

## SEED GERMINATION OF *KOCHIA SCOPARIA* UNDER SALINE CONDITIONS: RESPONSES WITH GERMINATION REGULATING CHEMICALS

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### Abstract

Seeds of *Kochia scoparia* were germinated at 25-35°C under 0, 300, 600 and 900 mM NaCl with or without some germination regulating chemicals. Most of the seeds germinated in distilled water and at 300 mM NaCl but germination decreased with a further increase in salinity and about 25% of the seeds germinated at 900 mM NaCl. Exogenous application of ethylene, fusicoccin, gibberellic acid, kinetin and thiourea completely alleviated the salinity effect. Betaine was partially effective while proline and nitrate had no effect. Rate of germination did not vary in comparison to the control by the application of all growth regulators up to 600 mM NaCl. However, at 900 mM NaCl the rate of germination was decreased substantially in the seeds treated with proline, betaine and nitrate.

### Introduction

*Kochia scoparia* (L.) Schrader (Summer-cypress) of the family Chenopodiaceae is an herb, distributed along the roadsides, canal banks, field margins and other waste places in salt marsh, sedge-rush, mountain brush, and Pinyon-juniper communities at 850 to 1985 m in Utah. Originally native to southern and Eastern Russia, it was introduced to North America from Europe. Because of low water requirement and resistance to diseases and insects, it has been grown as a drought-resistant forage crop and nicknamed as poor man's alfalfa. This is a highly palatable; nutritious, and productive forage species (Vavra *et al.*, 1977). However it may cause oxalate toxicity in livestock if fed over 60 days as the only feed. *Kochia scoparia* seeds show high salinity tolerance while stored in the soil and also during germination preferring warmer temperature regimes (Khan *et al.*, 2001).

Germination regulating chemicals like betaine, fusicoccin, gibberellins, kinetin, ethephon, nitrate, proline and thiourea have little effect on the innate dormancy of temperate halophytes like *Allenrolfea occidentalis* (Wats.) Kuntze (Gul & Weber, 1998), *Atriplex rosea* L. (Khan *et al.*, 2004b), whereas most of these chemicals have some effect on releasing innate dormancy in the case of *Ceratoides lanata* (Pursh) J. T. Howell, *Salicornia rubra* A. Nels., *Salicornia utahensis* Tidestr., *Suaeda moquinii* (Torr.) Greene and *Triglochin maritima* L. (Gul & Khan, 2003; Khan *et al.*, 2002; Khan *et al.*, 2004ab; Khan & Ungar, 2001c). Kinetin, GA, fusicoccin, ethephon, and thiourea had substantial effect in alleviating the salinity effects on seed germination while nitrate, proline and betaine were effective in some Great Basin species (Gul *et al.*, 2000; Gul & Khan, 2003; Khan *et al.*, 2002, 2004ab; Khan & Ungar, 2001c).

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The present study was designed to test the hypothesis that the germination of seeds of *Kochia scoparia* under saline conditions improved with the application of germination regulating chemicals.

## Materials and Methods

Seeds of *Kochia scoparia* which were collected during August from a salt marsh located 30 miles south of the Great Salt Lake at Faust Utah, were separated from the inflorescence and stored at 4°C. Germination studies were started in the following January. Seeds were surface sterilized using the fungicide Phygon. Germination was carried out in 50 x 9-mm (Gelman No. 7232) tight-fitting plastic Petri plates with 5 ml of 0, 300, 600, and 900 mM NaCl solutions supplemented with 10 mM ethephon, 5 µM fusicoccin, 3 mM gibberellic acid, 0.1 and 1 mM respectively proline and betaine, 20 mM nitrate, 10 mM thiourea or 0.05 mM kinetin. Four replicates of 25 seeds each were used for each treatment. These plates were placed in a growth chamber at an alternating temperature regime of 25-35°C, where the higher temperature coincided with the 12-hr light period (Sylvania cool white fluorescent lamps, 25 µM.m<sup>-2</sup>.s<sup>-1</sup>, 400 - 750 nm) and the lower temperature coincided with the 12-hr dark period.

