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Effect of Iso-Osmotic Salt and Water Stress on Germination and Seedling Growth of Two *Plantago* species

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Abstract: General responses to drought and salt stress was investigated in two Plantago species including *Plantago ovata* and *P. psyllium*. Seeds were treated with aqueous solutions of 0, -0.2, -0.4, -0.6 and -0.8 Mpa NaCl and iso-osmotic concentration of polyethylene glycol 8000. NaCl of -0.6 and -0.8 Mpa caused more delay in germination than the iso-osmotic solution of PEG. However, germination frequency was strongly affected by drought and salinity stress, such that only 20% of the *P. ovata* and 30% *P. psyllium* seeds germinated in -0.6 Mpa Osmotic Potential (OP) during the first week of incubation. The rate of germination was decreased with an increase in osmotic potential in both drought and salinity stress, however drought had much effect on the rate of germination. All treatment of NaCl and PEG were inhibitory to root and shoot elongation of seedlings in both species relative to the distilled water controls; however, NaCl affected seedling root and shoot elongation more severely specially in *P. ovata*. Recovery of their germination capacity after transferring the treated seeds to distilled water was determined and in all cases except in OP of -0.2 Mpa, high germination frequencies of more than 80% were observed after 1-2 week of incubation in distilled water.

Key words: *Plantago ovata*, *P. psyllium*, salinity, drought, germination

INTRODUCTION

With the increased frequency of drought years, the intensification of food production depends heavily on irrigation and the efficient management of limited water resources. The extension of irrigated agriculture and the intensive use of water resources combined with high evaporation rates have drawn attention to the problems of salinity in the soil and in ground water (Lambers, 2003). The most common solution to this problem is to increase the salt tolerance of conventional crop plants, but the gain in yield is generally low (Tester and Davenport, 2003). For example the water stress, generated by either drought or salinity, represents the most common severe environmental stress limiting plant growth and productivity. Terrestrial plants of saline habitats are often surrounded by low water potentials in the soil solution and atmosphere. Plant water loss has to be minimized under these circumstances, since biomass production depends mainly on the ability to keep a high net photosynthesis by low transpiration (Jefferies *et al*., 1979). The reduced water potential at saline habitats imposes a two-edged problem in plant: a corresponding water and ion stress. The uptake and accumulation of Na^+ and Cl^- into the different plant organs is highly controlled (Munns, 2002), salt-resistant species often possess special features to exclude NaCl from the cytoplasm, e.g., by compartmentation in the vacuole (Muhling and Lauchli, 2002). The osmotic component results from dehydration and loss of turgor induced by external solutes and are not specific for NaCl: other stresses such as drought and extreme temperatures also cause depletion of cellular water (Greenway and Munns, 1980). The genus *Plantago* L. appears to be a good model for comparative studies on the responses to salt stress, since it includes species with considerable differences in their degree of tolerance (Smekensa and Van Tienderen, 2001). Ungar (1995) reported that inorganic ions were not more inhibitory than mannitol and polyethylene glycol (PEG) in several halophytes, indicating that seeds are mainly affected by osmotic stress rather than specific ion toxicities (Koyro, 2006). Around 20 species in the Plantago genus are halophytic, or at least include genotypes able to grow in conditions of a certain salt stress, while others are typical glycophytes. For instance, *Plantago maritima* L. can withstand environmental

