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Full Length Research Paper

The effect of hydro and osmopriming on alfalfa seed germination and antioxidant defenses under salt stress

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Seeds of two alfalfa *(Medicago sativa* L) varieties, cv. Hamedani and Yazdi, were used to investigate the effects of osmo- and hydro-priming on seed germination, growth parameters, biochemical changes and antioxidant enzymes activities under high-level salt concentration (150 mM NaCl) stress. Seeds were primed with water and mannitol (4%) for 12 h at 25 ± 1°C. Ten-day-old seedlings obtained from seeds primed with mannitol (4%) and water showed more growth with respect to root and shoot length in comparison with seedlings obtained from non-primed seeds. The results showed that germination percentage was significantly higher than that of the unprimed seeds after priming. The priming treatment significantly enhanced the activities of catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and proline content and reduced the malondialdehyde (MDA) accumulation and electrolyte leakage under the salt stress condition. The results suggested that osmo- and hydro-priming were effective methods to enhance the ability of salt tolerance and to improve seed germination and seedling growth of alfalfa under high salt concentration stress condition. It seems that, these priming methods could be applied in alfalfa production in high saline soils in the future.

Key words: Alfalfa, antioxidant defenses, priming, salt stress.

INTRODUCTION

Saline soils are the most common abiotic stress that plants encounter. Globally, approximately 22% of the agricultural land is saline (FAO, 2004). Plants can resist osmotic stress by morphological changes, example by increasing the size of the root system or reducing leaf area (Guo et al., 2002; Han and Wang, 2005, Irigoyen et al., 1992) and by changing physiological and biochemical processes such as antioxidant defense systems, solute accumulation, etc. (Rogers et al., 1995; Hasegawa et al., 2000; Hsiao and Xu, 2000). It was reported that high salt condition caused penetration stress and ion toxin, damaged water potential balance and ion distribution of plants, and then caused assembling of reactive oxygen species (ROS). Lipid peroxidation mediated by activated toxic oxygen species should be accompanied by changes in activities of enzymes involved in oxygen metabolism. Injury of ROS to plant have different ways: (a) cause non-activity of enzyme; (b) damage structure of nucleic acid; (c) retard protein synthesis because of damaging DNA duplication; (d) start peroxidation and damage membrane system. Cooperation of protective enzymes such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) could eliminate ROS and keep a homeostasis between producing and cleaning of ROS and low level of free radicle. Finally, injury to cells could be declined or escaped (Hasegawa et al., 2000).

Alfalfa is the most important forage crop for the arid and semi-arid areas, where increased salinity of irrigated fields is one of the major abiotic stresses for agriculture. According to alfalfa biological and ecological characteristics and its salt and alkali tolerances, this species was the first selected plant for resuming vegeta-tion in derivative ecosystem. However, most alfalfa seeds could perform well only in middle or low saline-alkaline soils. If growing in a high-level saline soil, alfalfa has difficulties to complete its whole growth process because of serious stress condition. Low germination, non-uniform emergence, unsure seedling emergence and low seeding in spikes usually occur during alfalfa sowing and maturation and these problems could restrict planting area of alfalfa to a certain extent. Improving the ability of salt tolerance will be beneficial to using abundant saline-alkaline soil resource and to develop alfalfa production energetically (Yang, 2001).

Seed treatment technology before sowing can be used to enhance salt tolerance of plants. Priming of seeds in

osmoticums such as mannitol, polyethylene glycol and sodium chloride (osmo-priming) and in water (hydro-priming) has been reported to be an economical, simple and a safe technique for increasing the capacity of seeds to osmotic adjustment and enhancing seedling establishment and crop production under stressed conditions. This could be due to faster emergence of roots and shoots, lower incidence of resowing, more vigorous plants, better drought tolerance, early flowering, early harvest and higher grain yield under adverse conditions (Amooaghaie et al., 2010; Cayuela et al., 1996; Passam and Kakouriotis, 1994). In priming, seeds are exposed to restricted water availability under controlled conditions which allows some of the physiological processes of germination to occur and then, before germination is completed, the seeds are usually redried for short term storage before sowing (Halmer, 2000).

