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Seed Priming to Overcome Salinity Stress in Persian Cultivars of Alfalfa (*Medicago sativa* L.)

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Abstract

In order to investigate the effect of hydro-priming on seed germination of five alfalfa cultivars under salinity stress, an experiment was conducted as a factorial experiment based on the completely randomized design with three replications. Seven levels of hydro-priming and salinity of NaCl including prime and non-salinity, prime and 50 mM salinity, prime and 100 mM salinity, prime and 150 mM salinity, prime and 200 mM salinity, prime and 250 mM salinity and without prime and salinity and five alfalfa varieties, including 'Hamedani', 'Isfahani', 'Bami', 'Yazdi' and 'Ghareh Yonjeh' were used. The results showed that the main effect of prime, salinity and cultivars and their interaction in all studied traits were significantly affected at the 5% probability level. Priming treatments in non-salinity of all cultivars were the highest. In all cultivars, final germination percentage, length and weight of radicle, plumule and seedling, germination rate and time, relative radicle elongation, vigor index and stress index, were significantly improved in response to priming in salinity levels of 50-200 mM, compared to control. Radicle produced higher length and weight than the plumule in hydro- priming and salinity treatments. 'Hamedani' cultivar in most of studied characteristics had a better response than others. The lowest response to salinity stress and priming was observed in 'Yazdi' cultivar.

Keywords: hydro-priming, salinity, varieties

Introduction

Medicago sativa L. is one of the most important forage crops in the world. It has been originated from the Caucasus region (Michaud *et al.,* 1988). Alfalfa improves the yield and quality of the following crops by atmospheric nitrogen fixation (Bruulsema and Christie, 1987). Furthermore, it reduces diseases and weeds, increases soil organic matter contents and improve water infiltration (Cambel *et al.,* 1990). There is a considerable variation for salinity tolerance among and within the alfalfa populations. Alfalfa is sensitive to soil salinity at the germination stage, but after, during the early stages of growth, specific adaptations to soil salinity appear. Soil salinity is a major factor limiting plant productivity, affecting about 95 million hectares worldwide (Szabolcs, 1994). The UNEP (United Nations Environment Program) estimates that 20% of the agriculture and 50% of the cropland in the world are under salt stress (Flowers and Yeo, 1995). Salinity imposes serious environmental problems that affect grassland cover and the availability of animal feed in arid and semi-arid regions (El-Kharbotly *et al.,* 2003). Epstein *et al.* (1980) reported that salinity decreased seed germination, retarded plant development and reduced crop yield. Shokohifard *et al.* (1989) noted that salt stress negatively affected seed germination and either osmosis through reducing water absorption or ionization through the accumulation of Na^+ and Cl^- which caused imbalance in nutrient uptake and toxicity. Seed priming has proved to be a successful strategy to reduce the adverse effects of salt and drought stress, and improve the germination percentage and uniformity of emergence in grass, vegetable and field crops (Kaya *et al.*, 2006). Moradi *et al.* (2008) showed that, for the most evaluated germination parameters of corn seeds, priming was the effective treatment. Seed priming is a pregermination physiological method that improves seed performance and provides faster and synchronized seed germination. The improved seed performance could be attributed partially to osmotic adjustment, metabolic repair processes or to a buildup of germination metabolites during treatments (Haghpanah *et al.*, 2009). Hydro-priming, as proposed by Harris (1992), which is a low cost method with beneficial effects on many field crops, such as maize, rice, chickpea, soybean and sunflower (Ashraf and Foolad, 2005; Kaya *et al.*, 2006). When the seeds were primed and sown in the field, they showed a faster and more uniform germination comparing to non-primed seeds (Bourgne *et al.*, 2000). Hydro-priming of cereal rye and perennial ryegrass can significantly increase the rate percentage of germination (Snap *et al.*, 2008). Giri and Schilinger (2003) showed that the effect of hydro-priming with water is equal, and in some cases even more significant, than other priming environments. Liu and Wang (2008) reported that hydro-priming repaired the biological membrane, enhanced the stability of cellular structure and resistance to germination

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stress in alfalfa varieties. The objectives of the present study were to determine the effects of hydro-priming seeds on seed germination and seedling growth, and to establish possibilities to improve germination and overcome salinity stress.

