



## Alleviation of Seed Dormancy in the Desert Forb *Zygophyllum simplex* L. from Pakistan

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*Zygophyllum simplex* L. is a succulent annual that grows on the coastal and inland saline flats around Karachi, Pakistan. The seeds are moderately salt tolerant during germination. Germination of *Zygophyllum simplex* seeds under various salinity, proline, betaine, GA and kinetin treatments was determined. Proline (0.1 and 1 mM) and betaine (0.1 and 1 mM) alleviated the innate dormancy of seeds, and germination reached 60–70% compared to 12% in the control set. At low salinity compatible osmotica alleviated some effects of salinity, but at higher NaCl concentrations both proline and betaine were ineffective. Gibberellic acid (0.3 and 3 mM) and kinetin (0.05 and 0.5 mM) substantially alleviated both innate as well as salinity-induced seed dormancy. At higher salinity (125 mM), low concentrations of kinetin (0.05 mM) and high concentrations of GA (3 mM) were more effective. GA completely alleviated the effect of salinity at all concentrations used. © 1997 Annals of Botany Company

**Key words:** Betaine, desert, dormancy, forb, GA, germination, halophyte, kinetin, proline, seeds, *Zygophyllum simplex*.

### INTRODUCTION

*Zygophyllum simplex* L. (Zygophyllaceae), a succulent forb, is a characteristic species of the saline flat plains and coastal communities around Karachi, Pakistan. In Pakistan, it is distributed from the coastal areas of Sindh and Balochistan to the plains of Punjab (Stewart, 1972). It is found in moderately saline areas. From seed germination to the production of seeds in *Z. simplex* requires 75 to 80 d.

Germination of halophytes in subtropical arid environments occurs when the soil salinity is substantially reduced by monsoon rains (Khan, 1991; Zaman and Khan, 1992; Khan, 1993; Khan and Rizvi, 1994; Aziz and Khan, 1995; Khan and Ungar, 1995). Khan and Ungar (1996) reported that *Z. simplex* was moderately salt tolerant at the germination stage. No germination was recorded at NaCl concentrations higher than 125 mM, and exposure of seeds to high salinity substantially reduced germination when soil salinity was reduced (Khan and Ungar, 1996).

Growth regulators such as gibberellic acid (Ungar and Binet, 1975; Boucaud and Ungar, 1973; Ungar 1977, 1978; Khan and Ungar, 1985; Khan and Weber, 1986; Khan *et al.*, 1987; Kabar, 1987; Ungar, 1991; Khan and Rizvi, 1994), kinetin (Bozcuk, 1981; Ungar, 1982; Okusanya and Ungar, 1983; Khan and Ungar, 1985; Khan *et al.*, 1987; Kabar, 1987; Tirmizi, 1988; Ungar, 1991; Khan and Rizvi, 1994) and fusicosin (Ismail, 1990; Yaniv, Lisker and Corbineau, 1995; Pylar and Proseus, 1996) are known to alleviate the inhibitory effect of salinity on germination. Poljakoff-Mayber *et al.* (1994) reported that dry seeds

contain a significant amount of betaine but little proline, but during germination in the presence of NaCl the betaine content decreased, while the proline content increased suggesting a possible role of proline during germination. They found that both proline and betaine (10 mM) were ineffective in alleviating the effect of salinity stress on germination or in breaking innate dormancy.

This study was undertaken to determine whether the application of growth promoters such as GA and kinetin, and compatible osmotica such as proline and betaine could alleviate innate dormancy and dormancy induced by high salinity. The work of Poljakoff-Mayber *et al.* (1994) led us to believe that lower concentrations (0.1 and 1 mM) of compatible osmotica could be effective in removing innate or salt-induced dormancy.