Seed priming is now a widely used commercial process developed to help accelerate germination and improve seedling uniformity in many crops and ornamental plants, especially where they are grown under unfavorable environmental condition (Halmer, 2000). The priming may constitute a useful tool in overcoming these problems, assuring a high probability of successful establishment for each seed planted. Since some genotypes are more sensitive to salt than others, it is possible that priming could make these genotypes germinate as fast as the salt tolerant ones, and may consequently be a substitute for improvements in genetic background. Furthermore, it could also improve the performance of the salt tolerant ones (Kaya et al., 2006).

However, the effects of priming on alfalfa grown under high-salt stress conditions was not frequently reported, and osmo and hydropriming as procedures to improve alfalfa seed germination and growth in salt stress was also not documented.

In this study, the effects of osmo- and hydro-priming technology on alfalfa seed germination in high-level salt stress were investigated. The results offer theoretical bases for planting alfalfa in high-level saline soil. A better understanding of the mechanisms involved in the inhibition of plant growth by salinity may accelerate the introduction of environmental and genetic manipulations aimed at increasing crop salinity tolerance.

MATERIALS AND METHODS

Critical salt tolerance test

Alfalfa seeds *(Medicago sativa* L) from two varieties, (Hamedani and Yazdi cultivars) from Isfahan Agriculture research Center (Iran), were used in this experiment. Seeds were washed with water, dipped in 0.1% $HgCl_2$ for 5 min and again washed thoroughly with sterilized water under aseptic conditions. Salt tolerance of alfalfa seeds was tested by using solutions of 0, 50, 100, 150 and 200 mM NaCl concentrations. Alfalfa seeds were germinated in 9 cm Petri dishes containing two layers of moistened blotters with 4 ml above NaCl solution, respectively, in germination chamber at 20°C under alternating cycle of 12 h of light and 12 h darkness for 10 days.

Seed priming

The solutions of mannitol (4%) and distilled water were autoclaved for 15 min at 15 psi pressure for osmo- and hydro-priming of seeds. The washed alfalfa seeds were fully immersed (1:2, w/v) in these solutions under aseptic conditions and kept for 12 h at 25 ± 1°C. The seeds were then washed with distilled water and dried on a filter paper at room temperature.

Seed germination and seedling growth

Seeds of primed and unprimed (control) two alfalfa cultivars (Hamedani and Yazdi) were placed in 9 cm diameter Petri dishes containing two layers of moistened blotters with 5 ml 150 mM NaCl solution. After that, plates were placed in a growth chamber (model: PGV-36, Canada) with day/night rhythm: 12/12 h, temperature: 23/18°C, light intensity: 260 µmol/m^2·s, relative humidity: 65% for 10 days. In the first 5 days, 1 ml 150 mM NaCl solution was supplied in the Petri dishes. Three replicates of 50 seeds each for each treatment were used. Seeds were considered germinated when a 1 mm length radicle protruded through the seed coat. Final germination percentage, shoot and root length and seedling dry weight were calculated at the tenth day after sowing. The fresh and dry weights of the seedlings were measured immediately 10 days after the stress treatment. The dry weights were measured by drying the seedlings at 75°C, to give a constant weight.

Biochemical parameters

The content of malondialdehyde (MDA) and proline in shoots were measured at the tenth days after sowing. Proline was extracted in sulphosalicylic acid and was estimated by the method of Bates et al. (1973). MDA content was determined according to Zhang and Kirham (1994). Half a gram material from seedling shoots of alfalfa were ground in a mortar in 5.0 ml of 5% (w/v) trichloroacetic acid, and followed by centrifugation at 4000 ×g for 10 min at 4°C.

The supernatant extract (2.0 ml) was collected and mixed with 2.0 ml thiobarbituric acid. Samples were boiled for 10 min. After cooling down, samples were measured spectrophotometrically at wavelengths of 532, 600, and 450 nm,

Electrolyte leakage was determined as described by Dionisio-Sese and Tobia (1998). Fresh, shoot tissue (200 mg) were cut into pieces of 5 mm length and placed in test tubes containing 10 ml of double-distilled water. The tubes were incubated in a water bath at 25°C for 2 h and the initial electrical conductivity of the medium (EC_1) was measured. The samples were autoclaved at 121°C for 20 min to release all electrolytes and cooled to 25°C, after which the final electrical conductivity (EC_2) was measured. The electrolyte leakage (EL) was calculated using the formula: $EL = (EC_1/EC_2) \times 100$.

