

## ACTION OF COMPATIBLE OSMOTICA AND GROWTH REGULATORS IN ALLEVIATING THE EFFECT OF SALINITY ON THE GERMINATION OF DIMORPHIC SEEDS OF *ARTHROCNEMUM INDICUM* L.

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*Arthrocnemum indicum* Willd., a stem succulent perennial in the family Chenopodiaceae, is widely distributed along the coastal areas of Pakistan and forms an important component of the vegetation of salt marshes in the vicinity of mangrove swamps. Seed germination of halophytes is often inhibited by hypersaline conditions at these sites, which prevents the establishment of new populations (Ungar 1991). We studied the affect of growth regulators and compatible osmotica in alleviating the innate and salinity-induced dormancy in dimorphic (brown and black) seeds of *A. indicum*. Germination of both types of seeds decreased with an increase in salinity. Brown seeds germinated at the highest salinity concentration (1000 mM), whereas only a few black seeds germinated at 800 mM NaCl, and no germination was recorded at 1000 mM NaCl. The osmotica, proline and betaine, did not relieve salinity-induced dormancy in either black or brown seeds. Plant growth regulators, gibberellic acid (GA) and kinetin, significantly ( $P < 0.05$ ) alleviated the salinity-induced germination inhibition of both seed types but over different salinity ranges and to different degrees. Both growth regulators significantly increased ( $P < 0.05$ ) the rate of germination over most salinities, but the effect of GA was more pronounced than kinetin.

### Introduction

*Arthrocnemum indicum* is a perennial halophyte commonly found in tropical salt marshes that are frequently inundated with seawater. Karim and Qadir (1979) indicated that *A. indicum*, with its small shrub-like habit, occurs in almost pure patches in the coastal salt marshes of Karachi, Pakistan, but it occurs occasionally with other species such as *Limonium stocksii*, *Cressa cretica*, *Aeluropus insignis*, and *Suaeda monoica*. Flowering and seed set in this population of *A. indicum* from the Sands Pit Beach area of Karachi, Pakistan, occurs after monsoon rains (July–August). *Arthrocnemum indicum* plants produce many seeds, but recruitment through seeds is a rare event (Gul 1993). Therefore, reproduction is believed to be primarily asexual by means of rhizomes.

Seed dimorphism may play a significant role in determining the survival of populations in saline environments. The seed morphs of *Atriplex triangularis* Willd. from temperate marshes were found to have different levels of dormancy and salt tolerance (Khan and Ungar 1984). Large brown seeds were less dormant and more salt tolerant than were the small black seeds. *Arthrocnemum indicum* Willd. (Chenopodiaceae) was used in this experiment to determine if its two seed morphs also differed in their level of dormancy and the ability to germinate under saline conditions. The two seed morphs could provide multiple germination cohorts in saline habitats that provide several opportunities for *A. indicum* to establish populations in new habitats. Although halophytes are salt tolerant, germination of their seeds is often inhibited by increased salinity (Ungar 1991). The compatible osmotica proline and betaine have been hypothesized to increase

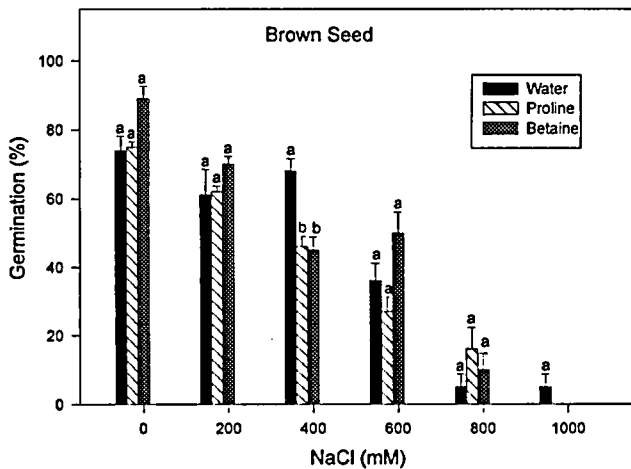
the salt tolerance of plants at the growth stage of development (Gorham 1995), but their effect at seed germination is poorly understood since their influence on seed germination has only been reported for a few species (Poljakoff-Mayber et al. 1994; Khan and Ungar 1996a). Growth regulators have been shown to stimulate germination of seeds under saline conditions in temperate salt marsh species, but no data is available concerning their effect on germination of subtropical halophytes (Ungar 1991; Plyler and Proseus 1996).