### MATERIALS AND METHODS

Seeds of *Zygophyllum simplex* collected during autumn 1994 from salt flats on the Karachi University campus were separated from the inflorescence and stored at 4 °C. These seeds were brought to Ohio University, USA, and germination studies were started in December 1995. Seeds were surface sterilized using the fungicide Phygon. Germination was carried out in 50 × 9 mm (Gelman No. 7232) tight-fitting plastic petri dishes with 5 ml of test solution. Each dish was placed in a 10 cm-diameter plastic petri dish as a further precaution against loss of water by evaporation. Four replicates of 25 seeds each were used for each treatment. Seeds were considered to have germinated upon emergence of the radicle.

Seeds were germinated in a growth chamber at an alternating temperature regime of 15–25 °C, where the

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higher temperature coincided with the 12-h light period (Sylvania cool white fluorescent lamps,  $25 \mu\text{mol m}^{-2} \text{s}^{-1}$ , 400–700 nm) and the lower temperature coincided with the 12-h dark period. GA concentrations of 0.3 and 3 mM, kinetin concentrations of 0.05 and 0.5 mM, proline and betaine concentrations of 0.1 and 1 mM, and NaCl concentrations of 0, 25, 75 and 125 mM were used. Percent germination was recorded on alternate days for 20 d. The rate of germination was estimated by using a modified Timson index of germination velocity:  $G/t$ , where  $G$  is the sum of the percentage of seed germination at 2-d intervals, and  $t$  is total germination period (Khan and Ungar, 1985). The maximum value possible using this index with our data was 50 (i.e. 1000/20). The higher the value, the more rapid the rate of 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 (SPSS, 1994).

## RESULTS

Proline applied at either 0.1 or 1 mM broke the dormancy of *Z. simplex* seeds in non-saline controls and in the low salinity treatment. The non-saline control treatment with 0.1 mM proline had 60% total germination in comparison to 12% in controls without proline (Fig. 1). However, at higher salinity treatments (75 and 125 mM NaCl) proline

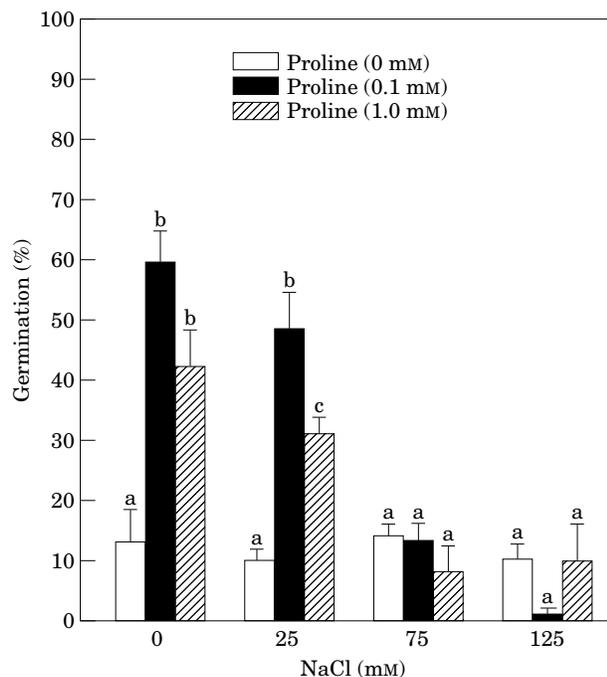


Fig. 1. Final germination percentages ( $\pm$  s.e.) of *Zygophyllum simplex* seeds in 0, 25, 75 and 125 mM NaCl and 0, 0.1 and 1.0 mM proline. Values at each proline concentration with the same letter are not significantly different ( $P > 0.05$ ).

TABLE 1. Result of two-way analysis of variance of characteristics by kinetin (K), salinity (S), GA (G), proline (P) and betaine (B) treatments

Dependent variable	Independent variable		
	K	S	K $\times$ S
Germination (%)	241.9***	22.2***	22.0***
Germination (velocity)	199.7***	18.3***	18.5***
	G	S	G $\times$ S
Germination (%)	124.3***	11.1***	5.3***
Germination (velocity)	85.6***	12.7***	88.3***
	P	S	P $\times$ S
Germination (%)	19.9***	37.1***	11.4***
Germination (velocity)	26.2***	42.6***	13.3***
	B	S	B $\times$ S
Germination (%)	17.6***	61.5***	15.6***
Germination (velocity)	24.1***	73.5***	17.6***

