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ORIGINAL ARTICLE

The Effect of Priming on Germination and Seedling Growth of Alfalfa (*Medicago sativa* L.) under Salinity Stress

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Salinity stress is an abiotic stress which has harmful effects on germination, growth and yield of alfalfa (*Medicago sativa* L.) in many parts of the world. Seed priming is a way of increasing salt tolerance of plants. In this study, the effect of seed priming on germination and growth of alfalfa seedlings, Bami cultivar, under salinity condition was investigated. According to germination percentage of seeds under *In vitro* conditions, salicylic acid, Vitamin B_{12} and distilled water were selected as primes under salinities of 0, 5 and 10 $ds.m^{-2}$ NaCl solution for further studies. These studies were carried out in a factorial experiment based on Completely Randomized Design (CRD) with three replications in greenhouse under controlled condition (25°C in day/night). Parameters like percentage and rate of germination, dry and fresh weight of seedling and leaf number were measured. Based on our research All of the treatments (priming, salinity and the interaction of priming with salinity) significantly ($p<0.01$) affected Hypocotyl length and radicle length and in low level of salt (0 $ds.m^2$) germination and seedling growth indexes enhanced and also with applying salicylic acid both hypocotyl and radicle lengths improved.

Key words: salinity stress/ hydropriming/ alfalfa/ salicylic acid and Vitamin B12

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Environmental pollution is a major global problem and plants are more and more subjected to a variety of abiotic stresses. Among these stresses, high concentrations of salt in the soil can result in severe detrimental factors, such as poor germination, seedling establishment and crop yield. This is mainly due to low soil water potential and an imbalance in the uptake of mineral nutrients and their accumulation within the plant. Seed priming is an efficient method for increasing seed vigor and synchronization of germination, as well as the growth of seedlings of many crops under stressful conditions (Carvalho *et al*., 2011). Generally priming would cause an effective invigoration of the dry seed which is the inception of metabolic processes that normally occur during imbibition and which are subsequently fixed by drying the seed (Hanson, 1973). The determination of optimum priming

levels is an essential prelude to proposed field trials (Bodsworth and Bewley, 1981). Various prehydration or priming treatments have been employed to increase the rate and synchrony of seed germination (Bradford, 1986). Two of common priming techniques include osmopriming and hydropriming or soaking seed in water (Ashraf and Foolad, 2006). The technique of osmopriming also known osmoconditioning involves the imbibition of seed in salt or polyethyleneglycol (PEG) osmoticum (Abernethy, 1987). Several investigations of seed germination under salinity stress have indicated that seeds of most species achieve their maximum germination in distilled water and are very sensitive to elevated salinity at the germination and seedling phases of development (Monirifar, 2008; Bhardwaj *et al*., 2010; Yarnia, 2011; Torabi *et al*., 2011). The best period of priming with solutions of PEG has obtained for maize, wheat, barley, sorghum and soybean (Bodsworth and Bewley, 1981).

Salicylic acid (SA) and polyamines as signal molecules may play a role in improving the growth and development of the crops (Krantev *et al*., 2008). Salicylic acid or ortho hydroxybenzoic acid belongs to a group of phenol compounds that are known as the mitigating factors of plant responses to environmental stresses (Senaratna *et al*., 2000). Alfalfa (*Medicago sativa*) is a perennial crop which is known as the queen of the crops. It is one of the earliest and most plentiful forage crops (Karimi, 1998). Alfalfa often germinates and its seedlings establish weakly in Iran because of the high concentration of salts in irrigation water and salinity of top layers of the soil (Torabian, 2010). Therefore, given the forage and medicinal value of alfalfa, the objective of the current research was to study the responses of alfalfa seedlings to salinity stress and to investigate the possibility of mitigating the effects of salinity stress on this crop by pretreating its seeds with salicylic acid, Vitamin B_{12} and distilled water.

MATERIALS AND METHODS

Seed Preparation:

Seeds of the alfalfa, Bami cultivar were surface sterilized with sodium hypochlorite solution.

