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Partitioning water potential and specific salt effects on seed germination of *Kochia scoparia*

Azam borzouei

Agricultural, Medical and Industrial Research School, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran, P.O.Box:31485-498, Karaj, Iran
aborzouei@gmail.com

Abstract

Salinity is one of the environmental factors that have a critical influence on the germination of halophyte seeds and plant establishment. Salinity affects imbibitions, germination and root elongation. However, the way in which NaCl exerts its influence on these vital processes (seed germination), whether it is through an osmotic effect or specific ion toxicity, is still not resolved. Seeds of the halophyte *Kochia scoparia* were treated with various iso-osmotic solutions of NaCl and polyethylene glycol 6000 (PEG) over the water potential range of 0 to -1.9 MPa. After 10d of treatment, the ungerminated seeds were transferred to distilled water for 3 d. The germination results revealed that both NaCl and PEG inhibited germination and seedling growth, but the effects of NaCl compared to PEG were less on final germination. The seeds of *kochia* differ in their response to salinity and failure of seeds to recover from high salinity when transferred to deionized water, revealed the toxicity of NaCl. In contrast, the increase in germination and during the recovery period after exposure of PEG suggested that PEG was not toxic. It was concluded that at low levels of salinity the main effect of NaCl was to reduce the rate of germination due to the reduce water potential and at a higher salinity NaCl had a toxic effect since many seeds failed to germinate even salinity stress was removed.

Keywords: *Kochia scoparia*, NaCl, Osmotic potential, Salinity, Seed germination.

Introduction

Germination is a crucial stage in seedling establishment and plays a key role in crop production (Khajeh-hosseini *et al*, 2003; Akbari ghogdi *et al*., 2012).Seed germination, seedling emergence, and early survival are particularly sensitive to substrate salinity (Mariko *et al*, 1992).Germination failures under saline soils are often the results of high salt concentrations in the seed planting zone because of upward movement of soil solution and subsequent evaporation at the soil surface (Kornejadi *et al*, 2004). Successful seedling establishment depends on the frequency and the amount of precipitation as well as on the ability of the seed species to germinate and grow while soil moisture and osmotic potentials decrease (Jamil *et al*, 2005).Salinity may affect the germination of seeds either by creating an osmotic potential external to the seed which prevents water uptake, or through the toxic of sodium and chloride ions on the germination seed (Khajeh-hosseini *et al*, 2003). Comparisons of the response of durum wheat (*Triticum durum*) cultivars to various iso-osmotic solutions of NaCl, and polyethylene-glycol (PEG) suggested that inhibition of germination could not be attributed to mobilization of reserves and that the main effect of PEG occurred via inhibition of water uptake while detrimental effects of NaCl may be linked to long-term effects of accumulated toxic ions.

Soil salinity is an important constraint to crop production, affecting about 95 million hectares worldwide (Bayuelo-Jiménez *et al*, 2002). One approach to reducing the deleterious effects of soil salinity on crop production is the development of salt-tolerant cultivars. In certain species, this may be achieved by exploiting intraspecific variability. However, when such variability is limited, as occurs in many crop species, genes may be transferred from closely related wild species adapted to high salinity (Bayuelo-Jiménez *et al*, 2002). Salinity tolerance is found in species of *Kochia*. It is reported that *Kochia* has been a rapid establishment in saline soil and could be used as a plant cover and alternative forage especially in the region with the shortage of forage production (Jami Al-Ahmadi *et al*, 2005). *Kochia* has a high germination rate in a favorite condition but it has a good tolerance to saline condition and also its germination is remarkable in low/medium levels of salinity. It has been detected that increasing salinity up to 10 dS/m did not have any significant effects on germination, but after that, the germination rate and percentage begun to reduce significantly (Jami Al-Ahmadi *et al*, 2005). The relative importance of osmotic or toxic effects of NaCl on seed germination of *Kochia* is not clear and this study was conducted to determine the effect of NaCl on seedling growth and germination, and to determine factors responsible for failure in seed germination under saline conditions comparing various levels of NaCl and PEG.