Numbers represent  $F$  values. \*\*\*,  $P < 0.0001$

was ineffective in alleviating the dormancy of seeds. A two-way ANOVA of percent germination indicated a significant ( $P < 0.0001$ ) main effect of salinity and proline and their interaction (Table 1). A one-way ANOVA of the rate of germination for each salinity level revealed that proline significantly increased the rate of germination of seeds at 0 mM NaCl ( $P < 0.0007$ ) and 25 mM NaCl ( $P < 0.0004$ ), while the effect of proline was not significant at 75 mM NaCl ( $P < 0.279$ ) or 125 mM NaCl ( $P < 0.279$ ).

The rate (speed) of germination in non-saline and low salinity media with proline was substantially higher than in controls (Table 2), but proline had no effect on the rate of germination in the high salinity treatments. A two-way ANOVA of the rate of germination indicated a significant ( $P < 0.0001$ ) main effect of salinity and proline and their interaction (Table 1). A one-way ANOVA of the rate of germination for each salinity level revealed that proline significantly increased the rate of germination of seeds at 0 mM NaCl ( $P < 0.0004$ ) and 25 mM NaCl ( $P < 0.0001$ ), while the effect of proline was not significant at 75 mM NaCl ( $P < 0.2653$ ) or 125 mM NaCl ( $P < 0.2653$ ).

Innate seed dormancy of *Z. simplex* seeds was significantly ( $P < 0.00001$ ) alleviated by both concentrations of betaine used (Fig. 2). In the 25 mM NaCl concentration, betaine (1 mM) significantly ( $P < 0.05$ , Bonferroni test) increased the percentage germination in comparison to the non-treated control. At higher salinities, betaine did not alleviate seed dormancy (Fig. 2). A two-way ANOVA indicated a significant ( $P < 0.0001$ ) main effect of salinity and betaine and their interaction (Table 1) on seed germination. A one-way ANOVA for each salinity level revealed that betaine significantly increased the percent germination of seeds at 0 mM NaCl ( $P < 0.0001$ ) and 25 mM NaCl ( $P < 0.0112$ ), while the effect of betaine was not significant at 75 mM NaCl ( $P < 0.4804$ ) or 125 mM NaCl ( $P < 0.7438$ ).

The rate of germination significantly increased ( $P < 0.05$ ) with betaine application in controls and in the lowest salinity treatments. However, there was no significant ( $P > 0.05$ ) effect of betaine at higher salinity concentrations

TABLE 2. Index of germination velocity (mean  $\pm$  s.e.) of *Zygophyllum simplex* seeds at various salinity, growth regulator, and osmotic treatments

Salinity (mM)	Water	Proline (mM)		Betaine (mM)		GA (mM)		Kinetin (mM)	
		0.1	1.0	0.1	1.0	0.3	3.0	0.05	0.5
0	5.8 $\pm$ 2.0 <sup>a</sup>	26.7 $\pm$ 2.5 <sup>a</sup>	33.4 $\pm$ 3.3 <sup>a</sup>	33.4 $\pm$ 3.3 <sup>a</sup>	30.2 $\pm$ 0.9 <sup>a</sup>	31.5 $\pm$ 1.5 <sup>a</sup>	26.9 $\pm$ 1.1 <sup>a</sup>	35.4 $\pm$ 1.3 <sup>a</sup>	35.0 $\pm$ 6.4 <sup>a</sup>
25	3.1 $\pm$ 0.5 <sup>a</sup>	21.8 $\pm$ 2.7 <sup>a</sup>	9.1 $\pm$ 2.1 <sup>a</sup>	9.1 $\pm$ 2.1 <sup>b</sup>	11.8 $\pm$ 0.6 <sup>b</sup>	22.0 $\pm$ 3.5 <sup>ab</sup>	21.2 $\pm$ 3.2 <sup>a</sup>	38.9 $\pm$ 3.4 <sup>a</sup>	33.4 $\pm$ 3.4 <sup>a</sup>
75	6 $\pm$ 1.1 <sup>a</sup>	5.9 $\pm$ 1.2 <sup>b</sup>	5.8 $\pm$ 3.0 <sup>b</sup>	5.8 $\pm$ 3.0 <sup>b</sup>	2.9 $\pm$ 0.8 <sup>b</sup>	17.9 $\pm$ 2.8 <sup>bc</sup>	21.1 $\pm$ 0.8 <sup>a</sup>	29.7 $\pm$ 2.4 <sup>ab</sup>	24.0 $\pm$ 1.8 <sup>a</sup>
125	4 $\pm$ 1.1 <sup>a</sup>	0.35 $\pm$ 0.2 <sup>b</sup>	4.3 $\pm$ 1.5 <sup>b</sup>	4.3 $\pm$ 2.4 <sup>b</sup>	3.4 $\pm$ 1.7 <sup>b</sup>	8.8 $\pm$ 2.7 <sup>c</sup>	20.0 $\pm$ 0.9 <sup>a</sup>	24.6 $\pm$ 2.3 <sup>b</sup>	18.6 $\pm$ 1.6 <sup>a</sup>