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concentrations of NaCl up to 250-300 mm, whereas *P. media* L. is salt sensitive and suffer significant damage, leading to plant death, at external concentrations of about 50 mm NaCl (Vicente *et al*., 2004). In addition, *P. coronopus* L., which occurs in habitats of variable salinity, shows an intermediate degree of salt tolerance, growing at concentrations of up to 150 mm NaCl (Koyro, 2006). Different aspects of the responses to salinity have been studied in a few Plantago species, especially in the halophytic *P. maritima*, often in comparison with non-halophytes from the same genus (Erdei and Kuiper, 1979; Sheehy Skeffingt and Jeffrey, 1985). We are not aware of any research in *P. ovata* and *P. psyllium*. These annual species is distributed in Iran plateau, Pakistan and Afghanistan. *Plantago ovata* is an important herb that has been used as a medicinal plant for many centuries in South Asia and its use is spread allover the world (Zahoor *et al*., 2004). The seed of *Plantago ovata* and *P. psyllium* contains mucilage, fatty oil, large quantities of albuminous matter a pharmacologically inactive glucoside, namely Aucubin and a pentose sugar (Rùnsted *et al*., 2000). The seed husk has the property of absorbing and retaining water which accounts for its utility in checking diarrhea. It is diuretic, alleviates kidney and bladder complaints, gonorrhea, arthritis and hemorrhoids (Zahoor *et al*., 2004).

The aim of this study was (I) to investigate the response of differentially two species of *Plantago* genus (*P. ovata* and *P. psyllium*) to osmotic stress during germination and seedling growth (ii) to differentiate the osmotic from the toxic effect through comparison of NaCl with the metabolically inactive osmotic agent polyethylene glycol (PEG 8000).

MATERIALS AND METHODS

Plant material: Seeds used in this study were obtained from the Jahad Medicinal Plant Research Center in Iran which was collected from the Khorasan Province in north east of Iran.

Germination tests under saline conditions: Four replicas of 25 seeds for each salt and drought treatments were placed on two layers of Whatman No. 2 filter paper in 90 mm Petri dishes. The filter paper was moistened with distilled water for the controls, or with aqueous solutions of 0, -0.2, -0.4, -0.6 and -0.8 Mpa NaCl and iso-osmotic Polyethylene glycol 8000 for the different salt and drought treatments. The concentration of PEG-8000 needed to produce proper osmotic potential was prepared according to Michel and Kaufmann (1973):

$$Qs = - (1.18 \times 10) C - (1.18 \times 10) C + (2.67 \times 10) CT + (8.39 \times 10) C T$$

Where, C is concentration of PEG-8000 in g LG of water, T is temperature in EC and Qs is osmotic potential in bars (converted to Mpa by dividing by 10). Water or fresh salt and drought solutions were added periodically to maintain the filter paper wet during the course of the experiment. Germination was carried out in a germination chamber with 12 h photoperiod and 25/15 day/night temperature. The number of germinated seeds was counted every 2 days for 1 month from the start of the experiment. Germination rates were calculated according to the modification by Khan *et al*. (1997) of the Timson's germination velocity index (Timson, 1965): $\Sigma G/t$; where G is the percentage of seeds germinated after 2 days intervals and t is the total time of germination (Vicente *et al*., 2004).

Seed recovery after salt pre-treatments: All seeds from the previous germination tests which did not germinate after 1 month at different salt and drought concentrations, were placed in new petri dishes with filter paper moistened with distilled water and incubated under the same conditions for additional 30 days. The recovery of germination was calculated following Pujol *et al*. (2000), using the relation:

The Recovery of germination = {(a-b)/(c-b)}*100

Where a is the total number of seeds germinated after being transferred to distilled water, b is the total number of seeds germinated in saline solution, c is the total number of seeds.

Effect of salinity on seedling development: Twenty seedlings of 1.5 cm length, in the cotyledonary stage, were placed in petri dishes with filter paper moistened with aqueous solutions of 0, -0.2, -0.4, -0.6 and -0.8 Mpa NaCl and iso-osmotic Polyethylene glycol 8000 and maintained in the germination chamber under the same conditions described above. After 30 days the seedlings were harvested and the root and shoot length and their dry weight were measured.

Statistics analysis: Germination and recovery of germination data were arcsine transformed before statistical analysis to ensure homogeneity of variance; data were analysed using the SAS program for Windows, v. 6.12.