Material and methods

Local study was carried out at the Faculty of Agriculture, University of Bu-Ali Sina, in Hamedan, Iran. *Medicago sativa* L. varieties were used as seed material. Seven levels of hydro-priming and salinity was used, including prime and non-salinity, prime and 50 mM salinity, prime and 100 mM salinity, prime and 150 mM salinity, prime and 200 mM salinity, prime and 250 mM salinity and without prime and salinity together with five alfalfa varieties, including 'Hamedani', 'Isfahani', 'Bami', 'Yazdi' and 'Ghareh Yonjeh'.

Germination tests

Three replicates of 100 seeds were germinated between double layered rolled Anchor germination papers with 5 ml of respective test solutions. The papers were replaced every 2 days to prevent accumulation of salts (Rehman *et al*., 1996). The rolled paper with seeds was put into sealed plastic bags to avoid moisture loss. Before the germination experiments, seeds were surface sterilized in 0.58% Clorox (sodium hypochlorite) solution for one minute. Seeds were allowed to germinate at 25±1 °C in the dark for 10 days. Germination was considered from the appearance of the radicles with a length of 2 mm. Germination percentage was recorded every 24 h for 10 days. NaCl was applied for different levels of salinity. For hydro-priming, seeds were immersed in distilled water at 25 °C for 10 h under dark conditions (according to the preliminary experiment). The hydro-priming duration was determined by controlling seed imbibition during germination. At the end of the germination period, final germination percentage, germination rate, mean germination time, length and dry weight of plumule and radical, relative radical elongation, vigor index, seedling dry weight, relative germination, stress index and ratio of radicle to plumule were recorded. Mean germination time (MGT) was calculated by using the following equation: $MGT=\sum(n\times d)/N$, where 'n' is the number of seeds that germinated on each day; 'd' is the incubation period in days; and 'N' is the total number of seeds that germinated in the treatment (Brenchley and Proberet, 1998).

Furthermore, the vigor index of seedling was calculated with the following equation (Dahindwal *et al*., 1991): Vigor index = (plumule length + radicle length) x germination percentage/100.

Likewise, relative seed germination percentage, radicle elongation and stress index were determined. Relative germination = (number of seeds germinated in test solution/number of seeds germinated in control) x 100, and relative radicle elongation = (mean radicle length in test solution/ mean radicle length in control) x 100 (Teaca and Bodirlau, 2008), and stress index = seedling dry weight in test solution / seedling dry weight in control.

Statistical analysis

The experimental design was a two factorial (7×5), arranged in a completely randomized blocks, with three replications with 100 seeds per replicate. The statistical analysis of the experimental data was done using SAS software. The differences between the means were compared using LSD values ($P < 0.05$).

Results and discussions

The results of the experiment showed that, the main effect of prime, salinity and cultivars and their interactions for all traits including final germination percentage, germination rate, mean germination time, length of plumule, length of radicle, relative radicle elongation, vigor index, radicle and plumule dry weight, seedling dry weight, relative germination, stress index and ratio of radicle to plumule were significantly affected at the 5% probability level (Table 1).

Final germination percentage

As it is shown in Table 2, in seed priming final germination percentage of cultivars were not markedly reduced to low salinity (≤ 150 mM), but were significantly inhibited by high salinity (250 mM). In 200 mM salinity of 'Hamedani', 'Isfahani', 'Bami' and 'Yazdi' cultivars wasn't observed significant decrease in final germination percentage but, in the same concentration, 'Ghareh Yonjeh' cultivar was reduced. In all cultivars a final germination reduction was observed (250 mM salinity) and the lowest was related to 'Yazdi' cultivar. Final germination percentage reached the 84.00 and 86.66% at 200 mM salinity and decreased the 70.66 and 80.00% at 250 mM salinity in 'Yazdi' and 'Ghareh Yonjeh' cultivars, respectively (Table 2). Priming can increase the germination and growth of seedling under salinity stress conditions in sunflower seeds (Kaya *et al*., 2006). The highest final germination percentage was seen in seed priming and non-salinity for 'Ghareh Yonjeh' cultivar (100%).

