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Research Article

The effect of different priming methods on seeds and seedlings of Lallemantia under salt stress

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Abstract

In order to investigate the effects of priming methods on the improvement of seed germination indices and seedling growth of Lallemantia (*Lallemantia iberica*) under different salinity levels, two factorial experiments in a randomized complete block design were carried out in the seed technology laboratory of Agricultural Sciences and Natural Resources University of Khuzestan in 2018-2019. In the first experiment, five levels of seed priming including: Control, hydropriming (for 24 hours), gibberellic acid (100 mg.l^{-1} for eight hours), and solutes of potassium nitrate and sodium chloride (NaCl**) (50 mg.l^{-1} for 6 hours) as the first factor and four levels of salinity stress, including 0 (control), 100, 200, and 300 mM of** NaCl**, as the second factor in four replications were examined. Also, a pot experiment was conducted to determine the effects of the studied treatments on some seedling growth indices in three replications. In general, indices of germination percentage, germination rate, radicle length, plumule length, emergence percentage, emergence rate, contents of chlorophyll a, b, carotenoids, and enzymatic activities of catalase and peroxidase were evaluated in this study. Results revealed that salinity stress, priming, and their interaction had significant effects on most of the studied traits. Also, increasing salinity concentrations led to decreases in all traits (except the activity of antioxidant enzymes). Generally, our findings showed that all seed priming treatments moderated the negative impacts of salinity stress and the application of** NaCl **could be a suitable treatment to increase the tolerance of Lallemantia seeds against high levels of salinity stress.**

Keywords: Antioxidant enzymes, Germination, Gibberellic acid, Photosynthetic pigments

Introduction

Lallemantia (*Lallemantia iberica*) is an aromatic annual plant of the Lamiaceae family (Mohammad Ghasemi *et al.*, 2020), which is used to treat some neurological, liver, and kidney disorders (Amanzade *et al.*, 2011, Omidi *et al.*, 2018). Also, its leaves, oil, essential oil, and seeds are widely used as stimulants and analgesics in traditional Iranian Medicine (Razavi *et al.*, 2017).

Climate change, uncontrolled population growth, and environmental stresses are among the most well-known challenges of humanity and modern agriculture. Meanwhile, saline stress, one of the most important abiotic stresses, has many effects on plants and can reduce the growth, development, and yield stages of most plant species (Nawaz *et al.*, 2010). This stress also causes the overproduction of reactive oxygen species (Khan and Panda, 2008; Liu *et al.*, 2021), lipid peroxidation, DNA damage, quantitative and qualitative changes in plant pigments, inhibition of photosynthesis, and disturbance in mineral absorption and transportation (Setayesh Mehr, 2013). Due to the high sensitivity of many medicinal plants to salinity, it is very important to study the effects of salinity stress on these plants (Hayouni *et al.*, 2008). All growth stages of medicinal plants such as seed germination, seedling establishment and vegetative growth and yield stages can be affected by salinity stress. In this regard, some studies have reported the adverse effects of salinity stress on the germination of medicinal plants, essential oils yield, and changes in the composition of active ingredients (Filippo *et al.*, 2002). In a study, the effects of sodium chloride (NaCl) were investigated on germination indices and physiological traits of stevia, and results showed that salinity stress had significant effects on germination percentage and rate, mean germination time, germination value, seedling length, seedling vigor, total chlorophyll content, proline content, and enzymatic activities of catalase and superoxide dismutase (Aghighi Shahverdi and Omidi, 2016). Also, Ezz El-Din *et al.* (2009) examined the effects of different levels of salinity stress on the medicinal plant of thyme (*Thymus vulgaris* L.) and found that high concentrations of NaCl significantly reduced plant height, number of branches, and fresh and dry mass. In addition, it was suggested that salt stress could decline the physical resistance of endosperm and membrane repair during imbibition and

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lead to the development of immature embryos (Ibrahim, 2016).