RESULTS

Drought and salt stress condition significantly ($p<0.05$) affected the germination percentage, germination rate and root and shoot length of *Plantago ovata* and *P. psylium* seeds (Table 1). Seeds used in this study were viable and most of them germinated on filter paper moistened with water. Over 70% of seeds in control

Table 1: Mean comparison of Species, salinity and drought on germination traits. With duncan test at the 5% level of probability

		Germination (%)		Root length (mm)		Shoot length (mm)		Main germination rate (mm)		Recovery germination rate	
Species		NaCl	PEG	NaCl	PEG	NaCl	PEG	NaCl	PEG	NaCl	PEG
Plantago ovata	-0.2 Mpa	53.5b	63.7b	20.6a	37.7a	22.0b	45.0b	0.23a	0.23ab	0.33a	0.33a
	-0.4 Mpa	32.2c	38.7c	12.2c	20.0b	11.0c	19.0c	0.18b	0.16b	0.32a	0.30b
	-0.6 Mpa	19.5d	24.5d	9.60c	13.6b	9.7c	13.0c	0.17b	0.14c	0.32a	0.29b
	-0.8 Mpa	7.00e	12.2e	9.30c	12.7b	9.2c	12.0c	0.15b	0.12c	0.30a	0.28b
	Control	78.5a	80.2a	19.3b	35.0a	29.0a	62.0a	0.26a	0.27a	0.26a	0.27b
Plantago psyllium	-0.2 Mpa	59.5b	67.2b	27.8a	59.0a	26.0b	56.0b	0.42ab	0.41ab	0.32b	0.33b
	-0.4 Mpa	38.7c	44.5c	15.7b	28.9b	15.0c	29.0c	0.39b	0.36b	0.30b	0.3bc
	-0.6 Mpa	26.7d	34.7d	10.2c	15.0b	10.0d	15.0d	0.29c	0.22c	0.31b	0.28b
	-0.8 Mpa	14.7e	16.7e	9.00c	12.1b	9.1d	12.0d	0.15d	0.15c	0.30b	0.3bc
	Control	92.0a	93.7a	27.3a	57.0a	41.0a	92.0a	0.45a	0.45a	0.45a	0.41a
P. ovata		53.5b	59.7b	18.6b	39.0b	25.9b	31.6b	0.33a	0.19b	0.33a	0.30a
P. psyllium		60.9a	68.4a	22.9a	58.5a	29.0a	44.8a	0.30a	0.32a	0.31a	0.32a