Germination rate

In all treatments, prime and non-salinity had the highest germination rate compared to the control (without prime and salinity) respectively, 0.48, 0.41, 0.35, 0.33 and 0.31 germination rate with priming in cultivars of 'Ghareh Yonjeh', 'Bami', 'Hamedani', 'Isfahani 'and 'Yazdi' increased.

Table 1. ANOVA analysis showing the germination parameters of the studied traits

S.O.V.	df	Final germination	Germination rate	Mean germination time	Radicle length	Plumule length	Relative radicle elongation	Vigor index
Prime-salinity	6	362.58**	0.75**	5.69**	4273.69**	3114.85**	52780.63**	14302.73**
Cultivar	4	209.79**	0.02**	0.21**	128.45**	100.45**	5611.36**	796.96**
Prime, salinity × cultivar	24	24.45*	0.01**	0.16**	49.75**	55.47**	1156.32**	86.78**
Error	70	16.61	0.002	0.01	2.40	2.30	26.44	18.45
CV (%)		4.40	7.00	7.68	5.33	5.94	5.09	8.13

ns,**,* Respectively non-significant and significant of 1 and 5 percent of probability

In other words, 'Ghareh Yonjeh' was the mostly affected cultivar in non-salinity. The germination rate with priming treatment increased to the level of 150 mM salinity and since then, priming effect on germination in salinity conditions (150 mM ≤) was not significant and germination rate was decreased (apart from 'Hamedani' cultivar that did not decrease to 200 mM salinity). Giri and Schilinger (2003); Finch-Savage *et al*. (2004) reported that the priming effect on germination rate was positive in comparison with the control.

Mean germination time

Hydro-priming treatments (without salinity) in all cultivars improved mean germination time in comparison to control, respectively 1.17, 0.79, 0.72, 0.56 and 0.47 germination time with priming in cultivars of 'Ghareh Yonjeh', 'Hamedani', 'Bami', 'Yazdi' and 'Isfahani' decreased. Hydro-priming was able improve mean germination time to 150mM salinity in cultivars of 'Isfahani', 'Bami' and 'Yazdi' and to 200 mM salinity in the following cultivars: 'Hamedani' and 'Ghareh Yonjeh'. In all cultivars at 250 mM salinity greatly increased mean germination time. The highest level of mean germination time under 250 mM salinity was observed in 'Yazdi' cultivar (3.601/day). Demir and Van de Vanter (1999) reported that osmo-priming of watermelon seeds caused the decrease of the mean germination time. These results were in agreement with Basalah (1991) who found that high levels of salinity could significantly inhibit and delay the seed germination and simultaneously affect the mean germination time.

Radicle and plumule length

The length of radicle and plumule in all treatments were reduced by increasing salinity concentration. Radicle produced higher length than the plumule in prime- salinity treatments. In contrast, plumule length was greater than radicle length under control treatments. Azhdari *et al*. (2010) reported that the length of radicle had a higher tolerance than of the plumule. Under priming and non-salinity condition, the length of radicle and plumule increased.

Table 2. Means interaction effects of hydro-priming-salinity and cultivars in studied traits