Extraction and determination of enzyme activities

The activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were calculated according to Gao (2000) and Zhang and Kirham (1994). 0.5 g shoot tissue material was ground in a mortar with pestle (0°C) containing a small amount of sand and 5.0 ml grinding media consisting of 50 mmol/l phosphate butter solution (PBS, pH = 7.8) and 1% PVP, followed by a 15 min centrifugation at 10000 ×g and 4°C. The supernatant extract was collected and stored at 4°C for all enzyme assays.

One enzyme unit of SOD activity is defined as the amount of enzyme required to cause 50% inhibition of the rate of NBT reduction measured at 560 nm. For POD, the oxidation of guaiacol was measured by the increase in absorbance at 470 nm. For CAT,

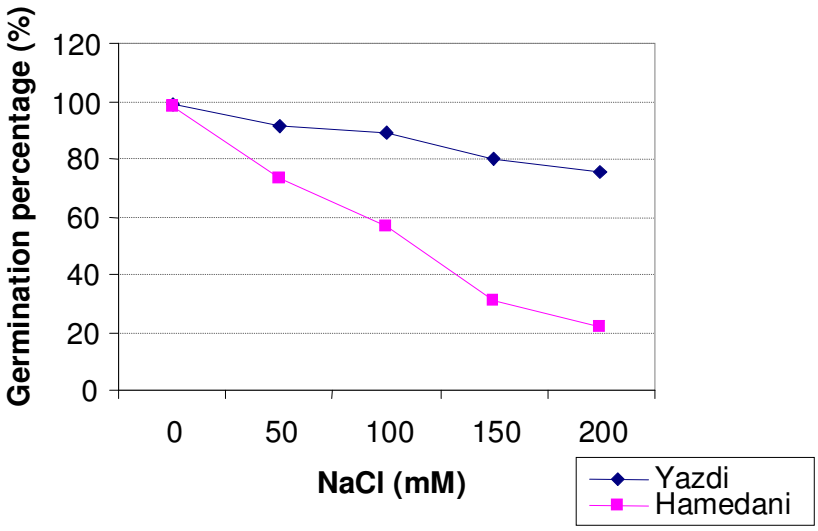

Figure 1. Changes of the germination percentage of alfalfa seeds under different NaCl concentration solution.

the decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. Activities for POD and CAT were expressed in enzyme units per mg fresh weight, where one enzyme unit was defined as a change of 0.01 absorbance min^{-1} caused by the enzyme aliquot.

Statistical analysis

All data were analyzed statistically by analysis of variance using MC-STAT software. The factorial experiments were carried out using a randomized complete design with 3 replications. Mean comparisons were performed using an ANOVA protected least significant difference (LSD) ($P < 0.05$) test.

RESULTS

Critical salt tolerance test

The ability of seed salt tolerance was indicated by the germination percentage, which showed the critical salt concentration for alfalfa seeds. Seeds germination of alfalfa was significantly affected ($P < 0.05$) by NaCl concentrations (Figure 1). The highest germination percentage was found in distilled water, followed by 50 mM NaCl. The highest NaCl concentrations (150 and 200 mM NaCl) showed substantial reduction in seed germination (Figure 1).

The differences between the variants were significant and mathematically proven ($P < 0.05$ - 0.01). However, none of the treatments had a statistically significant effect on seed germination of 'Yazdi' until 150 mM. But, seed germination decreased at salinity higher than 50 in 'Hamedani, (Figure 1). Yazdi had relatively steady levels until medium salt concentrations, but Hamedani cv. suffered a drastic decrease at 100 mM NaCl. These results show tha t 'Hamedani' is more sensitive than 'Yazdi.'

Seed germination and seedling growth

In unprimed seeds (controls), germination percentage in Hamedani cv was significantly lower than Yazdi cv in 150 mM NaCl solution. Germination percentage after osmo- and hydro-priming less than 150 mM NaCl solution was significantly higher than the controls in the two varieties (Table 1).

According to the analysis of data, shoot and root length of plants 10 days after treatment were significantly ($P = 0.05$) affected by the high salinity of both cultivars. There was a significant reduction in length shoots and a less decrease in root length (Table 1), leading to an increased root to shoot ratio.

