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EFFECT OF SALT AND WATER STRESS ON THE GERMINATION OF ALFALFA (*MEDICAGO SATIVA* L.) SEED

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Effect of salt and water stress on the germination of Alfalfa (*Medicago sativa* L.) seed. – Ghasem Ali Dianati Tilaki, Behzad Behtari, and Behnam Behtari. – The effect of three different levels of salt (*NaCl*) and polyethylene glycol 6000 on the germination of *Medicago sativa* L. seeds was studied. The electrical conductivity (EC) values of *NaCl* solutions were 0.0, 6.93, 11.55, and 16.94 μs/cm^{-1}. Drought conditions were induced by PEG 6000 at the same water potential of 0.0, -0.2, -0.4, and -0.8 MPa. The object of the study was to determine factors responsible for germination due to salt toxicity or osmotic effect. Our results revealed that *NaCl* and PEG treatments had significant effect on all the investigated characters. A lower germination percentage was obtained from PEG compared with *NaCl* at an equivalent water potential in each treatment. Non-germinated seeds under various *NaCl* treatments when transferred to distilled water recovered significantly, indicating little ionic effect of salinity on seed germination and viability. Germination inhibition, therefore, appears to be osmotic. A similar recovery response was noted when seeds from a PEG solution were transferred to water. PEG had no toxic effect since all the seeds germinated when PEG stress was removed.

Key words: Germination, *Medicago sativa*, Recovery, Salt and Drought stress.

Влияние солёности и засушливости на прорастание семян люцерны (*Medicago sativa* L.). – Хасем Али Дианати Тилаки, Бехзад Бехтари, Бехнам Бехтари. – Изучено влияние соли (*NaCl*) и полиэтиленгликоля (ПЭГ) 6000 на прорастание семян *Medicago sativa* L. Растворы *NaCl* имели электропроводность 0.0, 6.93, 11.55 и 16.94 мкс/см$^{-1}$. Засушливость имитировали введением ПЭГ 6000 при сохранении осмотического водного потенциала (0.0, -0.2, -0.4 и -0.8 МПа). Цель состояла в определении факторов, ответственных за нарушение прорастания (токсичность соли или эффект осмоса). Результаты показали, что обработка и *NaCl*, и ПЭГ оказывала существенное влияние на все исследованные объекты. Более низкий процент прорастания при каждой обработке наблюдался с ПЭГ по сравнению с *NaCl* при равном осмотическом водном потенциале. Непроросшие при обработке *NaCl* семена после переноса в дистиллированную воду восстанавливали прорастаемость, что указывает на слабое влияние солёности на прорастание семян и их жизнеспособность. Ингибирование прорастания, вероятно, имеет осмотический механизм. Подобное восстановление также отмечали, когда семена из раствора ПЭГ переносились в воду. ПЭГ не оказывал токсического эффекта, так как все семена проросли, когда его влияние было снято.

Ключевые слова: прорастание, *Medicago sativa*, восстановление, влияние соли и засушливости.

Alfalfa (*Medicago sativa* L.) is an important forage crop in many areas of the world where salinity and water stress limits crop productivity (Hulten, 1968; Hitchcock and Cronquist, 1973; Royer and Dickinson, 1999).

Alfalfa is best adapted to medium textured soils with a pH between 6 and 8. It requires a minimum of 10 to 12 inches of precipitation annually, at least half of which should be received as rain (opposed to snow). Alfalfa is highly drought and fire tolerant; but does not tolerate flooding, poor soil drainage, salinity, or shading. Alfalfa originates from southwestern Asia. It was first cultivated in Iran, and now has a worldwide distribution as an agricultural crop (Hultén, 1968).

Seeds of plants under natural conditions are subjected to salinity (usually *NaCl*). However, other chloride, sulfate and carbonate salts and their interactions play a significant role in affecting seed germination (Khan et al., 2002).

Seed germination in temperate salt marshes is the most critical phase for the survival because most plants are annuals whereas, in the sub-tropical environment, perennials use vegetative methods to induce new ramets and maintain continuity at a proximate scale. Salinity is a major environmental stress factor that affects seed germination (Khan et al., 2002) where salinity ranges from 0.8 to 2.4%. Saline sodic and non-sodic soils have a high salt content of sodium, calcium, and magnesium ions that could reach 8% (Waisel, 1972) in these soils while the more important anions are chloride, sulfate and bicarbonates (Bewley and Black, 1994).