Values in each column with the same superscript are not significantly different at  $P > 0.05$ , Bonferroni test.

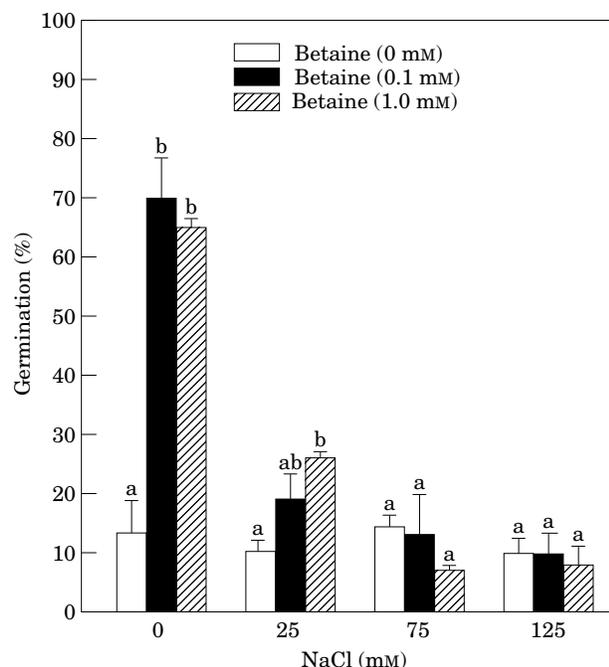


FIG. 2. Final germination percentages ( $\pm$  s.e.) of *Zygophyllum simplex* seeds in 0, 25, 75 and 125 mM NaCl and 0, 0.1 and 1.0 mM betaine. Values at each betaine concentration with the same letter are not significantly different ( $P > 0.05$ ).

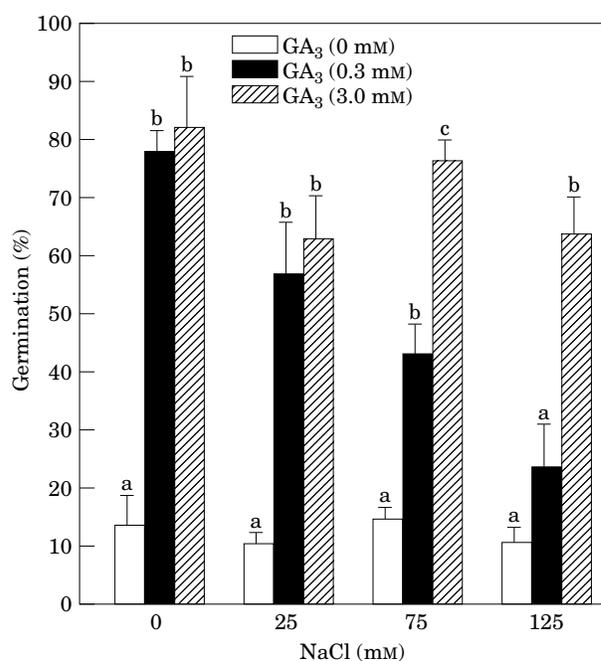


FIG. 3. Final germination percentages ( $\pm$  s.e.) of *Zygophyllum simplex* seeds in 0, 25, 75 and 125 mM NaCl and 0, 0.3 and 3.0 mM GA. Values at each GA concentration with the same letter are not significantly different ( $P > 0.05$ ).

