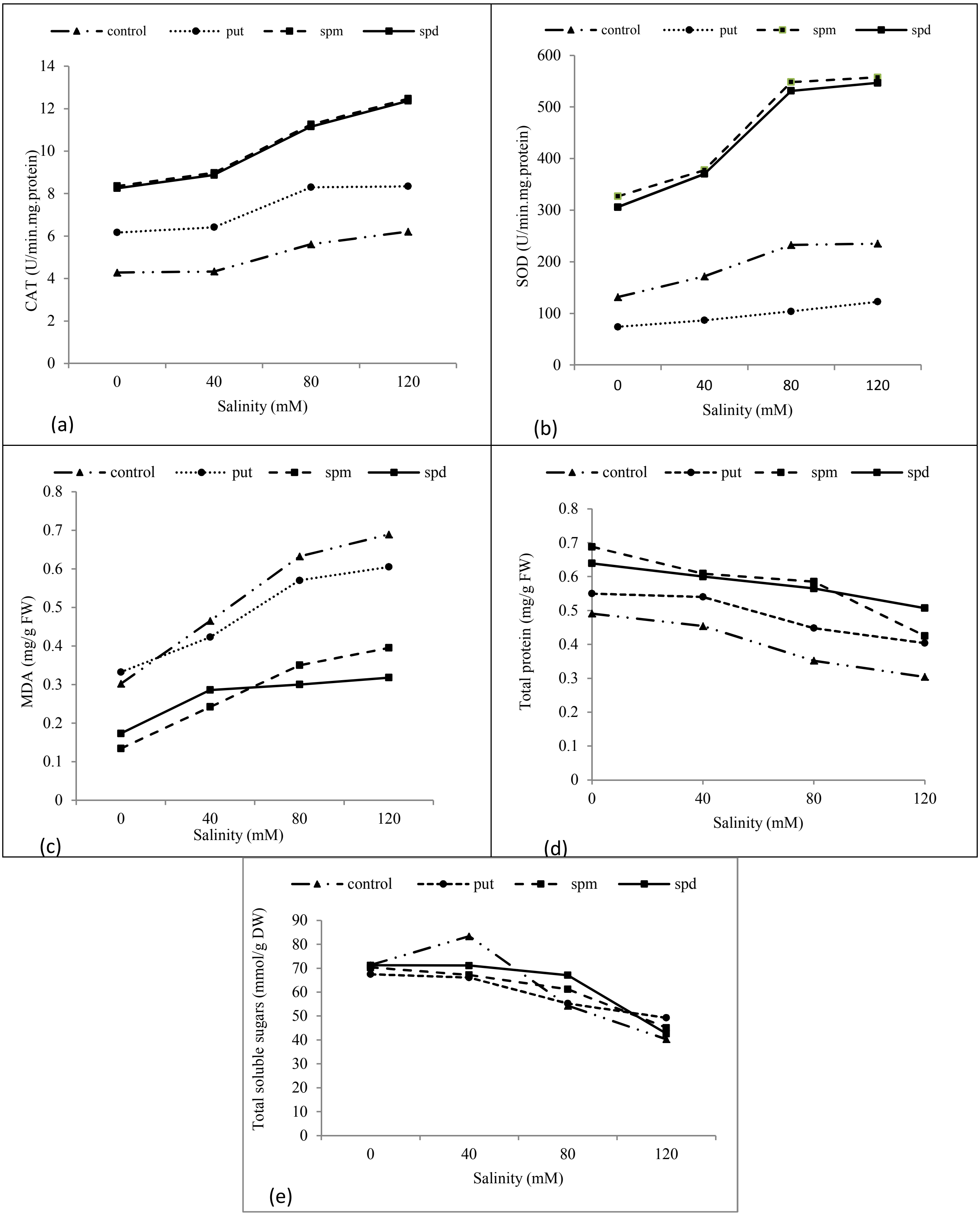

Fig. 1: Influence of seed priming with polyamines under saline condition on (a) catalase (CAT) activity, (b) superoxide dismutase (SOD) activity, (c) Malondialdehyde concentration, (d) total protein content and (e) Total soluble sugars of borage seedlings.

4. Conclusion

Results of this experiment indicated that priming with polyamines improved germination percentage and velocity, seedling growth of Borage (*Borago officinalis* L.) seeds under salt condition. Effect of all the polyamines was stimulatory but of the different priming agents used in the present study, Spm and/or Spd was found as the most effective technique. In the present study, under salinity, exogenous PAs elevated the activities of antioxidant enzymes, suppressed free radical production and membrane damage, and thereby mitigated the oxidative stress in borage seedlings. These results suggest that the free radical-scavenging and membrane protective properties of Spd and Spm that resulted in increasing the important primary metabolism materials necessary for seedling growth and development. In general, under semi-arid regions, borage seeds pretreatment with PAs (especially Spm) could be used to increase the borage cultivation area and finally production.

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