Salts can affect seed germination by either restricting the supply of water (an osmotic effect) or causing specific injury through their ions to the metabolic machinery (an ionic effect). Soil salinity may affect the germination of seeds either by creating an osmotic potential external to the seed preventing water uptake, or through the toxic effects of Na^+ and Cl^- ions on the germination seed (Khajeh-Hosseini et al., 2003). Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedling establishment (Almansouri et al., 2001). Under these stresses, there is a decrease in the water uptake during imbibitions and, furthermore, salt stress may cause excessive uptake of ions (Murillo-Amador et al., 2002).

Seed germination under isotonic solutions of PEG 6000 and *NaCl* had a similar effect on the seed germination of halophytes (Myers and Couper, 1989; Naidoo and Naicker, 1992; Ungar, 1995; Bajji et al., 2002).

Seeds of some plants when pretreated with salinity show the priming effect of salinity on germination, while others showed no effect of salinity and recover immediately after salinity stress is removed and still other plants failed to germinate when exposed to high salinity (Ungar, 1995; Keiffer and Ungar, 1995; Khan and Ungar, 1997).

Sodium chloride had no effect on the germinability of seeds and all of them germinated to the reference level when transferred to distilled water (Mohammed and Sen, 1990). The most important indices in tests for determining seedling vigor in response to salt and water stress are percentage and speed germination. High germination speed indicates seeds' relative ability to avoid deterioration caused by prolonged exposure to unfavorable biotic factors (Grabe, 1976).

Successful crop production is highly correlated to the uniformity and rate of stand established in the field. This, in return, shows a close correlation with test results on speed germination. The rate of germination is thus an important concept of vigor, and could possibly be useful in predicting crop stand establishment (Perry, 1978; Haastrup-Pederson et al., 1993).

The aim of the present study was to determine factors responsible for failure of germination of alfalfa seeds under saline conditions due to an osmotic barrier or the toxic effect of *NaCl* by comparing seed germination under a range of osmotic potentials due to *NaCl* and PEG.

MATERIAL AND METHODS

This study was carried out at Faculty of Natural Resources, Tarbiat Modares University, Noor, Iran. Alfalfa cultivar Sativa from Gene Bank of Natural Source of Iran, which is commonly cultivated in Iran, was used as seed material. Germination and early seedling growth (7 days) of the cultivar were studied using distilled water (reference) and under an osmotic potential of -0.2, -0.4 and -0.8 MPa, for *NaCl* or polyethylene glycol (PEG 6000). The *NaCl* concentration had electrical conductivity (EC) values of 0.0, 6.93, 11.55 and 16.94 $\mu s/cm^{-1}$.

Three replicates of 100 seeds were germinated on 3 sheets of a 9-cm diameter filter paper in 10 cm diameter Petri dishes with 10 ml of the solution. The paper was replaced every 2 days to prevent accumulation of the salts (Rehman et al., 1996). Germination was considered to have occurred when the radicles grew 2 mm long. Germination percentage was recorded every 24 h for 7 days. The mean germination time (MGT) and rate of germination (GS) were calculated to assess the rate of germination (Ellis and Roberts, 1980).

The percentage and rate of germination (GS) were recorded at 1 to 7 days in accordance with Kotowski (1926). The rate of germination was calculated by:

$$GS = \frac{\sum n}{\sum n(n \times Dn)} \times 100.$$

The experiment was carried out at 20±1°C and on 16h dark/8h light for 7 days. To determine the toxic effects of the solution on germination, the non-germination seeds in each treatment were transferred to distilled water and counted every 24 h for 4 days. The percent of recovery was calculated using the following index:

$$\%\text{Recovery} = \frac{a-b}{c-b} \times 100$$

where *a* is the total number of seeds germinated after being transferred to distilled water, *b* the total number of seeds germinated in the saline solution, and *c* the total number of seeds. High recovery germination percentages would indicate that previous seed germination was inhibited by an osmotic effect, whereas low germination would indicate specific ion toxicity (Khan et al., 2002). Statistical analyses were carried out using the MSTAT-C program (Michigan State University). A one-way ANOVA was carried out to determine the differences among treatment group means for percent germination, rate of germination, and recovery percent germination. The differences between the means were compared using orthogonal and LSD values ($P < 0.01$).