Material and methods

This study was carried out at the Department of Agronomy, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. The seeds of *kochia scoparia* obtained from Lorestan. 25 seeds were germinated in covered, sterilized disposable Petri dishes containing filter papers moistened once with 10 mL of distilled water or solutions containing NaCl or PEG. The NaCl solutions had concentrations of 61.5, 110.8, 184.6, 291.1 and 779.8 mmol NaCl resulting in water potentials of -0.15, -0.27, -0.45, -0.71 and -1.9 MPa respectively at 20 ^{0}C. PEG 6000 (Michel and Kaufmann, 1973) solutions were made up to same water potentials as the NaCl solutions .In order to prevent evaporation, each Petri dish was put into a sealed plastic bag. Seeds were allowed to germinate at

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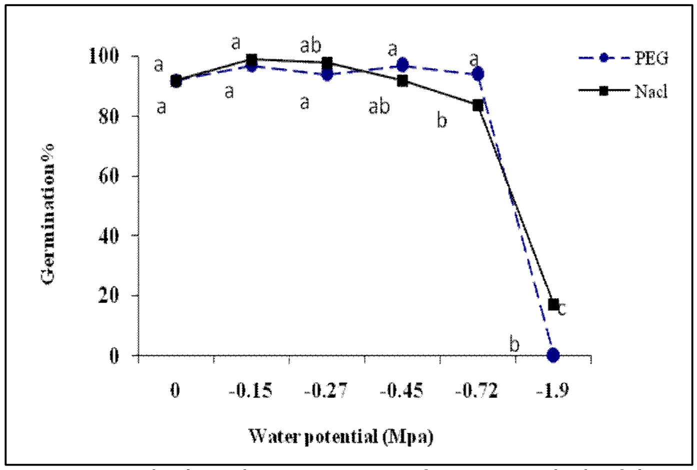
20 ± 1 ^{o}C in a dark place for 10 days. To determine the toxic effects of the solutions on germination, after completion of the germination test, non-germinated seeds in each treatment were transferred to distilled water and counted for an additional 3 days. A seed was considered to have germinated when the emerging radical elongated to 1 mm. Germination percentage was recorded every 24 h for 10 days. The numbers of normal and abnormal seedling were measured on the 10th day. The experimental design was arranged in a completely randomized design (CRD) with 4 replications and 25 seeds per replicate. Treatments contain solutions of both NaCl (61.5 to 779.8 mmol NaCl) and polyethylene glycol (PEG, 6000). The solutions of NaCl and PEG used had the same water potentials within the range -0.15 to -1.9 MPa. Data given in percentages were subjected to arcsine transformation before statistical analysis. For all investigated parameters, analysis of variance was performed using the MSTAT-C software package. Significant differences among the mean values were compared by LSD test ($P \leq 0.05$).

Results

Kochia scoparia retained high germination (>80%, Fig.1) up to 291.1 mmol NaCl (-0.73 MPa), with germination falling sharply above 291.1 mmol NaCl. There were more marked effects on the germination of this cultivar when it was germinated in contact with PEG solutions having the same water potentials as the NaCl solutions. At -1.9 MPa, the germination of seeds was less than NaCl at the same potential (779.8 mmol NaCl, Fig.1). Transfer of non-germinated seeds from PEG and NaCl solution to the distilled water (recovery period) resulted in an increase in germination within 1 to 2 days of transfer. A significant increase in germination following transfer from PEG conditions was observed only at -1.9 MPa (table1). It is evident that PEG was not toxic. As a result, although all seeds failed to germinate at -1.9MPa in PEG and NaCl solutions, but after the subsequent recovery period germination reached to 90 and 79% at 0

Fig.1. Germination percentage of Kochia scoparia at different water potential achieved in solutions of NaCl and PEG (polyethylene glycol). Each point is the mean of 4 replications of 25 seeds.



MPa respectively. Thus, recovery from a period of low water potential is greater when the reduced water potential resulted from PEG than from a saline solution. It seems that the response of seeds to salinity and their ability to recover from saline condition could be influenced by specific ion toxicity. This was examined with determine the number of normal and abnormal seedling after 8d in germination period.

Our results showed that decreasing water potential by NaCl and PEG caused a remarkable decrease in normal seedling number (Table 2). At -0.71 and -1.9 MPa both NaCl and PEG were inhibitory to early growth seedling; however seedling growth was more strongly affected by salinity solution. The numbers of abnormal seedling from NaCl and PEG treatments decreased with decrease in water potential of the solutions (Table 2).

Table 1. Germination percentage of Kochia scoparia in 8d of germination period in contact with NaCl and PEG (polyethylene glycol) solutions having different water potentials and in subsequent 8 days recovery period at 0 MPa.