(Table 2). A two-way ANOVA indicated a significant ( $P < 0.0001$ ) main effect of salinity and betaine and their interaction on the rate of germination (Table 1). A one-way ANOVA for each salinity level revealed that betaine significantly affected the rate of germination of seeds at 0 mM NaCl ( $P < 0.0001$ ) and 25 mM NaCl ( $P < 0.003$ ), while the effect of betaine was not significant at 75 mM NaCl ( $P < 0.4515$ ) or 125 mM NaCl ( $P < 0.835$ ).

Gibberellic acid treated seeds in non-saline control showed 80% germination compared 12% in controls (Fig. 3). At 0 and 25 mM NaCl treatments, increased GA concentrations had no significant ( $P > 0.05$ ) effect in alleviating seed dormancy. The inhibition of germination caused by NaCl salinity was progressively alleviated by increasing GA concentration (Fig. 3). A two-way ANOVA indicated a significant ( $P < 0.0001$ ) main effect of salinity and GA and their interaction on seed germination (Table 1). A one-way ANOVA for each salinity level revealed that GA signifi-

cantly affected the rate of germination of seeds at 0 mM NaCl ( $P < 0.00001$ ), 25 mM NaCl ( $P < 0.0008$ ), 75 mM NaCl ( $P < 0.00001$ ) and 125 mM NaCl ( $P < 0.0009$ ).

The rate of germination decreased with an increase in salinity (Fig. 4, Table 2). Addition of GA (0.3 mM) significantly ( $P < 0.05$ ) promoted the rate of germination at all NaCl concentrations. The higher concentration of GA (3.0 mM) almost completely alleviated the inhibitory effect of salinity on the rate of germination (Fig. 4). A two-way ANOVA indicated a significant ( $P < 0.0001$ ) main effect of salinity and GA and their interaction (Table 1) on the rate of germination. A one-way ANOVA for each salinity level revealed that GA significantly affected the rate of germination of seeds at 0 mM NaCl ( $P < 0.0001$ ), 25 mM NaCl ( $P < 0.0012$ ), 75 mM NaCl ( $P < 0.0006$ ) and 125 mM NaCl ( $P < 0.0015$ ).

Kinetin almost completely alleviated the innate dormancy in non-saline controls and the germination effect of salt in