Percent germination was recorded on every alternate day for 20 days and seeds were considered to be germinated with the emergence of the radicle. The rate of germination was estimated by using a modified Timson index of germination velocity =  $\sum G/t$ , where G is percentage of seed germination at 2-days intervals, and t is total germination period (Khan & Ungar, 1985). The maximum value possible using this index with our data was 50 (i.e., 1000/20). The higher values indicate rapid seed germination.

Germination data were transformed (arcsine) before statistical analysis. An ANOVA analysis was used to determine if significant differences were present among means. A Bonferroni test was carried out to determine if significant ( $p < 0.05$ ) differences occurred between individual treatments (Anon., 2001).

## Results

Germination and the rate of germination showed significant ( $p < 0.001$ ) effects of salinity, growth regulators and their interactions when analyzed using two-way ANOVA (Table 1).

Seeds of *K. scoparia* showed no sign of dormancy and almost all seeds germinated in non-saline control. Salinity reduced germination and only about 25% seeds germinated at 900 mM NaCl (Fig. 1). Application of ethephon, fusicoccin, gibberellic acid and kinetin completely alleviated salinity effects and these effects were more evident at the highest salinity concentration. Osmotica like glycinebetaine partially alleviated high salinity effects on seed germination while proline had little effect. Among nitrogenous compounds, nitrate had little effect in alleviating salinity effects on germination however; thiourea substantially alleviated the salinity effect (Fig. 1).

Rate of germination as indicated by the index of germination velocity was maintained by the application of all growth regulators up to 600 mM NaCl, however, at 900 mM NaCl, rate of germination was substantially reduced in the seeds treated with proline, betaine and nitrate (Table 2).

**Table 1. F-ratios and significance for the results of a two-way analysis of variance of germination responses by regulators and salinity treatments.**

Dependent variable	Salinity	Regulator	Salinity x Regulator
Germination	121***	74***	9***
Rate of germination	116***	81***	8***