RESULT AND DISCUSSION

A summary of the statistical analyses of Alfalfa seed germination, MGT, GS and Recovery percentage is given in Table 1. *NaCl* and PEG treatments had a significant effect on all the investigated characters ($P < 0.01$). Seeds of *M. sativa* showed 37.3% germination in a non-saline reference (Table 2). Seed germination decreased with an increase in the *NaCl* concentration.

Table 1

Analyses of variance for Alfalfa germination seeds, MGT, GS and Recovery

Source	d.f.	Germination	MGT	GS	Recovery
T	6	966.159**	4.157**	90.691**	456.222**
E	14	44.952	0.072	3.126	20.222
CV%	-	20.77	12.81	20.43	24.83

*, ** – Significant at 0.05 and 0.01 levels, respectively.

The mean germination time (MGT) increased with a decrease in the osmotic potential in both *NaCl* and PEG solutions, PEG increased it more compared to *NaCl* (Table 2). The mean germination time was delayed by stress conditions. Compared to *NaCl*, MGT for PEG was higher at an equivalent osmotic potential.

Table 2

Means of germination, MGT, GS and recovery of Alfalfa seeds treated with *NaCl*, PEG, and reference (untreated)

Treatments	Germination	MGT	GS	Recovery
Reference	37.33 a*	2.103 bc	11.09 ab	37.33 a
NaCl –2MPa	46.00 a	1.857 c	14.45 a	8.667 b
NaCl –4MPa	45.33 a	2.033 bc	12.86 ab	11.33 b
NaCl –8MPa	37.33 a	2.060 bc	10.69 ab	12.00 b
PEG –2MPa	45.33 a	2.640 b	9.507 b	9.333 b
PEG –4MPa	14.67 b	3.983 a	1.993 c	30.00 a
PEG –8MPa	0.0 b	0.0 d	0.0 c	37.33 a

* – Means followed by the same letter are not significantly different at the $P < 0.01$ level (LSD test).

Thus, the rate of germination had the most important effect on stand establishment and plan density under laboratory and greenhouse conditions. This agreed with the results of Ram and Wiesner (1987) and Alizadeh (1977) obtained the same result on wheat seed lots under unfavorable storage conditions.

As shown in Table 3, the orthogonal contrast for reference vs. others treatments for MGT was significant, but compared to PEG vs. *NaCl* treatments was non-significant. This result indicated that PEG and *NaCl* treatments affected MGT. The results are in line with the finding of Demir Kaya et al. (2006) in sunflower and Srinivasan et al. (1999) in mustard.

Table 3

Orthogonal comparison for germination, MGT, GS and Recover traits of Alfalfa seeds treated with PEG, *NaCl* and reference (untreated)

Sum of Square				
Orthogonal Contrast	Germination	MGT	GS	Recovery
Reference vs. Others	89.175^{ns}	0.002**	20.675*	37.97**
PEG vs. NaCl	2357.56**	0.227^{ns}	351.302**	997.556**

*, ** – and ^{ns} significant at 0.05, 0.01 levels and not significant, respectively.

The beneficial effects of *NaCl* on germination were found in this study. Seeds germination was always better in *NaCl* than in PEG at an equivalent water potential in line with the earlier observation made for soybean by Khajeh-Hosseini et al. (2003) and Demir Kaya et al. (2006). This may be due to the uptake of Na^+ and Cl^- ions by the seed, maintaining a water potential gradient allowing water uptake during seed germination. *NaCl* treated seeds compared to PEG treated seeds were allowed to imbibe water for a longer time and through the first stage of germination with out protrusion of the radicle. Akinola et al. (2000) reported that a higher duration of exposure to seed treatment resulted in a higher cumulative germination in wild sunflower. A lower germination percentage obtained in PEG compared with *NaCl* at an equivalent water potential in each treatment suggests that the adverse effect of PEG on germination was due to osmotic effect rather than specific ion accumulation. These results agree with those by Murillo-Amador et al. (2002) in cowpea, by Demir and Van De Venter (1999) in watermelon, they affirmed that drought or salinity may influence germination by decreasing the water uptake. Moreover, the present study revealed that PEG had no toxic effect since all the seeds germinated when PEG stress was removed (recovery treatment). Mehra et al. (2003) and Michel (1983); indicated that PEG molecules do not enter the seed and Khajeh-Hosseini et al. (2003) found that there was no toxicity of PEG. Under salt stress, Na^+ and Cl^- may be taken up by the seed and a toxic effect of *NaCl* might appear. Our findings revealed that inhibition of germination at an equivalent water potential of *NaCl* and PEG resulted from osmotic effect rather than salt toxicity. Both seed treatments gave better performance than reference (untreated) under salt and drought stresses.