The seed priming technique is one of the effective ways to reduce and modify the damage caused by salinity stress and refers to the process of controlled seed rehydration and subsequent restoration, which allows many physiological processes related to the early stages of germination and root emergence (Paparella *et al.*, 2015; Becerra-Vazquez *et al.*, 2020). In general, priming can increase germination percentage and speed, improve germination under adverse environmental conditions, and enhance vigor and seedling growth (Anwar *et al.*, 2021). Obviously, under these conditions, plants make maximum use of environmental inputs and ultimately have a higher chance of producing higher quality and quantity products compared to the untreated seeds (Ghiyasi, 2016). It has also been reported that priming can increase the physiological tolerance of seeds against stressful conditions, including salinity; Therefore, the effects of priming may become more apparent under adverse environmental conditions. In general, numerous techniques, including hydropriming, osmopriming, chemopriming, nutrient seed priming, and hormopriming, have been reported to prepare seeds and induce changes during seed germination (Paparella *et al.*, 2015). In this field, there are several reports on different plants. Rostami *et al.* (2018) investigated the effects of different priming treatments on germination, morphological, physiological, and biochemical indices of basil under salinity stress. They showed that the above traits were significantly affected by priming treatments so that the highest values for germination rate, antioxidant activities of leaf extracts, and total phenolic were obtained for seeds primed with distilled water. In addition, the highest content of chlorophyll a was obtained in the control treatment (without salt stress) and 50 mM salinity stress for plants raised from seed priming with distilled water (Rostami *et al.*, 2018). In another study, pre-treatment of rapeseed seeds with NaCl increased proline accumulation and decreased the toxic effects and nutrient deficiencies in salinity stress, which can be attributed to lower Na^+ accumulation and increased K^+ and Ca^{2+} accumulation in seedlings (Salari *et al.*, 2009). According to previous studies, salinity stress affects many physiological and morphological processes of plants and ultimately causes damage to germination, plant establishment and production. Therefore, the use of methods such as priming to reduce the effects of stress and increase plant tolerance to environmental stresses, especially salinity stress can be effective. Accordingly, this study was conducted to investigate different priming methods on improving germination and seedling indices of Lallemantia seeds under salinity conditions.

Materials and methods

This study was conducted in two stages to investigate the effects of different priming treatments on seeds and seedlings of Lallemantia under salinity conditions in the seed technology laboratory of Agricultural Sciences and Natural Resources University of Khuzestan during 2018-2019. Here, both experiments were performed using two-factor factorial based on the completely randomized design.

In the first experiment, five levels of seed priming, including control, hydropriming (for 24 hours), gibberellic acid at a concentration of 100 $mg.l^{-1}$ (for eight hours), and potassium nitrate and NaCl at a concentration of 50 $mg.l^{-1}$ (for 6 hours) as the first factor and four levels of salinity stress of 0 (control), 100, 200, and 300 mM of NaCl, as the second factor in four replications were considered. Hence, seeds were first placed in Petri dishes containing two layers of Whatman filter paper to apply priming treatments. Then, 10 ml of experimental treatments added to them, and the samples were kept in the dark at 20°C. Then, the examined seeds were washed with distilled water and used for germination tests following the reduction of surface water. In the next step, 50 seeds were placed in 9 cm glass Petri dishes on two layers of Whatman No.1 filter paper to perform the standard germination testing, and 5 ml of each salt concentration added to each Petri dish. The Petri dishes were then placed within plastic nylons to prevent evaporation of the experimental solutions and transferred to a seed germinator with an average temperature of 20°C and a light/dark cycle of 8:16. Finally, germinated seeds were counted daily based on radical emergence (two mm), and the produced seedlings were applied to assess growth indices following the end of the germination period.

It should be noted that germination percentage and germination rate were calculated respectively based on equations presented by Ikic *et al.* (2012) and Verma *et al.* (2005).

Equation (1)

$$Germination\ percentage\ (GP\%) = \frac{n}{N} \times 100$$

Where: n= Total number of germinated seeds and N= Total number of seeds.

Equation (2)

$$\text{Germination Rate} = \Sigma \frac{N_i}{T_i}$$

In which: “N” is the number of germinated seeds and “T” is the number of days after beginning the experiment.

10 samples were selected randomly from each petri dish at the end of the germination period, and their root and plumule length were measured using a ruler (Agraval, 2003).