In control conditions, there were small insignificant genotype differences in shoot length (Table 1). A well-defined difference trend occurred when the plants were exposed to high salt concentration. The difference at seedling shoot length between the two cultivars reached 52% at high salt (150 mM) concentration.

Our results showed that osmo- and hydro-priming improved shoots and roots length of two alfalfa varieties under high-salt stress of 150 mM NaCl,

Biochemical parameters

An increase of MDA accumulation following salinity stress was observed in both the control and the treated variants, that is, a state of oxidative stress is induced related to membrane damage (Table 2). The MDA content in primed seedlings was reduced to 60% of its value in controls ($P < 0.05$).

Table 1. The effect of osmo and hydro-priming on some growth parameters of alfalfa cultivars as compared to the control (un-primed) under 0 and 150 mM NaCl.

Cultivar	Treatment	Salinity (mM)	Final germination (%)	Root length (cm)	Shoot length (cm)	Shoot to root ratio
Yazdi	Control	0	98.6^{a}	5.21^{acd}	5.57^{a}	0.93^{c}
		150	75.2^{c}	2.90 ^{e}	2.76^{c}	1.05^{ab}
	Osmopriming	0	99.1^{a}	5.46^{ac}	5.68^{a}	0.96^{bc}
		150	89.7^{b}	4.46^{ab}	4.16^{b}	1.07^{a}
	Hydro priming	0	98.9^{a}	5.51^{a}	5.74^{a}	0.95^{bc}
		15	86.9^{b}	4.44^{ab}	4.09^{b}	1.08^{a}
Hamedani	Control	0	96.3^{a}	4.15^{b}	5.15^{a}	0.80^{d}
		150	25.3^{e}	1.1^{8f}	1.45^{d}	0.81^{d}
	Osmopriming	0	97.9^{a}	4.41^{bc}	5.42^{a}	0.81d
		150	61.4^{d}	3.40^{b}	3.96^{b}	0.85^{cd}
	Hydro priming	0	99.5^{a}	4.37^{bd}	5.33^{a}	0.82^{d}
		150	59.8^{d}	3.45^{b}	3.97^{b}	0.86^{cd}
LSD			10.1	1.1	1.06	0.11

Different letter among treatments of each column mean significant difference at P ≤ 0.05 LSD.

MDA content showed rapid increase in Hamedani and gentle increase in Yazdi under salinity stress, and accumulated (1.82 and 2.78 times of the controls in Yazdi and Hamedani, respectively) at high salt concentration. After priming, MDA content exhibited slight decrease in Yazdi and dramatic decrease in Hamedani (Table 2). These results shows that the antioxidative abilities in Yazdi and Hamedani were affected differently by salinity and priming.

An increase of proline accumulation following salinity stress was observed in both the control and the treated variants. The proline content in shoots of two alfalfa varieties after osmo- and hydro-priming was higher than that of the controls. Proline content increased by osmo- and hydro-priming (45.23, 40.47 in Hamedani and 12.79, 19.76 in Yazdi) (Table 2).

Our results showed that osmo- and hydro-priming also reduced, EL under high-salt stress of 150 mM NaCl, in shoots of two alfalfa varieties (Table 2).

Enzyme activities

At control conditions, there were small insignificant genotype differences in the activity of SOD, POD and CAT. SOD, POD and CAT activities were stronger in Yazdi than Hamedani. The activity of SOD, POD and CAT significantly changed under salinity stress which, as shown in Table 2, were 1.22, 1.18 and 1.45 times of the controls in Hamedani and 1.42, 1.57 and 2.16 times of the controls in Yazdi, respectively. The different change of SOD, POD and CAT activities might result from different sensitivities of Yazdi and Hamedani to salt stress.

After exposure to stress, SOD, POD and CAT activities increased in both the primed and unprimed seedlings (Table 2). At the same time, the differences between unprimed and primed plants were considerable. The activities of POD, CAT and SOD were significantly increased after osmo- and hydro-priming as compared with non-primed plants in the two varieties.