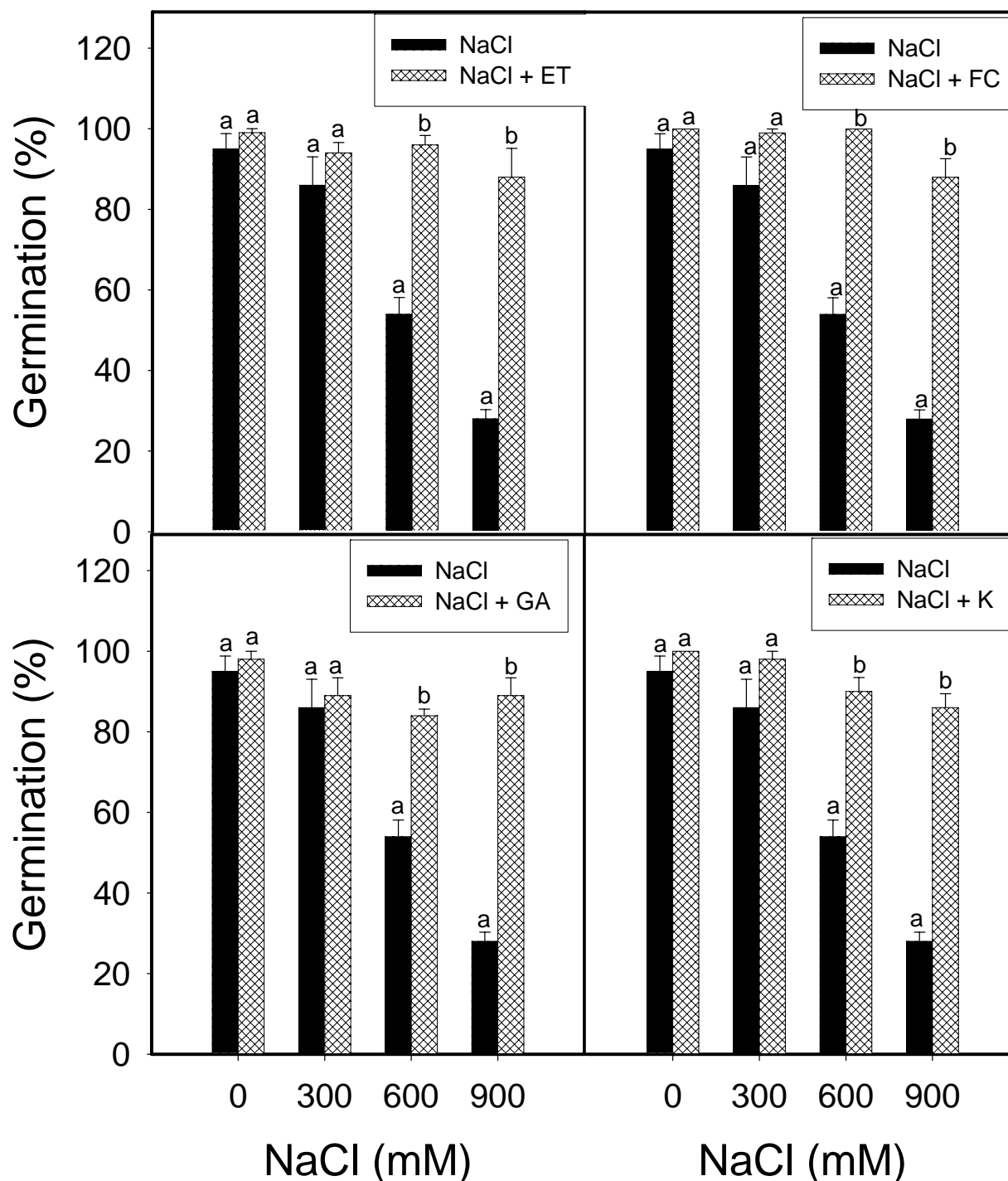
\*\*\* =  $p < 0.0001$ 

Fig. 1. Percent germination of *Kochia scoparia* seeds in NaCl, ethephon (ET), fusicoccin (FC), gibberellic acid (GA), and kinetin (K). Value for dormancy regulating chemicals having the same letter are not significantly different from the control (Bonferroni test).

Table 2. Rates of seed germination of *Kochia scoparia* under various concentration of salinity and germination regulating chemical.

NaCl (mM)	Water	Proline	Betaine	Germination regulating chemicals				Fusicoccin	Ethephon
				GA <sub>3</sub>	Kinetin	Thiourea	Nitrate		
0	40 ± 1.2 <sup>a</sup>	39 ± 2.5 <sup>a</sup>	48 ± 0.9 <sup>b</sup>	49 ± 1.0 <sup>b</sup>	50 ± 0.0 <sup>b</sup>	45 ± 2.4 <sup>ab</sup>	35 ± 2.0 <sup>c</sup>	50 ± 0.0 <sup>b</sup>	49 ± 0.8 <sup>b</sup>
300	37 ± 2.2 <sup>a</sup>	37 ± 2.2 <sup>a</sup>	39 ± 1.0 <sup>a</sup>	44 ± 2.4 <sup>b</sup>	49 ± 1.0 <sup>c</sup>	49 ± 0.8 <sup>c</sup>	31 ± 1.7 <sup>a</sup>	50 ± 0.0 <sup>c</sup>	46 ± 1.4 <sup>c</sup>
600	34 ± 1.7 <sup>a</sup>	31 ± 1.0 <sup>a</sup>	38 ± 4.0 <sup>a</sup>	42 ± 0.8 <sup>b</sup>	43 ± 2.0 <sup>b</sup>	41 ± 2.4 <sup>b</sup>	26 ± 1.3 <sup>c</sup>	50 ± 0.0 <sup>d</sup>	48 ± 1.3 <sup>d</sup>
900	13 ± 1.3 <sup>a</sup>	7 ± 1.3 <sup>b</sup>	23 ± 3.1 <sup>c</sup>	44 ± 2.3 <sup>d</sup>	42 ± 2.2 <sup>d</sup>	36 ± 4.0 <sup>d</sup>	6 ± 2.7 <sup>b</sup>	43 ± 2.8 <sup>d</sup>	44 ± 3.6 <sup>d</sup>