In the second study, a pot experiment was also performed to determine the effect of the treatments in the first stage on seedlings in the research greenhouses of Agricultural Sciences and Natural Resources University of Khuzestan in three replications. In the second stage of the experiment, cultivation was done in seedling trays (containing two parts soil, one part manure, and one part sand) and irrigated once every fifth day using saline solutions. After two weeks, emergence percentage and emergence rate indices were

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calculated using equations presented by Ikic *et al.* (2012) and Verma *et al.* (2005), respectively.

Equation (3)

$$Emergence\ percentage\ (\%) = \frac{\text{Total number of emergenced seeds}}{\text{Total number of seeds}} \times 100$$

Equation (4)

$$\text{Emergence rate index} = \Sigma \frac{N_i}{T_i}$$

In which: N and T refer to the number of emerged seedlings and the number of days, respectively.

In this experiment, contents of chlorophyll a, b, and carotenoids in the seedling stage were assessed by Arnon (1949) method. Also, catalase activity by Aebi (1983), peroxidase activity by Hemeda and Kelin (1990) method were measured.

The data were analyzed by SAS software (version 9.3), and the LSD test was used at P< 0.05 to compare the mean of the data for both Petri dish and pot experiments. Also, the data were normalized using Minitab Statistical Software (version 18).

Results and discussion

Percentage and rate index of germination: Results revealed that different priming, salinity stress, and interaction of priming × salinity levels had significant effects on both germination percentage and germination rate indices at P <0.01 (Table 1). The results of the mean comparison showed that the highest germination percentage (96%) was recorded for control (without salinity stress) × hydropriming, but there was no significant difference with the data obtained from control and hormopriming treatments. Also, under the application of salinity concentrations of 100, 200, and 300 mg.l^{-1}, the highest germination percentages (with averages of 94, 82, and 56%, respectively) were obtained under the application of hormopriming treatment, which had no significant differences companed with the data obtained from priming with NaCl× salinity levels of 100 and 300 mM. In contrast, the lowest values for this trait were achieved at concentrations of 100 and 300 mM salinity × control (unprimed conditions) and 200 mM salinity × priming with potassium chloride (Table 2). Okcu *et al.* (2005) affirmed that reducing germination percentage at high salinity levels might be due to the negative (toxicity) effects of chlorine and sodium ions on plant metabolism, water uptake, and consequently seed swelling. Various experiments have also shown that impaired water uptake delayed root emergence and reduced seed germination rate (Ebadi and Kamel, 2009). In a study, a decrease in the germination percentage of safflower cultivars was reported under the influence of different levels of NaCl, and the researchers attributed this finding to the reduction of water flow to induce metabolic reactions related to seed germination. Also, this research showed that salinity stress resulted in increasing abscisic acid concentration in seeds and inhibiting seed germination (Laleh *et al.*, 2012). According to the slicing results in treatments without salinity, the highest germination percentage was obtained under the application of the hydropriming treatment (Table 2). One of the reasons for the superiority of hydropriming may be related to the intermittent priming of seeds and increases of the accumulation of germination activating enzymes compared to the other pre-treatments (Mansour *et al.*, 2005). Pedram *et al.* (2019) demonstrated that hydropriming treatment with water had a significant effect on germination percentage compared to the control treatment. In another study, it was reported that hormopriming and hydropriming improved the germination percentage of stevia seeds under different levels of salinity stress (Aghighi Shahverdi *et al.*, 2017).

Evidence of the present study showed that germination rate had a direct proportion with increasing salinity levels up to 100 and 200 mM under all priming treatments (except priming with NaCl); However, germination rate decreased at higher salinity levels. Also, although the highest germination rate was obtained without application of NaCl (with an average of 0.069 buds per hour), no significant differences were observed in germination rates of seeds treated with hydropriming treatment. In addition, at 100, 200, and 300 mM salinity levels, the highest germination rate was achieved for seeds treated with NaCl, which increased by 67, 58, and 100%, respectively, compared to the unprimed conditions. In addition, there were no significant differences between the germination rate of seeds treated with hydropriming and priming with potassium chloride and NaCl at 100 mM salinity (Table 2). Evidence indicated that a higher potential of water uptake in pre-treated seeds compared with unprimed seeds had positive influences on germination rate (Ghana and Schillinger, 2003). Patanea *et al.* (2009) confirmed that pre-osmopriming of sweet sorghum seeds increased germination rate and reduced negative impacts of salinity stress that attributed to higher water uptake in pre-treated seeds compared to the control treatment. The positive effects of pre-osmopriming with increasing salinity levels might be due to salt-induced salinity, which not only accelerated the germination process in Lallemantia seeds but also enhanced the tolerance of germinated seeds against salinity conditions. Also, some researchers demonstrated that potassium cation reduced the harmful effects of NaCl and increased seed germination rate (Ashraf and Rauf, 2001). Hence, the better reaction of potassium chloride than other treatments can be justified.