After exposure to stress, increase of the activities of SOD, POD and CAT was observed after priming in both cultivars (1.19, 1.18 and 1.23 times of un-primed plants in Yazdi and 1.34, 1.69 and 2.04 times of un-primed plants in Hamedani, respectively).

DISCUSSION

Critical salt tolerance

In this trial, the salt stress was simulated with NaCl and the critical salt concentration for alfalfa seeds was detected by seed germination. General and steady decrease in seed germination occurred upon exposure to increasing salt concentrations (Figure 1). The explanation of the reduction in germination percentage by the effects of NaCl may be that the decrease in water potential gradient between seeds and their surrounding media

Table 2. The effect of osmo- and hydro-priming on MDA and proline content, EL, CAT, POD and SOD activity of alfalfa cultivars as compared to the control (un-primed) under 0 and 150 mM NaCl

Cultivar	Treatment	Salinity (mM)	MDA (µmol.g FW^{-1})	Proline (µg.g FW^{-1})	EL (%)	CAT (u.g FW^{-1} min^{-1})	POD (u.g.FW^{-1}. h^{-1})	SOD (u.g FW^{-1}. h^{-1})
Yazdi	Control	0	2.8^{f}	35.1^{d}	28^{d}	195.1^{f}	364.5^{c}	282.0^{g}
		150	5.1^{b}	86.3^{b}	56^{b}	423.4^{c}	575.1^{c}	401.3^{c}
	Osmopriming	0	1.6^{g}	39.2^{d}	16^{c}	257.2^{e}	401.2^{d}	313.2^{e}
		150	3.9^{e}	97.1^{a}	43^{c}	514.1^{a}	699.1^{a}	465.4^{a}
	Hydro priming	0	1.8^{g}	43.4^{d}	15^{c}	255.0^{e}	415.3^{d}	325.1^{de}
		150	4.1^{de}	103.2^{a}	48^{c}	522.3^{a}	684.4^{a}	478.1^{a}
Hamedani	Control	0	3.2^{f}	26.5^{e}	32^{d}	163.2^{g}	297.2^{g}	269.5^{h}
		150	8.9^{a}	42.3^{d}	76^{a}	237.5^{d}	352.1^{e}	329.2^{d}
	Osmopriming	0	1.9^{g}	38.1^{d}	21^{c}	229.3^{d}	323.4^{f}	297.0^{f}
		150	4.3^{ce}	61.6^{c}	54 ^{b}	473.1^{b}	587.1^{bc}	431.1^{b}
	Hydro priming	0	1.7^{g}	41.2^{d}	24^{c}	238.2^{d}	317.6^{d}	316.6^{dc}
		150	4.7^{c}	59.7^{c}	58^{b}	485 .0^{b}	596.2^{b}	442.2^{b}
LSD			0.5	8.2	12	15.3	17.2	14.1

Different letter among treatments of each column mean significant difference at P ≤ 0.05 LSD. CAT, Catalase; POD, peroxidase; SOD, superoxide dismutase.

adversely affect seed germination. Thus, the mobilization of stored reserves and synthesis of proteins in germi-nating embryos are not able to begin subsequent growth processes (Ramagopal, 1990).

In this study, the germination significantly decreased with increasing salinity. Non-significant differences in germination were recorded when plants were grown in up to 150 mM NaCl in 'Yazdi' and 100 mM NaCl in 'Hamedani' (Figure 1). The differences in seed germination were non-significant for the second (50 mM) salinity level in 'Yazdi,' whereas the alternate salinity level in the increasing order was observed to depress significantly the seed germination in the Hamedani. This result indicates that the Hamedani is more sensitive to salinity than Yazdi, and that its growth was sharply retarded.

Seed germination and seedling growth

All results showed that the studied growth parameters were affected by the salt stress, which like all other abiotic stressors, slowed down the growth of the plants (Table 1). This slowing may be an adaptive response to stress (Zhu, 2001). Other researchers also reported that salinity inhibited seed germination (Hosseini et al., 2002) as well as seedling growth and development (Rogers et al., 1995). Regardless of the cause (ion toxicity, water deficit, and/or nutritional imbalance), high salinity in the root zone severely impedes normal plant growth and development, resulting in reduced crop productivity or crop failure. Reduced growth of plants subjected to gradual increase in salinity in the growth media could be due to an osmotic effect and/or ion toxicity (Munns, 2002). The salt treatment significantly affected the shoot length (Table 1). Root length was significantly lower under salt treatment though the most affected parameter is

the germination percentage.