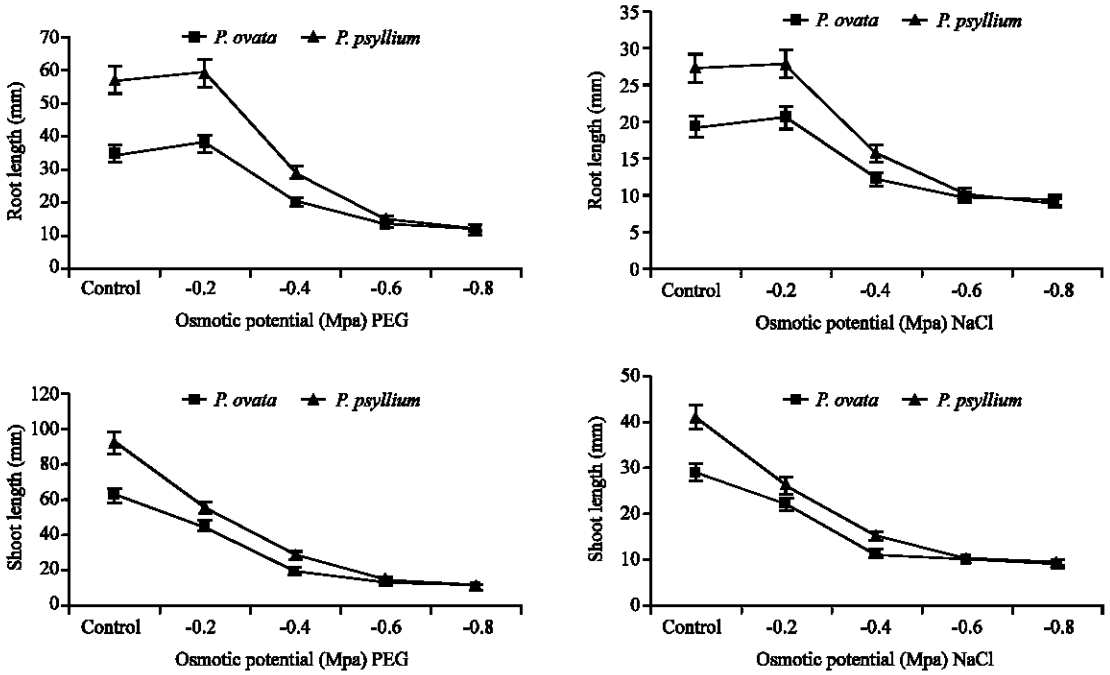

Fig. 1: Root and shoot length of *P. ovata* and *P. psyllium* in 0, -0.2, -0.4, -0.6, -0.8 Mpa OP NaCl and iso-osmotic concentration of PEG

samples germinated in 1 week in both species. This percentage increased to a maximum of 85 and 95% in *P. ovata* and *P. psyllium* during the second week, respectively. -0.6 and -0.8 Mpa NaCl treatments delayed germination longer than the iso-osmotic solution of PEG (Fig. 2a-d). Germination frequency was strongly affected by both drought and salinity stress, such that only 20% of the *P. ovata* and 30% *P. psyllium* seeds germinated in -0.6 NaCl Mpa Osmotic Potential (OP) during the first week of incubation (Table 1). No further germination was observed over the course of the experiment. Germination was strongly inhibited by OP -0.8 Mpa in both species but it was more noticeable in *P. ovata*.

The rate of germination decreased with an increase in osmotic potential in both drought and salinity stress, however drought had a much negative effect on the rate of germination (Table 1). All treatments of NaCl and PEG were inhibitory to seedling root and shoot elongation in both species (Table 1), although, NaCl affected seedling root and shoot elongation more severely especially in *P. ovata* (Fig. 1). The rate of germination was lowest at -0.8 Mpa OP in drought and salinity stress and highest germination rate was recorded at -0.2 Mpa and control (Table 1). The rate of germination was completely similar at all Ops in drought and salinity stresses in seed recovery germination condition. There were no significant