Radicle and plumule length: Results of the analysis of variance in table 1 showed that the radicle and plumule length of Lallemantia seeds were affected by priming, salinity, and their interaction. Results of table 2 showed that the application of hydropriming treatment at salinity levels of 0 and 300 mM had the highest radicle length with values of 3.06 and 0.49 cm, respectively. Also, results showed that NaCl (2.63 cm) and hormopriming (1.34 cm) had the maximum and minimum radicle length at 100 mM salinity, respectively. However, at a salinity of 200 mM, the

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Table 1. Analysis of variance germination percentage, germination rate and radicle and plumule length of Lallemantia under salinity and priming

Source of variation	df	Mean of squares			
		Germination percentage	Germination rate	Radicle length	Plumule length
Salinity (s)	3	23791.20**	0.00504**	20.45**	28.60**
Priming (p)	4	1792.50**	0.00382**	0.96**	2.02**
Interaction (s×p)	12	443.03**	0.00040**	0.84**	0.74**
Error	60	15.33	0.00002	0.04	0.04
C.V (%)	-	6.07	13.09	14.82	11.06

**** are significant at a probability level of 1%**

Table 2. The effects of priming and Salinity (mM) on germination percentage and germination rate, radicle and plumule length of Lallemantia under salinity and priming

Characteristics / Treatment	Germination (%)	Germination rate (seeds per day)	Radicle length (cm)	Plumule length (cm)
0×No prime (control)	95^{a}	0.024^{d}	2.49^{b}	3.17^{a}
0×Hydroprim	96^{a}	0.068^{a}	3.06^{a}	2.22^{b}
0×Hormonprim	95^{a}	0.053^{c}	1.35^{c}	3.23^{a}
0×Haloprim (potassium chloride)	88^{b}	0.061^{b}	2.93^{a}	3.23^{a}
0×Haloprim (NaCl)	79^{c}	0.069^{a}	2.58^{b}	3.23^{a}
100×No prime (control)	83^{c}	0.024^{c}	2.21^{b}	2.68^{b}
100×Hydroprim	88^{bc}	0.069^{a}	1.94^{c}	1.69^{c}
100×Hormonprim	94^{a}	0.055^{b}	1.34^{d}	3.14^{a}
100×Haloprim (potassium chloride)	84^{c}	0.069^{a}	1.81^{c}	3.14^{a}
100×Haloprim (NaCl)	89^{ab}	0.068^{a}	2.63^{a}	3.28^{a}
200×No prime (control)	70^{b}	0.025^{cd}	1.09^{ab}	1.45^{a}
200×Hydroprim	70^{b}	0.021^{d}	1.04^{ab}	1.02^{b}
200×Hormonprim	82^{a}	0.028^{c}	1.24^{a}	1.59^{a}
200×Haloprim (potassium chloride)	59^{c}	0.048^{b}	0.63^{c}	1.59^{a}
200×Haloprim (NaCl)	74^{b}	0.065^{a}	0.94^{b}	1.44^{a}
300×No prime (control)	0^{c}	0.000^{d}	0.00^{b}	0.00^{b}
300×Hydroprim	12^{b}	0.020^{bc}	0.49^{a}	0.54^{a}
300×Hormonprim	56^{a}	0.017^{c}	0.05^{b}	0.52^{a}
300×Haloprim (potassium chloride)	2^{c}	0.025^{ab}	0.16^{b}	0.47^{a}
300×Haloprim (NaCl)	60^{a}	0.028^{a}	0.16^{b}	0.34^{a}