It is important that drought and salinity resistance in variety is characterized by small reduction of shoot growth in drought stressed conditions. In this study, greater reduction in shoot length was obtained under same NaCl concentration in Yazdi cv. Root to shoot length ratio increased 150 mM NaCl concentrations (Table 1). The results showed more inhibition of shoot growth than root growth in 150 mM NaCl (Table 1). Increase of root to shoot length ratio under stress condition depends on low water uptakes and results in reduction of shoot growth more than root growth. The decrease of shoot/root ratio indicates that plants favour growth and allocate resources to roots. Our findings confirm the observation of Hsiao and Xu (2000) who reported that shoot growth was often more reduced than root growth by salinity, a phenomenon common with dry soil. It seem that selection in variety for root length and root-to-shoot length ratio under osmotic stress could be instrumental in predicting the salinity tolerance of genotypes. There were no significant differences in root to shoot length ratio between primed seed and untreated. Osmo- and hydro-priming lowered the negative effects of NaCl on root and shoot. But the increase in shoot growth with primed seed was higher when compared to root growth.

Overall, increasing salt concentrations inhibited growth of both alfalfa cultivars but, the inhibition was genotype dependent. However, the growth of Yazdi cv. was not repressed greatly by high salt. Since these cultivars grow better than Hamedani cv. It could be the best for field applications. Therefore, 'Hamedani' suffered more from the salinity than 'Yazdi.' and they have different developmental responses against salinity stress. These results support previous findings. The decreasing shoot and root weight was the result of osmotic and ionic effects; different plants have developed different mechanisms to cope with these effects (Munns, 2002). Alfalfa is a pasturage plant with some ability of salt and alkali tolerance; however, this characteristic is influenced by the genotype (Yang et al., 2001).

Seeds are more prone to stress from sowing to seedling emergence. Procedures to shortening this period may contribute to improve seed performance (Halmer, 2000). Our results showed that osmo- and hydro-priming could enhance seed germination and seed vigor and improve shoot and root growth in alfalfa under high-salt stress of 150 mM NaCl concentration. Kaya et al. (2006) have previously reported that seed priming enhanced root length at osmotic stress of water and NaCl when compared to untreated seeds in sunflower. This may be related to rapid water uptake in priming techniques such as mannitol and hydropriming treatments in comparison with untreated seed. Therefore, higher water uptake ability in seed primed with mannitol and water led to higher germination percentage in osmotic stress of NaCl. Kaya et al. (2006) reported that primed seeds had more rapid water uptake abilities than untreated seeds in sunflower.

Other researches also showed that seed priming could increase the seed tolerance to stress and improve adversity threat. Cayuela et al. (1996) reported that priming of seeds with NaCl enhanced salt tolerance of tomato. The results of Passam and Kakouriotis (1994) showed that seed osmo conditioning treatment with NaCl increased the germination and emergence of cucumber under saline conditions. Another research reported that NaCl priming increased seedling dry weight and germination, and increased proline and soluble sugar content in melon under saline conditions (Sivritepe et al., 2003).

Biochemical parameters

Our results showed that osmo- and hydro-priming reduced MDA accumulation and EL under high-salt stress. The MDA content is a reflection on the level of lipid peroxidation action on cell membrane. Osmo- and hydro-priming could restrain lipid peroxidation and improve the ability of salt tolerance in alfalfa. Therefore, the priming treatment revealed its effects on physiological and biochemical metabolism. If the plasmalema is damaged, the cell contents may leak out, and the plant will die. Under saline conditions, plasma-membrane leakage (an indicator of cell plasma-membrane integrity) increases. In fact, there is a linear relationship between external salinity and membrane-leakage rate (Orcutt and Nielsen, 2000).

Our result showed that increasing salinity damaged cell membrane stability, and electrolyte leakage in 'Hamedani' more than in 'Yazdi' (Table 2). Therefore, impairment of membrane permeability was lower in the shoots of the tolerant variety than in those of the sensitive one.