***In vitro* studies:**

In vitro treatments included salinity at five levels (0, 5, 10,15 and 20 $ds.m^2$) and Seed priming in solutions of vitamin B12, polyethylene glycol (PEG), and 5 mM salicylic acid (SA). Distilled-deionized water was used for hydropriming and non-primed (NP) seeds which had not received any priming treatment were used as control. After soaking in priming solutions, seeds were surface dried on filter paper and then allowed to air dry for 12 h at 25 °C. Air-dried seeds following priming with SA, Vitamin B_{12}, PEG and water only and non-primed seeds were placed in covered Petri dishes containing one layer of Whatman filter papers moistened with 4 ml distilled water. Plates were placed in a germination chamber at 25°C with 12 h fluorescent light and 12 h dark. Counts of germinating seeds were made daily, starting on the first day of imbibition and terminated when maximum germination was obtained. Normal seedlings were recorded for calculating germination percentage (GP) at last count.

In vivo studies:

After priming and air drying of the seeds as mentioned above, different salinities were applied to the soils of the pots and seeds were sowed in treated soils. On the 10th day, the germination percentage, germination rate, radicle length, hypocotyl length, Seedling Fresh and dry weight and leaf number were calculated.

Statistical analysis:

For statistical analysis, a factorial experiment with completely randomized design (CRD) with three replications was used. Analysis of Variance was based on ANOVA procedure by software SAS. Differences among the means of the treatments were estimated using the Duncan's multiple range tests at the 1% probability level.

RESULTS

***In vitro* studies:**

Regarding germination percentage of seeds, three priming factors (vitamin B12, distilled water and salicylic acid) and three level of salinity (0, 5 and 10 $ds.m^2$) were selected for *In vivo* studies:

***In vivo* studies**

The results of analysis of variance showed that priming and the interaction between priming and salinity didn't significantly affected Germination percentage and Leaf number however salinity significantly ($p<0.01$) affected them. Priming and the interaction between priming and salinity treatments led to significant ($p<0.01$) increasing in Seedling Fresh and dry weight and Germination rate (table 1). The comparison of the means of the traits as affected by different EC levels indicated that the values of all of the means except of germination rate in low salinity (o $ds.m^2$) were more than in higher salinities (5 and 10 $ds.m^2$) (table 2).

Salicylic acid treatment and hydropriming enhanced hypocotyl length and radicle length more than vitamin B_{12}. Salicylic acid treatment increased hypocotyl length more effective than hydropriming but there was not any significant difference between Salicylic acid treatment and hydropriming about radicle length (table 3).

There was no significant difference between priming with salicylic acid and distilled water with 0 $ds.m^2$ on hypocotyl length. The interaction effects of priming with salinity on radicle length were in higher level than other traits. About radicle length different priming factors had no significant difference in 0 $ds.m^2$ and hydropriming showed the best results in 5 $ds.m^2$. The most Seedling dry and fresh weight obtained of interaction effects of salicylic acid with 0 $ds.m^2$ and hydropriming with 0 $ds.m^2$, respectively (table 4).

Table 1. Analysis of variance for germination and seedling growth parameters of alfalfa

Source of variation	Germination percentage	Leaf number	Hypocotyl length	Radicle length	Seedling Fresh weight	Seedling dry weight	Germination rate
Prime	3.5^{ns}	3.1^{ns}	52.2^{**}	25.58^{**}	2.05^{ns}	3.51^{ns}	2.05^{ns}
EC	40.35^{**}	23.61^{**}	46.3^{**}	28.9^{**}	16.4^{**}	17.05^{**}	59.2**
Prime ×EC	2.3^{ns}	10.5^{ns}	11.52^{**}	5.15^{**}	4.55^{**}	7.26^{**}	9.01**

**: significant at 1% probability levels. ns: non-significant.

Table 2. Classification of means simple effect of salinity stress levels on germination and seedling growth.