Treatments		Final germination (%)	Germination rate (1/day)	Mean germination time (day)	Radicle length (mm)	Plumule length (mm)	Relative radicle elongation	Vigor index
Cultivar	Prime salinity (mmol.L^{-1})							
'Hamedani'	0	98.66	0.86	1.16	53.93	47.17	161.00	99.75
	50	97.33	0.84	1.21	47.57	38.10	141.99	83.39
	100	97.33	0.83	1.20	42.87	34.27	1.27.97	75.08
	150	96.66	0.80	1.25	38.13	30.17	113.83	66.01
	200	96.00	0.63	1.86	10.10	6.17	30.15	15.62
	250	89.33	0.39	2.42	6.20	11.00	18.51	15.37
	Control	96.00	0.51	1.95	33.50	36.30	100.00	67.01
'Isfahani'	0	96.00	1.00	1.00	54.53	47.63	163.76	98.07
	50	96.00	0.85	1.18	42.40	36.77	127.33	76.00
	100	96.00	0.84	1.19	40.03	32.93	120.22	70.04
	150	94.67	0.80	1.25	26.17	25.23	78.58	48.66
	200	92.00	0.50	1.98	11.67	4.50	35.04	14.88
	250	88.00	0.46	2.32	6.93	2.00	20.82	7.86
	Control	92.00	0.67	1.47	33.30	47.50	100.00	74.34
'Bami'	0	96.00	0.96	1.08	50.20	43.93	218.26	90.36
	50	95.33	0.88	1.13	50.27	32.53	218.56	78.93
	100	93.33	0.84	1.24	35.27	31.53	153.33	62.35
	150	93.00	0.74	1.41	33.67	20.37	146.38	50.53
	200	89.33	0.53	1.88	6.83	9.33	29.71	14.44
	250	86.66	0.36	2.73	5.00	4.97	21.74	8.64
	Control	90.00	0.55	1.80	23.00	43.50	100.00	59.85
'Yazdi'	0	94.66	0.91	1.09	48.57	36.03	198.23	80.08
	50	92.00	0.85	1.18	41.70	36.17	170.20	71.64
	100	90.33	0.85	1.18	31.37	22.60	128.03	48.75
	150	90.00	0.80	1.20	19.93	16.53	81.36	36.46
	200	84.00	0.49	2.04	6.83	13.00	27.89	16.66
	250	70.66	0.26	3.60	4.67	3.00	19.05	5.42
	Control	90.00	0.60	1.65	24.50	33.00	100.00	51.75
'Ghareh Yonjeh'	0	100.00	0.92	1.08	47.57	39.37	145.47	86.94
	50	97.66	0.92	1.08	47.37	35.57	144.85	80.99
	100	97.33	0.92	1.08	35.10	31.30	107.34	64.63
	150	97.33	0.86	1.17	26.43	20.70	80.84	45.87
	200	86.66	0.44	2.10	8.43	6.80	25.78	13.19
	250	80.00	0.38	2.43	6.17	2.17	18.86	6.67
	Control	96.00	0.44	2.25	32.70	38.80	100.00	68.64
LSD 5%		6.36	0.08	0.19	2.52	2.47	8.37	6.99

Radicle length to 150 mM salinity in 'Hamedani' and 'Bami' cultivars and 100 mM salinity of 'Isfahani', 'Yazdi' and 'Ghareh Yonjeh' cultivars were improved with hydro-priming. In this study, the length of radicle and plumule decreased sharply with the increase of salinity from 150 to 200 mM in all varieties (Table 2). 'Isfahani' cultivar had the highest radicle length in priming and non-salinity (54.53 mm) across cultivars. The smallest radicle length was related to priming and 250 mM salinity, in 'Yazdi' cultivar (4.67 mm). In snap beans (*Phaseolus vulgaris*), hydro-priming resulted in improved germination and seedling emergence and growth (Suzuki and Khan, 2001).

Relative radicle elongation

The calculated data of radicle elongation showed that hydro-priming (in non-salinity) compared to control enhanced the relative radicle elongation of all cultivars (Table 2). Also, priming improved the length of the radicle to 150 mM salinity concentrations for cultivars of 'Hamedani' and 'Bami' but, in other cultivars this increase was seen only to concentration of 100 mM salinity. It can be concluded that realative radicle elongation was a more sensitive test than relative seed germination. Similar findings were reported by Teaca and Bodirlau (2008).

Vigor index

The vigor index (Table 2) increased with hydro-priming in non-salinity treatmeants. The vigor index in all treatments was reduced by increasing salinity concentrations. 'Hamedani' cultivar had the highest vigor index in non-salinity (99.75) and also, concentration of 250 mM salinity (15.37) across cultivars. Moreover, the lowest vigor index in non-salinity (80.08) and 250 mM salinity (5.42) was observed in 'Yazdi' cultivar (Table 2). 'Hamedani' cultivar in this trait was more successful comparing to other cultivars. Hydro-priming resulted in a significant improvement of germination and seedling vigor and a decrease in leakage of electrolytes from germinating seeds (Srinivasan *et al*., 1999). Recently, Afzal *et al.* (2008) observed that priming induced salt tolerance was associated with improved seedling vigor, metabolism of reserves as well as enhanced K^+ and Ca^{2+} and decreased Na^+ accumulation in wheat plants.