Changes in general metabolism of the seedlings and mature plants under the influence of stress factors may be responsible for their tolerance rate. When plants are exposed to a high concentration of salt, some tolerate the salinity stress by producing organic solute in the cytoplasm to lower the osmotic potential. The osmotic adjustment, that is, reduction of cellular osmotic potential by net solute accumulation, has been considered an important mechanism of salt tolerance in plants. This reduction of osmotic potential in salt-stressed plants can be as a result of compatible organic solute accumulations (Hasagawa et al., 2000).

Proline content in the shoot 10 days after treatments was increased with increasing salt in the nutrient solutions. These results show that proline production is a response of alfalfa to salt stress (Table 2). We found that proline content was increased by salinity and the sensitive variety had less proline content.

Among the organic cytosolute, proline appears to be the most widely distributed osmolyte that accumulates

under stress conditions in plants. It has been suggested that proline accumulation under stress plays several roles, namely, as an osmoticum, as a nitrogen and carbon reservoir for post-stress conditions, hydroxyl-radical scavenger, as a redox buffer means of reducing the acidity and as a compatible solute that protects enzymes and organelles (Hasagawa et al. 2000).

The present research showed that there was difference in proline accumulation between the two varieties that had difference in salt tolerance, with and without priming. It indicated that osmo- and hydro-priming improved salt tolerance by accumulation of proline under high salt stress condition.

Enzyme activities

One consequence of exposure to salinity stress is the generation of reactive oxygen species (ROS), which in turn have a negative oxidative stress effect on cellular structures and metabolism. To be able to endure oxide-tive damage, plants must possess efficient antioxidant system. Plants possess antioxidant systems in the form of enzymes such as: SOD, APX, GR, DHAR, CAT and metabolites viz., ascorbic acid, glutathione, α-tocopherol, etc.

These antioxidant enzymes and metabolites are reported to increase under salinity stress as well as comparatively higher activity has been reported in tolerant cultivars (species) than the susceptible ones (Wang et al., 2009; Sekmen et al., 2007), suggesting that higher antioxidant enzymes activity have a role to impart tolerance to these cultivars against salinity stress. Our results confirm this hypothesis.

The activity of SOD, POD and CAT significantly changed under salinity stress (Table 2) differently in both cultivars. Zhu et al. (2004) reported a similar result in different kinds of soybean seedlings. Robioa et al. (2002) reported the increase of SOD activity in transgenic alfalfa leaves subjected to water stress. Some of the free radical scavenging enzymes is reported to increase in wheat (Zhang and Kirham, 1994), plantago (Sekmen et al., 2007) and soybean (Zhu et al., 2004) plants under osmotic stress. However, Irigoyen et al. (1992) reported that SOD activity was unchanged and POD activity declined gradually during water stress in alfalfa leaves. It has been reported that several environmental factors such as drought and salinity can cause an excess or high toxic oxygen-free radicals (Hasegawa et al., 2000; Munns 2002).

Our results showed that osmo- and hydro-priming enhanced the activity of protective enzyme (POD, CAT and SOD). The higher activity of antioxidant enzymes in the primed seedlings (Table 2) suggests, that priming probably prepares the cell to meet and overcome stress by stabilizing membranes and forming a potential of higher antioxidant capacity.

Hu et al. (2006) reported that sand priming enhanced the free radical scavenging capacity of treated alfalfa plants including the levels of POD, CAT and SOD under salinity stress.

Conclusion

Our results showed that salt stress causes growth reduction, membrane disorganization, generation of reactive oxygen species and biochemical change. The present results suggested that osmo- and hydro-priming method could enhance the ability of salt tolerance in alfalfa seeds by improving seed germination under high-salt stress condition by using two cultivars, each representing one seed. Our results on 'Hamedani' suffered more from the salinity than 'Yazdi' because they have different mechanism to overcome salinity stress. More seed lots in the experiment will be of benefit to explain the results.

Our result showed that positive effects of osmo- and hydro-priming for seed germination under high-salt stress were similar. But probably, osmopriming methods were not suitable for application in field crop production in large scale, because of its high cost. On the other hand, its usage may be difficult for farmers because it needs special condition. But hydropriming as an ideal priming method is cheap, reusable and easy to use. Hydropriming can process a large number of seeds at a time, and this method seems to be possible for application in alfalfa production in high salt soil in the future.

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