Salt Tolerance EC ($ds.m^2$)	Germination Percentage	Leaf number	Hypocotyl length	Radicle length	Seedling Fresh weight	Seedling dry weight	Germination rate (number in day)
0	68(a)	3(a)	3.5(a)	3.5(a)	0.04(a)	0.03(a)	66(a)
5	34.5(b)	2(b)	1.88(b)	2.25(b)	0.025(b)	0.013(b)	27.5(b)
10	24.5(b)	2(b)	1.75(b)	2(b)	0.02(b)	0.012(b)	16.5(c)

Means with similar letter(s) in each trait is not significantly different at 1% probability level according to Duncan's Multiple Range Test.

Table 3. The comparison of the means of the measured traits as affected by different primes (distilled water, salicylic acid and vitamin B_{12}).

Prime	Hypocotyl length	Radicle length
Distilled water	2.33(b)	2.3(b)
Salicylic acid	4.1(a)	2.8(a)
Vitamin B_{12}	2(c)	2.1(a)

Means with similar letter(s) in each trait is not significantly different at 1% probability level according to Duncan's Multiple Range Test.

Table 4. Classification of means simple effect of interaction effects of priming with salinity stress levels on germination and seedling growth.

Interaction effects	Hypocotyl length	Radicle length	Seedling Fresh weight	Seedling dry weight
Distilled water×0 ds.m^2	2.61(a)	3.7(a)	0.1(a)	2.75(a)
Distilled water×5 ds.m^2	2(bc)	2.6(b)	0.07(b)	2.4(b)
Distilled water×10ds.m^2	1.3(c)	2.22(b)	0.05(bc)	2.35(b)
Salicylic acid×0 ds.m^2	1.8(b)	3.89(a)	0.09(a)	3.1(a)
Salicylic acid×5 ds.m^2	1.54(bc)	2.4(b)	0.06(b)	2.49(b)
Salicylic acid×10 ds.m^2	1.4(c)	2.3(b)	0.05(bc)	2.4(b)
Vitamin B_{12}×0 ds.m^2	1.43(c)	3.9(b)	0.06(b)	2.6 (b)
Vitamin B_{12}×5ds.m^2	3.3(c)	2.5(b)	0.05(bc)	2.33(b)
Vitamin B_{12}×10 ds.m^2	1.3(c)	2(b)	0.04(c)	2.29(b)

Means with similar letter(s) in each trait is not significantly different at 1% probability level according to Duncan's Multiple Range Test.

DISCUSSION

Among interaction effects of priming (as affected by distilled water, salicylic acid and vitamin B_{12}) with salt tolerance (0, 5, 10 ds.m^2), the maximum hypocotyl length obtained of hydropriming with 0 ds.m^2. Adequate water absorption and performance of metabolic and biochemical process in phases I and II germination may be reasons of germination and weight improvement. Noorbakhshian *et al.* (2011) have examined the priming agents (PEG 6000, KNO_3 and $CaCl_2$) in different osmotic potentials to determine the hydro and osmopriming effects on seed germination of sainfoin and resulted that the most germination of treated pods was obtained in the hydroprimed seeds (0 Mpa = hydropriming).

Based on results in low level of salt (0 ds.m^2) germination and seedling growth indexes enhanced and with applying salicylic acid both hypocotyl and radicle lengths improved. It can be concluded that pre-treatment with SA induced adaptive responses in alfalfa plant under salinity stress and consequently, encouraged protective reactions in biotic membranes which improved the growth of alfalfa seedlings. Torabian (2010) have investigated the effect of SA pre-treatment of seeds (at three levels of 0, 0.1 and 0.5 mmol) on germination and growth of seedlings of alfalfa under salinity stress conditions (at three levels of 0, 10 and 15 ds.m^{-1}). According to those researches, SA pre-treatment have improved growth and resulted in higher resistance of plants to salinity, so that it have increased germination percentage, seed vigor index and growth parameters of the seedlings. So salicylic acid is an effective priming factor and increases some of the growth parameters.

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