The same letters in a column indicate no significant difference at the 5% probability level (LSD test)

maximum radicle length was observed under hormopriming treatment (Table 2). In addition, results indicated that at salinity levels of 0, 100, and 200 mM, the highest plumule length were recorded respectively in hormopriming and halopriming and the lowest plumule length was registered in hydropriming. At 300 mM salinity, the longest plumule (0.54 cm) was obtained in the hydropriming treatment while without any significant difference with hormopriming and halopriming (Table 2). There have been numerous reports of adverse effects of salinity on plant growth. Osmotic potential increased with increasing salinity and water potential will be reduced and thus less water is available to seed. Less water absorption will reduce the inflammation of seed embryonic cells. Finally, radicle growth is reduced by reducing the available water and swelling of the seeds (Cavalcanti *et al.*, 2007).

However, priming treatments moderate the adverse effects of salinity stress to some extent. Torabi Chafjiri *et al.* (2019) examined the effects of different priming treatments on seed germination characteristics of some endemic populations of Chamomile and reported that the maximum values for radicle and plumule length were obtained under the application of the osmopriming and hormopriming treatments, respectively, which was consistent with the results of the present study. In addition, impaired nutrient transport from the cotyledons to the embryo is another cause of reduced plumule length under salinity conditions. It was also reported that reduced water uptake by seeds under salinity conditions could lead to decreased hormone secretion and enzymatic activities and impaired seedling growth (Kaafi *et al.*, 2005). Jahanban *et al.* (2016) investigated the efficiency of three seed priming methods toward improving safflower tolerance to salinity stress at germination and early growth stages and found that salinity stress decreased seed vigor compared to the control treatment while halopriming treatment presented opposite effects.

The pot experiment

Emergence percentage and rate: Results of pot experiment (Table 3) showed that priming, salinity stress, and their interaction had significant effects at

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Table 3. Analysis of variance percentage and rate of emergence, content of chlorophyll a, b, carotenoids and activity of catalase and peroxidase of Lallemantia under salinity and priming

Source of variation	df	Mean of squares						
		Percentage of emergence	Rate of emergence	content of chl a	Content of chl b	Content of carotenoids	Activity of catalase	Activity of peroxidase
Salinity (s)	3	4875.35**	0.0036**	0.05**	0.07**	0.02*	0.0024**	0.000003^{ns}
Priming (p)	4	498.74**	0.0049**	0.11**	0.02**	0.36**	0.0012**	0.000049**
Interaction (s×p)	12	274.95**	0.009^{ns}	0.01*	0.01**	0.05**	0.0007**	0.000007**
Error	40	75.44	0.0008	0.0021	0.0025	0.01	0.0001	0.000002
C.V. (%)	-	13.32	10.55	6.96	16.77	19.20	18.93	22.91

*** and ** are significant at a probability level of 5 and 1%, ns: non significant**

P<0.01 on the germination percentage of Lallemantia seeds while germination rate was only affected by priming and salinity stress. Results of salinity × priming interaction revealed that although mild salinity had no significant effects on seed germination percentage, severe salinity levels significantly reduced the above trait. On the other hand, seed priming facilitated the seedling emergence and moderated adverse effects of salinity stress. Furthermore, there were no significant differences between different types of priming and control treatment under natural (without salinity) conditions. However, higher levels of salinity led to a significant effects between different priming conditions so that the highest values for the percentage of germination (Table 5) were recorded for interactions of salinity of 300 mM × hormopriming (34%) and salinity of 300 mM × halopriming of NaCl (33%). Comparison of the main effects of different priming conditions showed that the fastest emergence rate was achieved under the application of hormopriming with gibberellic acid. However, there was no significant difference in the emergence rate of seeds under hormopriming and hydropriming treatments. Also, it was observed that there was no significant difference in the application of NaCl and potassium chloride in terms of the emergence rate index. In addition, the lowest emergence rate (Table 4) was obtained under unprimed conditions (control treatment). Based on the results of table 4 (comparison of mean values), salinity stress reduced the emergence rate index of seedlings so that the highest and the lowest values assessed equal to 0.28 and 0.24 buds per day for control treatment and 300 mM salinity, respectively.