Ungerminated seeds at all the salt concentrations when transferred to distilled water germinated less than at the non-saline reference (Table 2). Iso-osmotic solutions of *NaCl* and PEG have similar effect on seed germination at similar concentrations (Fig. 1) and seed germinated at or above -0.4 MPa PEG decreased. When recovery of germination from various salts and PEG were compared at -0.4 MPa and -0.8 MPa PEG, 37.3% seeds germinated at -0.8 MPa in PEG solution same as reference (Fig. 2). Comparison of recovery of germination when treated with various concentrations of *NaCl* and PEG had no similar pattern showing germination at the highest osmotic potential (Fig. 2).

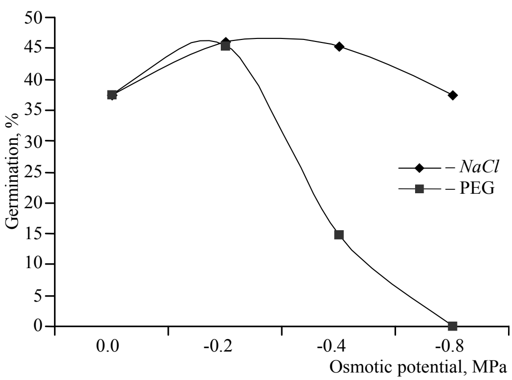

Fig.1. Seed germination of *Medicago sativa* inat different concentrations of *NaCl* and PEG solutions

Yuying et al. (1999) reported that after the stress was removed, the seed germination and early seedling growth were higher than those of the untreated seeds were. *Medicago sativa* seeds showed a priming effect when treated with high salt concentrations. Seed of Alfalfa when pretreated with salinity showed a priming effect of salinity on germination. Similar results were reported by Ungar, 1995; Keiffer and Ungar, 1995; Khan and Ungar, 1997.

It shows that exposure to sodium chloride had a priming effect and germination was significantly increased in comparison to reference (Table 2 and 3). There was no specific ion toxicity, so the osmotic effect limited germination as in other plants (Mohammed and Sen, 1990; Egan et al., 1997; Pujol et al., 2000). Other species are also reported to show osmotic effects on germination rather than specific ion toxicity and saline pretreatment stimulated germination (Macke and Ungar, 1971; Williams and Ungar, 1972; Khan and Ungar, 1998).

When seeds were transferred to a non-saline medium after 7 days of exposure to salinity, there was a substantial recovery of germination. However, seed germination under natural conditions is more complicated and influenced by many factors such as salinity, drought, light, and temperature. Future studies would focus on the interactive effects of these factors. *M. sativa* is an important plant for forge production in Iran that could be used to improve the quality of degraded saline land as well as a high protein diet for animals.

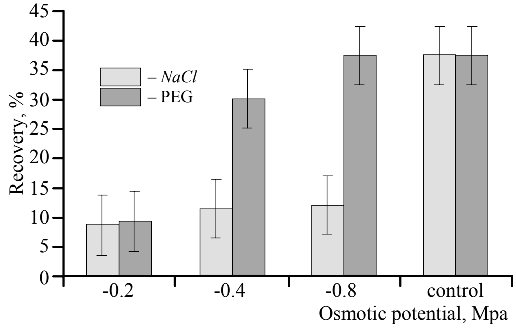

Fig.2. Recovery of seed germination in *Medicago sativa* with different concentrations of *NaCl* and PEG

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