Values in rows for different growth regulating chemicals at each salinity levels having the same letter are not significantly different ( $p > 0.05$ ) from control. Bonferroni test.

## Discussion

*Kochia scoparia* seeds germinated readily in distilled water but exhibited progressive inhibition with increase in salinity. Germination regulating chemicals had variable effect on alleviating this salinity induced response (Fig. 1, Table 2).

Fusicoccin (FC), initially identified as a fungal metabolite, was later found to occur in higher plant roots (Babakov *et al.*, 1995) and other tissues (Bartova, 1997) as well. It is now known to promote seed germination (Evidente *et al.*, 2006; Khan, 1977) and has also been reported to alleviate salinity effect on seed germination. The responses range from positive in some halophytes e.g., *Zygophyllum qatarensis* (Ismail, 1990), *Allenrolfea occidentalis* (Gul & Weber, 1998), *Atriplex stocksii* (Khan & Ungar, 2000), *Sporobolus arabicus* (Khan & Ungar, 2001a), *Halopyrum mucronatum* (Khan & Ungar, 2001c), *Salicornia rubra* (Khan & Gul 2002), *Halogeton glomeratus* (Khan & Gul, 2006) to almost no effect in certain others eg., *Triglochin maritima* (Khan & Ungar, 2001b), *Atriplex rosea* (Khan *et al.*, 2004b).

The salinity effect on the seed germination of *K. scoparia* was completely alleviated by the application of FC. The response of FC system appears to be species specific which changes during seed imbibition and germination (Antipova *et al.*, 2003). It is suggested that prior to the cell elongation, endogenous FC is synthesized and activates the preformed proton ATPase after binding to a preformed FC receptor situated at the inner side of plasmalemma. This complex system starts functioning because of the hydration of imbibing axis tissues up to the threshold level of 68% which triggers acid-induced cell elongation and as a result, radicle protrusion (Obroucheva, 1999).

The application of ethephon (an ethylene releasing compound) completely alleviated the salinity-induced inhibition in the germination of *K. scoparia* seeds. Application of ethylene has been reported to relieve dormancy in seeds of several species (Corbineau *et al.*, 1989; Whitehead & Nelson, 1992) and reverse the inhibitory effect of abscisic acid and osmotic stress (Karssen, 1976; Schonbeck & Egley, 1981). However, seeds of many plants do not respond to ethylene (Ismail, 1982) or some of the alleviating effects are not substantial. Zapata *et al.*, (2004) consider ethylene production a consequence of the seed germination while some others (Matilla, 2000; Petruzelli *et al.*, 2000; Rinaldi, 2000) suggest that it is a requirement for seed germination. Increase in ethylene production during germination under saline conditions has been reported in different species (Chrominski *et al.*, 1986; Datta *et al.*, 1998). It has also been suggested that ethylene and abscisic acid may modulate the physiological effects induced by salinity (Gomez-Cadenas *et al.*, 1998).