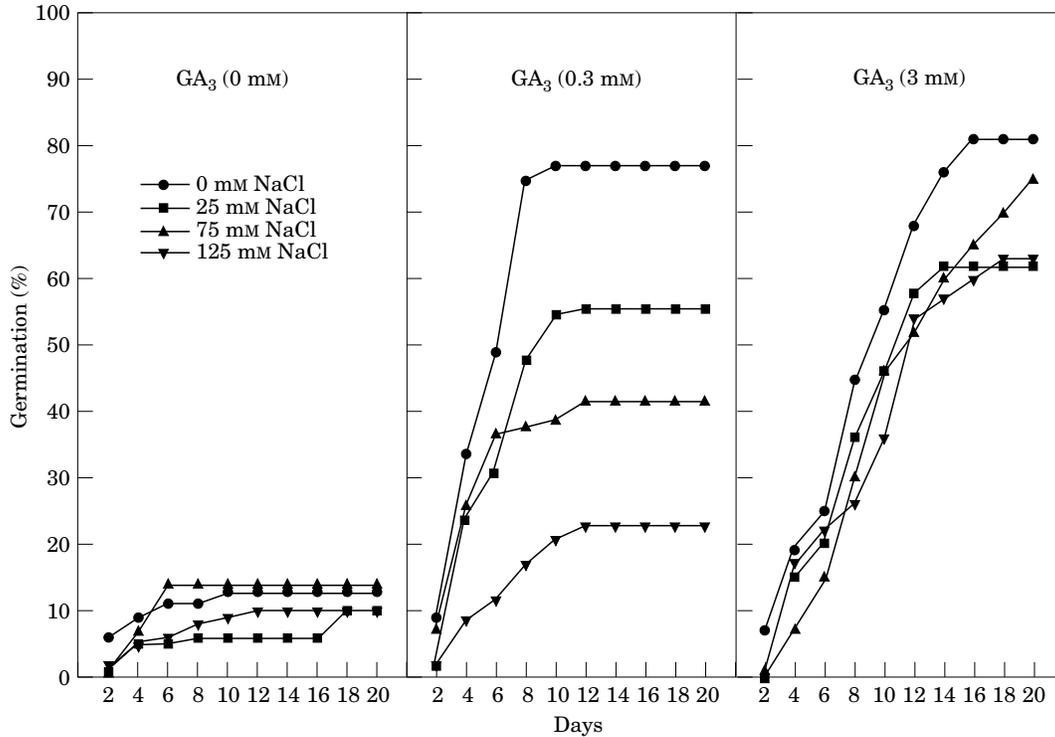


FIG. 4. Rate of germination of *Zygophyllum simplex* seeds in 0, 25 and 75 and 125 mM NaCl and 0, 0.3 and 3.0 mM GA<sub>3</sub>.

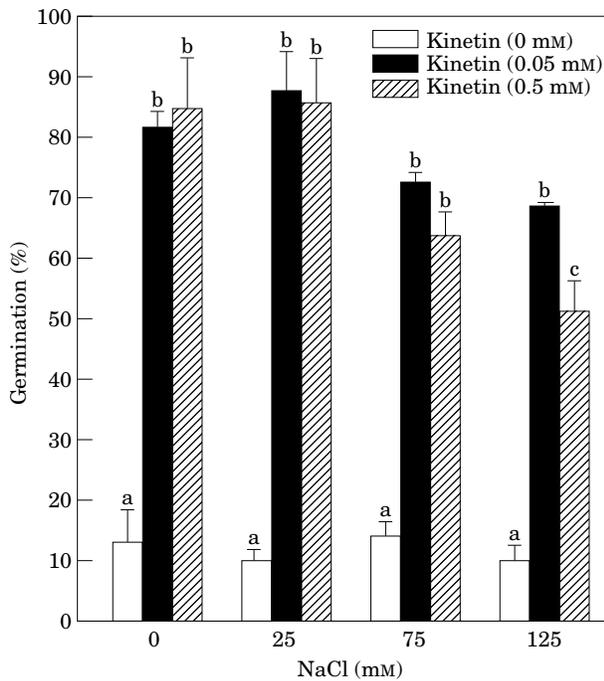


FIG. 5. Final germination percentages ( $\pm$  s.e.) of *Zygophyllum simplex* seeds in 0, 25, 75 and 125 mM NaCl and 0.05 and 0.5 mM kinetin. Values at each kinetin concentration with the same letter are not significantly different ( $P > 0.05$ ).

the low salinity (25 mM) treatment (Fig. 5). At 75 mM NaCl both concentrations of kinetin induced about 70% germination, compared to 14% in the non-treated control.

However, at 125 mM NaCl, the low kinetin concentration (0.05 mM) was more stimulatory than the high kinetin concentration (0.5 mM). A two-way ANOVA indicated a significant ( $P < 0.0001$ ) main effect of salinity and kinetin and their interaction (Table 2) on seed germination. A one-way ANOVA for each salinity level revealed that kinetin significantly affected the rate of germination of seeds at 0 mM NaCl ( $P < 0.00001$ ), 25 mM NaCl ( $P < 0.00001$ ), 75 mM NaCl ( $P < 0.00001$ ) and 125 mM NaCl ( $P < 0.00001$ ).