It was reported that decreasing emergence percentage and rate indices under increasing salinity stress could be due to the more negative osmotic potential of the root environment compared to the plant root cells and the accumulation of high amounts of salt in soil solution (Emam *et al.*, 2013). Soltani *et al.* (2007) indicated that seed priming increased the percentage and rate of seed germination. Ghaderifard *et al.* (2014) also investigated the effects of priming on wheat seeds and found that hormonpriming and halopriming treatments increased both emergence percentage and rate indices of wheat seedlings under both salinity and normal conditions. Other researchers have stated that germination percentage and rate of savory seedlings had a negative correlation with increasing salinity levels; Nevertheless, seed priming was introduced as an alternative technique to increase both germination percentage and rate indices (Pourreza and Omidi, 2018).

Photosynthetic pigments: Results of table 3 indicated significant effects of priming, salinity stress, and the interaction between priming and salinity stress on the content of photosynthetic pigments, e.g., chlorophyll a and b, and carotenoids content (Table 3). Findings showed that although salinity levels reduced the content of chlorophyll a in Lallemantia seedling leaves, the application of priming improved the destructive consequences of salinity stress to some extent. The content of chlorophyll a was calculated equal to 0.63 mg.g^{-1} fresh weight under unprimed conditions (control) and no salinity stress (normal conditions) but limited to 0.49 mg.g^{-1} fresh weight at the salinity concentration of 200 mM. It should be noted that the salinity of 300 mM in unprimed seeds resulted in complete inhibition of the emergence index in Lallemantia seedlings (there was no data to calculate chlorophyll a). Also, results revealed that the highest content of chlorophyll a (0.85 mg.g^{-1} fresh weight) was obtained in the interaction of hydropriming × normal conditions (without salinity stress), and similar results were observed at salinity levels of 100, 200, and 300 mM (Table 5). Although changes in chlorophyll b content were the same as chlorophyll a, chlorophyll b was more sensitive to salinity conditions and the severity of the destructive effects of salinity stress was more prominent on this index. Under normal conditions (without salinity stress), the highest content of chlorophyll b (0.47 mg.g^{-1} fresh weight) was obtained under hydropriming and the lowest content value was observed under priming treatment with NaCl. However, contrary to the results obtained from chlorophyll a, salinity-induced changes for chlorophyll b content in priming with NaCl was negligible (Table 5). Also, results of table 5 showed that carotenoids had an increasing trend in mild stress conditions, but high levels of salinity led to a decrease in their contents. On the other hand, the application of different priming conditions had positive and significant effects on the carotenoid contents. In general, NaCl and then potassium chloride had the highest effects on this trait at all stress levels. It was also found that the use of hormopriming had non-significant effects on carotenoid contents compared to the control treatment (Table 5).

Concerning the effects of salinity stress on

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Table 4. Mean comparison of germination rate of Lallemantia under salinity and priming

Treatment	Germination rate (Seeds per hour)
Salinity (mM)	
0	0.283[a]
100	0.270[b]
200	0.258[c]
300	0.247[c]
Priming (p)	
No prime (control)	0.245[c]
Hydroprim	0.271[ab]
Hormonprim	0.296[a]
Haloprim (potassium chloride)	0.255[b]
Haloprim (NaCl)	0.255[b]

The same letters in a column indicate no significant difference at the 5% probability level (LSD test)

Table 5. The effects of priming and salinity (mM) on germination percentage and germination rate and root and shoot length of Lallemantia under salinity and priming