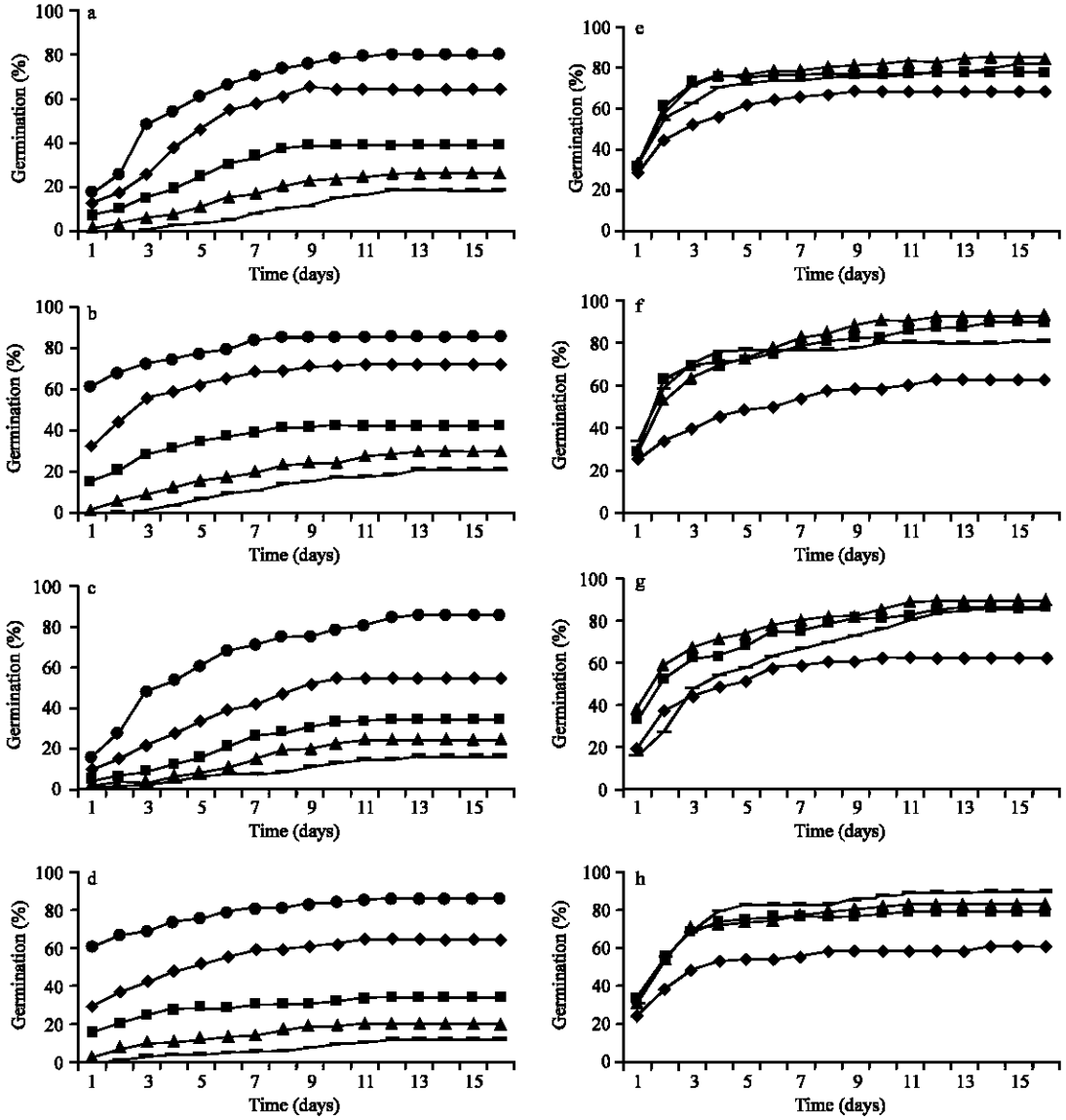

Fig. 2: Germination of *P. ovata* and *P. psyllium* in drought and salinity. (a, b, c and d) Seeds were germinated in Petri dishes on filter paper wetted with distilled (●), with -0.2 Mpa NaCl or Drought (◆), with -0.4 Mpa (■), with -0.6 Mpa (▲) and -0.8 Mpa (–); the values shown are the means of germination frequencies determined, days, for four replicas of 25 seeds per treatment. (e, f, g and h), all seeds which did not germinate after 1 month in the experiment shown in (a, b, c and d), in the presence of solutions that mentioned above, were transferred to fresh Petri dishes and germinated in water and recovery of germination capacity was determined as before

differences between NaCl and PEG solution of water potential treatments in Recovery Germination Rate (RGR) in both species in relation to the control, whereas it was significantly different in the Main Germination Rate (MGR) (Table 1). Regardless of all treatments, MGR was lowest in seeds of *P. ovata* compare to *P. psyllium*. Significant effect of OP were observed in root and shoot length so that increasing OP from -0.2 to -0.8 Mpa progressively decreased root and shoot lengths, although the decline in root length from -0.6 to -0.8 Mpa OP was not statistically significant and root elongation of both species had no significantly difference in -0.2 Mpa NaCl and PEG (Fig. 1). The root and shoot length of the *P. psyllium* was significantly more than that of *P. ovata* species in all treatments (Table 1 and Fig. 1).