Kinetin and GA<sub>3</sub> both completely alleviated the salinity effect on the germination of *K. scoparia* seeds under saline conditions. The role of both in alleviating salinity effects on the seed germination has been well established in many halophytes (Khan & Gul, 2006; Ungar, 1991, 1995). Seed germination of halophytes like *Atriplex stocksii* and *Zygophyllum simplex* (Khan & Rizvi, 1994; Khan & Ungar, 1997) are almost completely alleviated under saline conditions and partial reversal was also reported for *Allenrolfea occidentalis*, *Arthrocnemum macrostachyum*, *Atriplex triangularis*, *Chrysothamnus nauseosus*, *Cressa cretica*, *Polygonum aviculare*, *Salicornia rubra*, and *Salicornia utahensis* (Gul & Weber, 1998; Khan & Gul, 2006; Khan & Ungar, 1985; Khan & Ungar, 1998; Khan & Weber, 1986; Khan *et al.*, 1987) while species such as *Ceratoides lanata*, *Triglochin maritima*, *Sporobolus ioclados*, *Urochondra setulosa*, *Suaeda fruticosa*, *Salsola imbricata* and *Haloxylon stocksii* failed to respond to any gibberellic acid treatment to alleviate salinity effects on germination (Gulzar & Khan, 2002; Khan & Ungar, 2000; Khan *et al.*, 2004a).