The germination rate was substantially higher in kinetin treatments in comparison to non-treated controls (Table 2). At higher salinity concentrations, the lower kinetin concentration (0.05 mM) induced a higher rate of germination. A two-way ANOVA indicated a significant ( $P < 0.0001$ ) main effect of salinity and kinetin and their interaction (Table 2) on the rate of germination. A one-way ANOVA for each salinity level revealed that kinetin significantly affected the rate of germination of seeds at 0 mM NaCl ( $P < 0.00001$ ), 25 mM NaCl ( $P < 0.0001$ ), 75 mM NaCl ( $P < 0.0001$ ) and 125 mM NaCl ( $P < 0.0001$ ).

## DISCUSSION

Seeds are a critical component of the life history of annual desert forbs because, as dormant dispersal units, they permit the species to avoid stress over the course of its life cycle. *Zygophyllum simplex* seeds germinated when temperature and salinity were reduced due to rainfall (Khan and Ungar, 1996). Most seeds remain ungerminated in the

soil even in non-saline conditions. Dormancy of seeds, whether innate or induced by high salt concentration is alleviated in some species by the application of GA (Boucaud and Ungar, 1973; Ungar and Binet, 1975; Ungar, 1978, 1984; Khan and Ungar, 1985; Khan and Weber, 1986; Kabar and Baltepe, 1990; Khan *et al.*, 1987; Khan, 1991; Khan and Rizvi, 1994), kinetin (Khan and Ungar, 1985; Khan *et al.*, 1987; Kabar and Baltepe, 1990; Tirmizi, 1988; Khan and Rizvi, 1994), or a mixture of GA and kinetin (Tirmizi, 1988; Kabar, 1990; Kabar and Baltepe, 1990; Ungar, 1991).

Gibberellic acid and kinetin were found to be very effective in alleviating innate as well as salinity induced dormancy in *Z. simplex* seeds. In the high salinity treatment (125 mM) a low concentration of kinetin (0.05 mM) and a high concentration of GA (3 mM) were most effective in alleviating seed dormancy.

Effects of NaCl on seed germination are both ionic and osmotic (Ungar, 1991). Compatible osmotica such as proline and betaine which acclimatize plants to high salt concentrations in their tissues may be of significance in seeds. Poljakoff-Mayber *et al.* (1994) studied the proline and betaine level in *Kosteletzkya virginica* seeds during the process of germination. They found low concentrations of proline and a significant amount of betaine in dry seeds. However, when seeds were germinated in a saline media, proline content increased while betaine concentration decreased. They suggested that proline could be the compatible osmoticum for germinating seeds. External application of proline and betaine to *K. virginica* seeds in both control and saline medium had no effect on seed germination.

Our study indicates that both proline and betaine could alleviate the innate dormancy of *Z. simplex* seeds. Proline application substantially promoted seed germination from 12 to 60%, while betaine promoted germination from 12 to about 70%. At low salinity (25 mM NaCl), 0.1 mM proline was more effective than 1 mM proline in alleviating salinity induced inhibition of germination (from 10 to 50%). The higher concentration of betaine (1 mM) only increased germination from 10 to 25%. Both proline and betaine failed to show any effect on alleviating dormancy at higher salinity concentrations. Our results are similar to those of Poljakoff-Mayber *et al.* (1994), indicating no effect of compatible osmotica at high salinity, but at 0 or 25 mM NaCl both proline and betaine substantially promoted germination. These differences in results could be attributed to the lower concentration of compatible osmotica (0.1 to 1 mM) used, with higher concentrations of compatible osmotica being less effective.

*Zygophyllum simplex* produces abundant seeds at the end of autumn and beginning of winter. After dispersal, seeds remain in the seed bank until the next monsoon rains which not only reduce the ambient temperature but also provide much needed moisture for germination. However, not all of the seeds germinate. Dormancy of seeds could be either innate or salt induced. These data indicate that the compatible osmotica overcome drought or ionic stress in seeds, while growth regulators stimulate germination directly.

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