Seed germination recovery: Seed from the previous tests which had not germinated after 1 month in the presence of salt and drought stress, were transferred from NaCl and

PEG medium to Petri dishes with filter paper wetted with distilled water and 'Recovery' of their germination capacity was determined. In all cases except in -0.2 Mpa OP, high germination frequencies of more than 80% were observed after 1-2 weeks of incubation in distilled water (Fig. 2c-h). Irrespective of osmotic potentials used in the previous tests, germination rates also did not vary significant samples, as shown by similar value of the germination rate for each batch of seeds (Table 1). These data clearly indicate that, although NaCl strongly inhibited seed germination in *P. ovata* and *P. psyllium*, it did not affect seed viability or germination capacity at least up to OP of -0.8 Mpa salinity and drought stress.

DISCUSSION

Salt and drought tolerant is developmentally regulated and the responses to salt and drought stress maybe quite diverse at different stage of plant development (Lauchli and Epstein, 1990). NaCl and PEG solution of the same osmotic potential affected the seeds of two tested species differentially which indicates that the effects of NaCl on seeds of these species were not solely osmotic. Besides drought stress, seeds under NaCl solution also experienced toxicity of salt ions. Variation in recovery responses have been demonstrated in a few halophytic species. Khan *et al.* (2000) reported that species vary greatly in their germination recovery responses when exposed various salinity and drought stress. Seeds of some halophytes are reported to tolerate high salinity during the period when they are dormant in soil and subsequently germinate when soil salinity are reduced (Khan *et al.*, 1997). Since viability and germinated capacity of the both species were not affected by pre-treatments with up to -0.8 Mpa NaCl and PEG and while this is also a common feature of the seed of halophytes, which usually survive long periods of exposure to high salt conditions in the soil prior to rainfall which causes a reduction in the salinity of the soil surface layers. Salt-marsh species are also subjected to fluctuations in salinity and soil moisture through out the year (Keiffer and Ungar, 1997). Elevated salinity slows down water uptake by seeds, thereby inhibits germination and root elongation. At the low osmotic potential both NaCl and PEG inhibited the processes of imbibitions, germination and root elongation of *P. ovata* and *P. psyllium*, however this was more severely in *P. ovata* (Fig. 1). Lower rate of water uptake by *P. ovata* and *P. psyllium* when they soaked in high NaCl and PEG concentration is probably caused by the decrease in water potential gradient between the seeds and their surrounding media (Osmond *et al.*, 1980; Simon, 1984). For all of the treatments, NaCl was found to be inhibitorier to water uptake, especially at high concentrations than iso-osmotic solutions of PEG. According to this knowledge that The first phase of water uptake by the seeds involves movement of water into the free space (apoplast) which does not depend on the osmotic potential of the surrounding solution (Simon, 1984) and the second slower linear phase of water uptake involves the movement of water across cell membranes into the cells of the seeds whose rate is determined by the difference between the osmotic potential of the seed and that of the medium (Simon, 1984), we can realize that unlike PEG, NaCl may readily cross the cell membrane into the cytoplasm of the cells unless an active metabolic pump prevents accumulation of the ions. In some cases, NaCl in the cytoplasm can result in toxic accumulation of a particular ion or decreased availability of some essential nutrients (Tester and Davenport, 2003). Moreover, the presence of Na^+ and Cl^- ions in the cells may induce changes in protein activity since ions affect the structure of the hydration water which surrounds the protein molecule (Tester and Davenport, 2003).

According to statistic, overall these results can be drowning that:

- There is genetic variation for germination and seedling traits between *P. ovata* and *P. psyllium* species.
- Species *P. psyllium* was more tolerant of lower OP than *P. ovata*
- Salinity stress significantly reduced root and shoot length and MGR than drought stress in both species, however such a treat was not observed in germination percentage and Recovery Germination Rate (RGR).
- The rate of germination was completely similar at all OPs in drought and salinity iso-osmotic stress in seed recovery germination experiment.
- In General, to gain over 50% seed germination OP of culture medium should not be over -0.2 Mpa.

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