Radicle and plumule dry weight

Data on mean radicle and plumule dry weight of different prime-salinity is shown in Table 4. Radicle and plumule dry weight decreased gradually with increasing salinity concentration in all varieties. This reduction was smaller inlow levels of salinity. These results are similar to radicle and plumule length. In 150 mM salinity of 'Bami' and 'Hamedani' cultivars no significant decrease was observed in radicle dry weight but, in the same concentration, cultivars of 'Isfahani', 'Yazdi' and 'Ghareh Yonjeh' were reduced. The lowest radicle dry weight in 250 mM salinity was observed in 'Yazdi' and 'Ghareh Yonjeh' cultivars (Table 4). Plumule dry weight to 50 mM salinity concentrations increased in 'Hamedani' and 'Yazdi' cultivars in comparison to control group. Apparently, prime-salinity has been more effective on radicle dry weight. Based on this study, it reveals that hydro-priming treatment has a positive effect on radicle dry weight and also on plumules dry weight in germination stages. Moradi and Younesi (2009) reported that osmo-priming and hydro-priming of *Sorghum*, increased radicle length and radicle dry weight of the seedlings obtained.

Seedling dry weight

The seedling dry weight in hydro-priming treatments increased to the level of 150 mM salinity in 'Hamedani' cultivar. Hydro-priming effect on germination in salinity conditions (≤150 mM) was not significant but seedling dry weight decreased. Likewise, hydro-priming treatment was able to improve the seedling dry weight to 100 mM salinity in 'Bami' cultivar and to 50 mM salinity in 'Isfahani', 'Yazdi' and 'Ghareh Yonjeh' cultivars. In the mean comparison, seedling dry weight altered from 0.18 mg in 250 mM prime-salinity treatments in 'Yazdi' cultivar to 2.36 mg in prime-non salinity treatment of 'Hamedani' cultivar. Kaya *et al.* (2006) showed that sunflower seed priming under salinity stress, causes improved germination and increases seedling dry weight.

Relative germination

Compared to the control, hydro-priming seeds in low and non-salinity treatments had a positive effect on relative germination in all cultivars. Comparison among treatments in 250 mM salinity condition decreased relative germination. The highest relative germination percentage across 250 mM salinity conditions was observed in 'Bami' cultivar (96.28), respectively in 'Isfahani' (95.65), 'Hamedani' (93.06), 'Ghareh Yonjeh' (83.33) and 'Yazdi' (78.51). Relative germination percentage reached 93.33 and 90.28 at 200 mM salinity and decreased the 78.51 and 83.33 at 250 mM salinity in 'Yazdi' and 'Ghareh Yonjeh' cultivars. Several reports show that seed priming had positive effects on germination rate of maize and cotton seeds (Ashraf and Rauf, 2001; Murungu *et al*., 2003).

Ratio of radicle to plumule

Data obtained from different levels of salinity showed that hydro-priming caused an increase in ratio of radicle to plumule. Table 3 indicates that the ratio of radicle to plumule weight increased by increasing salinity stress. The ratio of radicle length to plumule length (or dry weight) is a flexible trait which increased as the result of salinity stress (Hamidi, 2000). Sadrabadi (1989) in a study on alfalfa plant argues that in this plant dedication of carbohydrates to root and leaves is prior. Leaves and roots have the priority to absorb water and to perform photosynthesis. Therefore the increase in ratio of radicle length to plumule length was decreased in the growth of aerial organs of the plant (Safarnejad, 2008).

Stress index

Stress index for various treatments are summarized in Table 3. The analysis of stress index showed that alfalfa cultivars have different reactions to different salinity levels. Decreasing trend of stress index with an increase in salinity stress has been observed in all cultivars. Hydro-priming was able to improve the stress index to 50 mM salinity in 'Isfahani' and 'Ghareh Yonjeh' cultivars and to 100 mM salinity in 'Bami' and 'Yazdi' cultivars and to 150 mM salinity in 'Hamedani' cultivar. 'Hamedani'