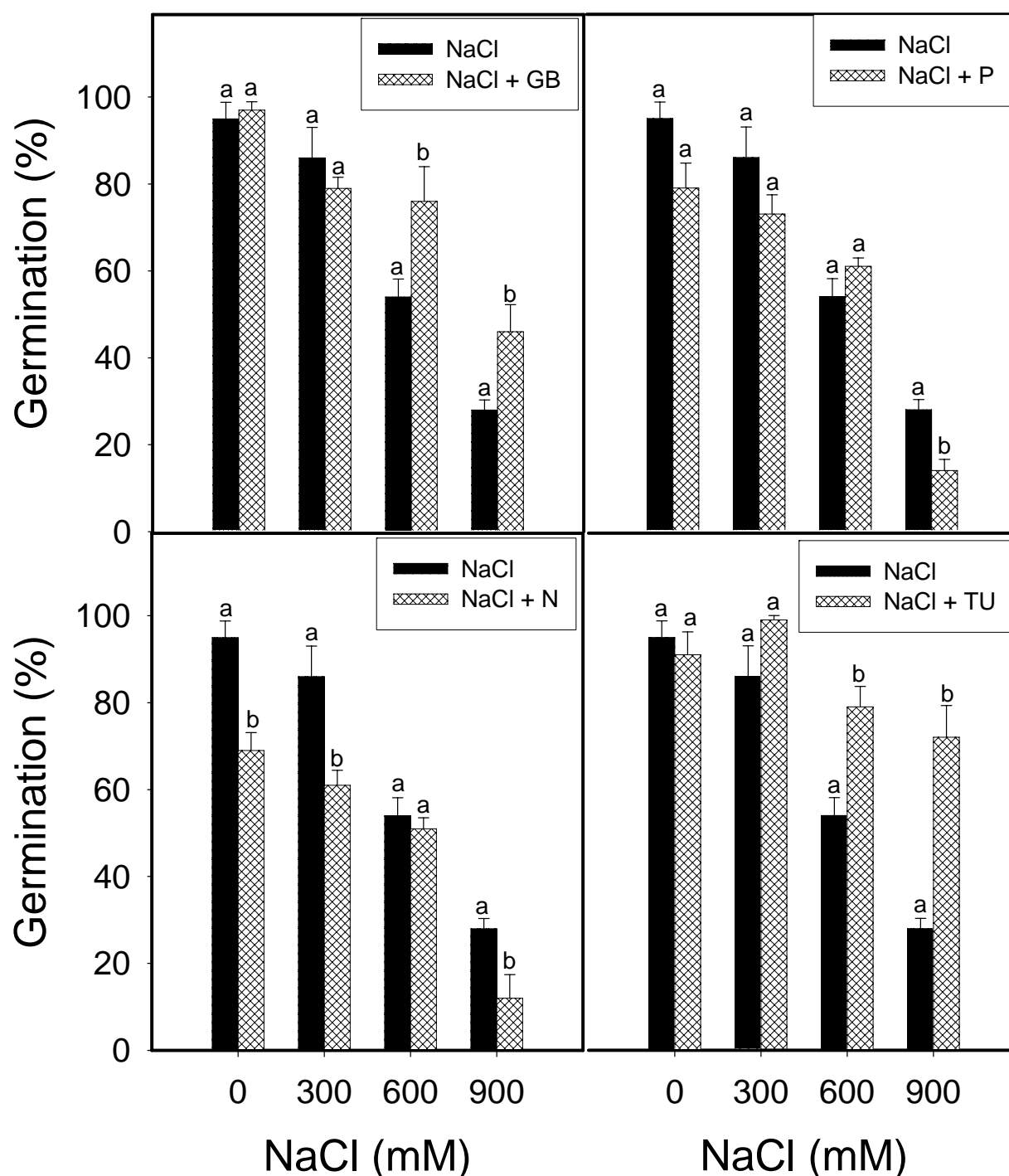


Fig. 2. Percent germination of *Kochia scoparia* seeds in NaCl, Glycinebetaine (GB), Nitrate (N), Proline (P), and Thiourea (TU). Value for dormancy regulating chemicals having the same letter are not significantly different from the control (Bonferroni test).

The nitrogenous compounds used in this study had a variable effect on germination of *K. scoparia* seeds where thiourea was stimulatory but nitrate had no effect under saline conditions. Khan & Gul (2006) reported that thiourea alleviates seed germination of a number of species under saline condition. Some nitrogenous compounds such as nitrate, nitrite and thiourea are known to stimulate the germination of seeds (Aldasaro *et al.*, 1981; Esashi *et al.*, 1979; Yoshiyama *et al.*, 1996). Thiourea counteracts the effect of ABA and reduces the level of cytokinins in plant tissues (Kabar & Baltepe, 1990). These adverse hormonal changes occur when plant tissues are subjected to water stress induced

by drought, salinity or high temperatures (Kabar & Baltepe, 1990). Treatment with thiourea is highly effective in alleviating the inhibition of germination by salinity or high temperatures (Aldasaro *et al.*, 1981; Esashi *et al.*, 1979; Gul & Weber, 1998).

Proline failed but betaine partially alleviated the inhibitory effect of salinity on the seed germination *K. scoparia*. Proline and GB have been shown to provide tolerance to environmental stress by preserving osmotic balance and stabilizing the quaternary structures of complex proteins, membranes and many functional units like oxygen evolving PS-II complex (Demiral & Turkan, 2006; Rajasekaran *et al.*, 1997; Thakur & Sharma, 2005). Therefore, external application of these compounds may help seeds to negotiate high saline stress. Proline was effective in alleviating salinity effect on the seed germination of *Allenrolfea occidentalis*, *Halogeton glomeratus*, *Salicornia utahensis*, and *Triglochin maritima* but failed in *Atriplex rosea*, *Salicornia rubra*, *Sarcobatus vermiculatus* and *Salsola iberica* (Gul & Khan, 2003; Gul *et al.*, 2000; Khan & Gul, 2006; Khan & Ungar, 2001c; Khan *et al.*, 2002, 2004ab), while betaine alleviated seed germination in *A. occidentalis* but showed no effect on other Great Basin species (Khan & Gul, 2006).

*Kochia scoparia* is distributed in the playas of Great Basin desert and produces numerous seeds which after dispersal form a persistent seed bank. These seeds are prevented from germination during early spring due to cooler temperatures but they begin to germinate with the rise in temperature. Higher salinity, a consequence of evaporation, prevents or delay seed germination. Our results indicate that application of germination regulating chemicals like gibberellic acid, kinetin, fusicoccin, ethylene and thiourea completely alleviated the salinity effects on the germination of *K. scoparia* seeds. This suggests that germination under salinity stress may have been prevented by the imbalances in germination regulators. *Kochia scoparia* is a species of significant medicinal importance and could also be used as forage crop. Emergence under field conditions may be substantially increased with the pretreatment of seeds with germination regulating chemicals.

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