NaCl (mmol)	Water potential (-Mpa)	NaCl		PEG	
		Initial germination period	Recovery period	Initial germination period	Recovery period
0	0	92 ^{ab}	92 ^{ab}	92 ^{a}	92 ^{a}
61.5	-0.5	99 ^{a}	99 ^{a}	99 ^{a}	97 ^{a}
110.8	-0.27	98 ^{a}	98 ^{a}	94 ^{a}	94^{a}
184.6	-0.45	92 ^{ab}	96 ^{ab}	97 ^{a}	99 ^{a}
291.1	-0.71	84 ^{b}	88 ^{b}	94 ^{a}	94 ^{a}
779.8	-1.9	17 ^{c}	79 ^{c}	0.0 ^{b}	90 ^{a}
LSD ($P \leq 0.05$)		12.5	8.91	8.46	9.34

** Germination in both the initial germination and recovery period is expressed as a percentage of the total number of seeds initially set to germinate. * Means followed by the same letter(s) are not significantly different at $P \leq 0.05$*

Discussion

NaCl and PEG adversely affected the germination and seedling growth of *Kochia Scoparia* but PEG had a greater inhibitory effect than NaCl. These results are similar with Okcu *et al* (2005).They reported that NaCl had a lesser effect on the germination and seedling growth of pea than PEG. The drop in the rate of water uptake by the seeds of *K. scoparia* when they were soaked in NaCl and PEG solutions is probably caused by the decrease in water potential gradient between the seeds and their surrounding media (Katembe *et al*, 2005). Increasing salinity concentrations in germination often cause osmotic and/or specific toxicity which may reduce or retard germination percentage (Almansouri *et al*, 2001). Khajeh-Hussein *et al* (2003) suggested that the seeds take up Na^{+}/ and or Cl^{-} from the solution, hence lowering the osmotic potential of cells and

Table 2. The number of normal seedlings and abnormal seedlings of Kochia scoparia at different osmotic potentials of NaCl and PEG

Treatment	Type of seedling	Water potential (-Mpa)					
		0	0.15	0.27	0.45	0.71	1.9
PEG	Normal	21 ^{ab}	23.5 ^{a}	21.25 ^{ab}	20.5 ^{ab}	18.5 ^{b}	0 ^{c}
	Abnormal	2 ^{bc}	0.75 ^{c}	2.25 ^{abc}	3.75 ^{ab}	5 ^{a}	0 ^{c}
NaCl	Normal	21 ^{ab}	22.25 ^{a}	21.25 ^{ab}	19.25 ^{ab}	17.5 ^{b}	0 ^{c}
	Abnormal	2 ^{a}	2.5 ^{a}	3.25 ^{a}	3.75 ^{a}	3.5 ^{a}	4.25 ^{a}

** Means followed by the same letter(s) are not significantly different at P≤ 0.05*

maintaining a water potential gradient that enables the continued water uptake at reduced external water potential. Whilst this may be advantageous in maintaining germination at low/ medium salinities, this uptake of Na^+ and/ or Cl^- could lead to the toxic effect observed at high salinities and the failure of seed to germinate. In fact, our finding showed that the decreasing of some seeds to recover from the effects of high salinity when they were returned to 0 MPa, revealed that poor germination in saline conditions resulted from toxic effect of NaCl. Katembe *et al* (1998) suggested that NaCl in the cytoplasm can result in toxic accumulation of a particular ion or decreased availability of some essential nutrients. Specific ion toxicity of the Na^+ and Cl^- ions on the cell membrane, cytoplasm and/or nuclei of the cells of halophyte seeds may partly be responsible for the fact that NaCl is inhibitorier to the germination than concentrations of PEG. Moreover, the presence of Na^+ and Cl^- ions in cells may changes protein activity because ions affect the structure of the hydration water which surrounds the protein molecule. NaCl may be inhibitory to the activities of some enzymes that may play critical roles in seed germination. Our results confirm the finding of Khajeh-hosseini *et al* (2003) in soybean and those of Murillo-Amador *et al* (2002) in cowpea.

Although differences in numbers of normal and abnormal seedling revealed that the toxic effects of Na^+ and Cl^- ions on the germinating seed. Reduction of root growth under high salinity is also well documented (Okcu *et al*, 2005). The shoot and root length are the most important parameters for salt stress because roots are in direct contact with soil and absorb water from soil and shoot supply it to the rest of the plant. For this reason, root and shoot length provides an important clue to the response of plants to salt stress. The reason for reduced shoot and root development may be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings. High salinity may inhibit root and shoot elongation due to slowing down the water uptake by the plant (Jamil *et al*, 2005) may be another reason for this decrease. Similar observations have been reported in *phaseolus* species (Bayuelo-Jiménez *et al*, 2001), chick pea (*Pisum sativum*), (Okcu *et al*, 2005), and *Atriplex* species (Katembe *et al*, 1998). Similar observations have been reported by Jamil *et al* (2005) in *brasscia* species that salinity significantly reduced the root and shoot length at all salinity treatments. In conclusion, at low levels of salinity the main effect of NaCl was to reduce the rate of germination due to reduce water potential and slower rate of imbibitions. At a higher salinity NaCl had a toxic effect since many seeds failed to germinate even salinity stress was removed

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