Table 3. ANOVA analysis showing the germination parameters of the studied traits

S.O.V.	df	Radicle dry weight	Plumule dry weight	Seedling dry weight	Relative germination	Radicle: plumule ratio	Stress index
Prime-salinity	6	3.64**	0.80**	7.56**	403.81**	1.71**	3.54**
Cultivar	4	0.14**	0.02**	0.36**	124.79**	0.20**	0.13**
Prime, salinity x cultivar	24	0.02**	0.01**	0.05**	29.62*	0.13**	0.03**
Error	70	0.002	0.0005	0.004	18.10	0.03	0.001
CV (%)		6.23	5.98	5.15	4.27	9.10	4.89

ns,**,* Respectively non significant and significant of 1 and 5 percent of probability

Table 4. Means interaction effects of hydro-priming-salinity and cultivars in studied traits

Treatments		Radicle dry weight (mg)	Plumules dry weight (mg)	Seedling dry weight (mg)	Relative germination (%)	Radicle: plumule ratio	Stress index
Cultivar	Prime salinity (mmol.L^{-1})						
'Hamedani'	0	1.59	0.77	2.36	102.78	2.07	1.52
	50	1.38	0.61	1.99	101.39	2.26	1.28
	100	1.24	0.54	1.78	101.39	2.29	1.14
	150	1.16	0.50	1.66	100.34	2.29	1.07
	200	0.29	0.12	0.41	100.00	2.41	0.26
	250	0.22	0.10	0.32	93.06	2.20	0.21
	Control	0.97	0.58	1.55	100.00	1.67	1.00
'Isfahani'	0	1.56	0.76	2.32	104.35	2.05	1.34
	50	1.23	0.59	1.82	104.35	2.09	1.05
	100	1.16	0.53	1.69	104.35	2.20	0.98
	150	0.76	0.40	1.16	102.90	1.89	0.67
	200	0.26	0.11	0.37	100.00	2.36	0.21
	250	0.15	0.06	0.21	95.65	2.50	0.12
	Control	0.97	0.76	1.73	100.00	1.27	1.00
'Bami'	0	1.46	0.70	2.16	106.66	2.08	1.58
	50	1.30	0.55	1.85	105.92	2.36	1.36
	100	1.02	0.50	1.53	103.70	2.04	1.12
	150	0.88	0.40	1.28	103.33	2.20	0.94
	200	0.25	0.12	0.37	99.25	2.08	0.27
	250	0.16	0.08	0.24	96.28	2.00	0.17
	Control	0.67	0.70	1.36	100.00	0.96	1.00
'Yazdi'	0	1.41	0.57	1.98	105.17	2.47	1.59
	50	1.21	0.58	1.79	102.22	2.09	1.44
	100	0.91	0.36	1.27	100.36	2.53	1.03
	150	0.58	0.26	0.84	100.00	2.20	0.68
	200	0.21	0.11	0.32	93.33	1.91	0.26
	250	0.14	0.05	0.18	78.51	2.82	0.15
	Control	0.71	0.53	1.24	100.00	1.35	1.00
'Ghareh Yonjeh'	0	1.37	0.62	1.74	104.17	2.20	1.11
	50	1.37	0.57	1.94	101.72	2.42	1.24
	100	1.02	0.50	1.52	101.39	2.03	0.97
	150	0.77	0.33	1.10	101.39	2.33	0.70
	200	0.24	0.11	0.35	90.28	2.20	0.23
	250	0.14	0.06	0.20	83.33	2.33	0.12
	Control	0.95	0.62	1.57	100.00	1.53	1.00
LSD 5%		0.08	0.04	0.10	6.92	0.31	0.06

cultivar had a better response than the others. Liu and Wang (2008) indicated with hydro-priming increases resistance to germination stress in alfalfa.

Conclusions

The current study shows that salinity has been affecting the germination process in all studied alfalfa varieties, although their classification regarding to salinity tolerance was depending on the considered parameter. In addition, hydro-priming of alfalfa seeds before planting improved germination, seedling growth and stress index under salinity stress. This can be considered a general response of alfalfa seeds to hydro-priming under salinity stress, as a protective response against it. The magnitude of these effects could be related to different levels of salt stress in alfalfa varieties. As a conclusion, 'Hamedani' cultivar in most of studied characteristics had better responses than other cultivars. Therefore, selecting the 'Hamedani' cultivar of *Medicago sativa* seeds with priming in reclamation and restoration projects could lead to positive results in arid and semi-arid areas, where salinity has a strong effect on vegetation development.

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