Tharacteristics / Treatments	Percentage of emergence	Content of chl a	Content of chl b	Content of carotenoids	Activity of catalase	Activity of peroxidase
		(mg/g fresh weight)			Standard unit in mg of protein	
0×No prime (control)	69.5[b]	0.63[c]	0.42[b]	0.48[b]	0.038[cd]	0.0035[c]
0×Hydroprim	74.4[a]	0.85[a]	0.47[a]	0.53[ab]	0.051[c]	0.0041[bc]
0×Hormonprim	78.2[a]	0.76[ab]	0.39[b]	0.44[b]	0.023[d]	0.0049[b]
0×Haloprim (potassium chloride)	80.6[a]	0.70[b]	0.39[b]	0.50[b]	0.068[b]	0.0053[b]
0×Haloprim (NaCl)	79.0[a]	0.73[b]	0.30[c]	0.61[a]	0.086[a]	0.0071[a]
100×No prime (control)	68.3[b]	0.57[c]	0.31[ab]	0.59[b]	0.055[b]	0.0058[b]
100×Hydroprim	80.6[a]	0.77[a]	0.35[a]	0.56[b]	0.057[b]	0.0069[b]
100×Hormonprim	80.6[a]	0.70[b]	0.24[b]	0.23[c]	0.039[c]	0.0065[b]
100×Haloprim (potassium chloride)	83.3[a]	0.69[b]	0.25[b]	0.55[b]	0.050[bc]	0.0066[b]
100×Haloprim (NaCl)	71.7[ab]	0.72[b]	0.29[ab]	0.67[a]	0.069[a]	0.0092[a]
200×No prime (control)	50.0[c]	0.49[c]	0.23[b]	0.24[d]	0.057[b]	0.0052[c]
200×Hydroprim	72.2[a]	0.76[a]	0.27[a]	0.34[c]	0.064[a]	0.0058[bc]
200×Hormonprim	72.0[a]	0.62[b]	0.17[c]	0.19[d]	0.066[a]	0.0067[b]
200×Haloprim (potassium chloride)	66.7[b]	0.58[b]	0.22[b]	0.54[b]	0.040[c]	0.0084[ab]
200×Haloprim (NaCl)	65.0[b]	0.70[ab]	0.26[a]	0.67[a]	0.056[b]	0.0093[a]
300×No prime (control)	0.0[c]	0.00c	0.00d	0.0[c]	0.000[c]	0.0000[c]
300×Hydroprim	21.4[b]	0.68[a]	0.26[a]	0.27[b]	0.029[b]	0.0035[c]
300×Hormonprim	34.4[a]	0.59[b]	0.12[c]	0.03[c]	0.048[a]	0.0072[b]
300×Haloprim (potassium chloride)	19.4[b]	0.57[b]	0.20[b]	0.63[a]	0.041[ab]	0.0091[ab]
300×Haloprim (NaCl)	33.3[a]	0.67[a]	0.21[b]	0.59[a]	0.028[b]	0.0100[a]

The same letters in a column indicate no significant difference at the 5% probability level (LSD test)

photosynthetic pigments, the high chlorophyll content in leaves is one of the critical factors in maintaining photosynthetic capacity and plant growth. The effect of stress on chlorophyll content varies according to the duration of stress and plant growth stage. Production of different types of reactive oxygen species and subsequent lipid peroxidation and chlorophyll degradation are the most well-known factors in decreasing chlorophyll during exposure of plants to salinity stress (Sairam and Tyagi, 2004). Jamal Omidi *et al.* (2018) reported that stress-induced decreasing chlorophyll is probably due to the effects of chlorophyllase and peroxidase enzymatic activities, phenolic compounds, and subsequently chlorophyll decomposition. Also, the decrease in chlorophyll b content under stressful conditions is attributed to disorders in chloroplasts and changes in the ratio of proteins and lipids involved in pigment formation (Khalil *et al.*, 2010). Salinity stress destroys chloroplasts by increasing carbonate ion concentrations and increasing acidity, thereby reducing chlorophyll content (Valdez-Aguilar and Reed, 2008). It has been suggested that salinity stress could precipitate magnesium ions and prevent chlorophyll synthesis. In addition, this stress may increase the enzymatic activity of chlorophyllase and lead to chlorophyll degradation (Shi and Sheng, 2005). EL-Tayeb (2005) found that priming could increase photosynthetic pigments in plants under salinity stress through protective effects on photosynthesis, chlorophyll index, and photosynthetic pigments. There have been several reports regarding the positive effects of hydropriming on growth indices and

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photosynthetic pigments, which were in line with the results of the present study. Azad *et al.* (2018) reported that hydropriming increased photosynthesis and plant growth and development by increasing the chlorophyll content in leaves, which were in the early stages of the aging process. It seems that carotenoids in Lallemantia seedlings act as an antioxidant under salinity conditions and help improve seedling growth and development. An increase in the content of carotenoids under stressful conditions is a mechanism to protect chlorophylls against light oxidation. Pourreza and Omidi (2018) investigated the effects of different levels of salinity stress on plant photosynthetic pigments and have reported that decreasing chlorophyll contents is negatively correlated with salinity levels, but the contents of carotene and xanthophyll increased under salinity stress. Previous researchers have attributed the increase in carotenoids to the inhibition of chlorophyll photooxidation. In addition, some researchers suggested that carotenoids increased the antioxidant capacity of plants, and hydropriming induced the synthesis of carotenoids and xanthophylls in different plants (Bekhrad *et al*., 2015). The increase in carotenoid contents under the application of hydropriming treatment in the present study was in line with the findings of Valipour Dahnoo *et al.* (2018) in fenugreek.

Enzymatic activities of peroxidase and catalase: Analysis of variance of antioxidant enzyme activities demonstrated that peroxidase and catalase activities were affected by main treatments of priming and salinity stress and the interaction of salinity stress × priming at $P<0.01$ (Table 3). Comparison of mean values indicated that peroxidase activity increased under the application of mild levels of salinity stress; however, high levels of salinity stress decreased its activity. On the other hand, the application of different priming treatments enhanced plant response systems of the studied seedlings by improving the activity of this enzyme. According to the results, it was observed that seeds primed with NaCl and potassium chloride had the highest impacts on peroxidase activity among different types of priming used in the present study. In another words, the highest enzyme activities of peroxidase were obtained in the stress-free conditions (control treatment) × NaCl interaction (0.086 standard unit in mg of protein) followed by in the control treatment × potassium chloride interaction (0.068 standard unit in mg of protein). Findings also determined that salinity stress reduced the positive effects of potassium chloride while increased the role of gibberellic acid in improving the activity of the peroxidase enzyme, and its highest activity (0.048 standard unit in mg of protein) was obtained under the interaction of hormopriming with gibberellic acid × 300 mM salinity (Table 5). Also, results related to catalase activity revealed that both treatments of salinity stress and seed priming increased the activity of this enzyme. In addition, the highest catalase activity at all salinity levels was achieved under the pre-treatment of seeds with sodium chloride. Studying interactions between different levels of salinity × priming with gibberellic acid showed that the highest and lowest enzymatic activities of catalase (Table 5) were recorded for 300 mM salinity × priming with NaCl (0.01 standard unit in mg of protein) and normal conditions × unprimed conditions (0.0035 standard unit in mg of protein).

In confirmation of the results of the present study, Karamian and Ataei Barazande (2013) stated that NaCl increased enzymatic activities of catalase as well as peroxidase in the sainfoin plant. Findings presented by Rostami *et al.* (2018) also confirmed that increasing the concentration of NaCl up to the four $dS.m^{-1}$ increased enzymatic activities of both catalase and peroxidase in Lallemantia while their activities decreased at higher salinity levels. Peroxidase and catalase enzymes are two enzymatic antioxidant defense systems in plants against peroxidative damage of cell wall and oxidative stress (Venkatesan and Sridevi, 2009). Catalase can scavenge hydrogen peroxide, the main product of the activity of superoxide dismutase. In general, catalase is a general oxidoreductase reagent that catalyzes the decomposition of hydrogen peroxide into water and oxygen molecules (Asish and Anath, 2005).

Conclusion

Results of this study showed that increasing salinity reduced all the studied traits except the activity of antioxidant enzymes. Salinity stress reduced seedling germination and growth traits by damaging the physiological activities of seedling such as photosynthetic pigments and enzyme activity. This study showed that all seed priming methods are effective in reducing the effects of salinity stress due to increasing moisture absorption capacity, accumulation of germination activating enzymes and activation of antioxidant enzymes. No significant difference was observed between the effects of different primings, at low salinity concentrations (100 mM), but NaCl priming showed better results in most traits when salinity stress increased. Priming with potassium chloride was a suitable treatment to increase the tolerance of Lallemantia seedlings against high levels of salinity stress. Hence, since potassium has the potential to reduce the harmful consequences of NaCl and consequently the effects of salinity stress, the better reaction of potassium chloride compared to the other treatments can be attributed to the presence of potassium in the mentioned solute.

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