Poljakoff-Mayber et al. (1994) reported that dry seeds of *Kosteletkya virginica* contain a significant amount of betaine and proline. Betaine content decreased during germination in the presence of NaCl, while proline content increased, indicating a possible role of proline during germination. They also found that both proline and betaine (10 mM) were ineffective in alleviating the effect of salinity stress on germination or in breaking innate dormancy. Khan and Ungar (1996a) studied the effect of proline, betaine, GA, and kinetin on the reversal of salinity-induced dormancy in seed of *Zygophyllum simplex* L. seeds. Proline and betaine alleviated the innate dormancy of seeds, and germination reached 70% as compared to 12% in control. Osmolytes had little effect on germination under saline conditions, but GA and kinetin substantially alleviated both innate and salinity-induced dormancy in *Z. simplex* seeds. Growth regulators like kinetin and GA are reported to alleviate the inhibitory effect of salinity on germination (Boucaud and Ungar 1973; Ungar and Binet 1975; Ungar 1977, 1982, 1984; Bozcuk 1981; Okusanya and Ungar 1983; Khan and Ungar 1985; Khan and Weber 1986; Kabar 1987; Khan et al. 1987; Tirmizi 1988; Ismail 1990; Khan 1991; Khan and Rizvi 1994; Yaniv et al. 1995; Plyler and Proseus 1996).

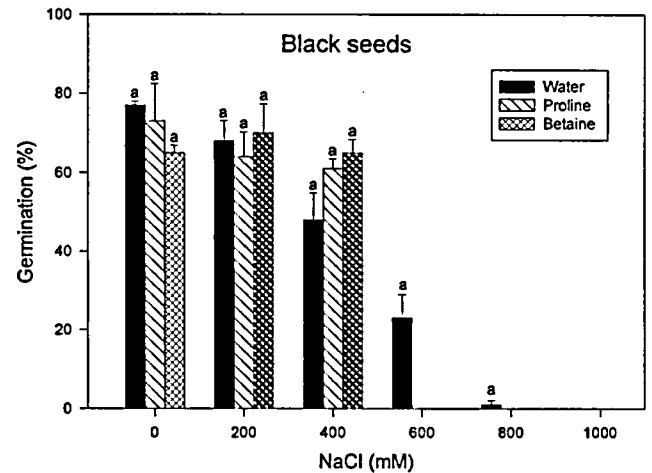
The purpose of this investigation was to determine the role of the growth promoters GA and kinetin and

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**Fig. 1** Final germination percentages of *Arthrocnemum indicum* brown seeds in 0, 200, 400, 600, 800, and 1000 mM NaCl, 1.0 mM proline, and 1.0 mM betaine. Values at each NaCl concentration having the same letter are not significantly different ( $P > 0.05$ ).



**Fig. 3** Final germination percentages of *Arthrocnemum indicum* black seeds in 0, 200, 400, 600, 800, and 1000 mM NaCl, 1.0 mM proline, and 1.0 mM betaine. Values at each NaCl concentration having the same letter are not significantly different ( $P > 0.05$ ).

compatible osmotica such as proline and betaine in alleviating the seed dormancy in *A. indicum*.

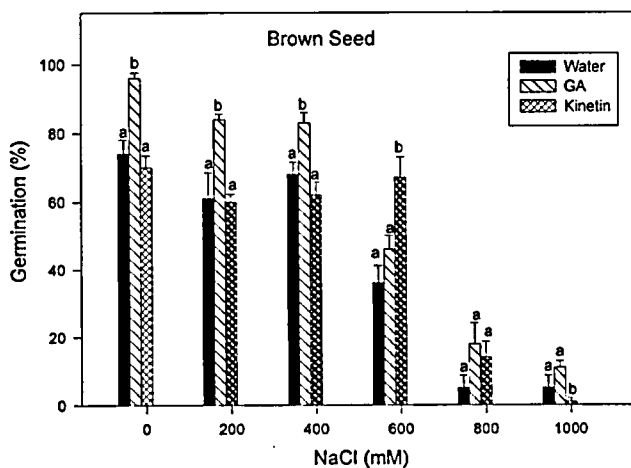
### Material and Methods

Seeds of *Arthrocnemum indicum* were collected during the fall 1994 from salt flats situated on the Sands Pit Beach, Karachi, Pakistan. Seeds were separated from the inflorescence, sorted into black and brown seeds, and stored at 4°C. These seeds were brought to Ohio University, U.S.A., where 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-diam plastic petri dish as an added precaution against loss of water by evaporation.

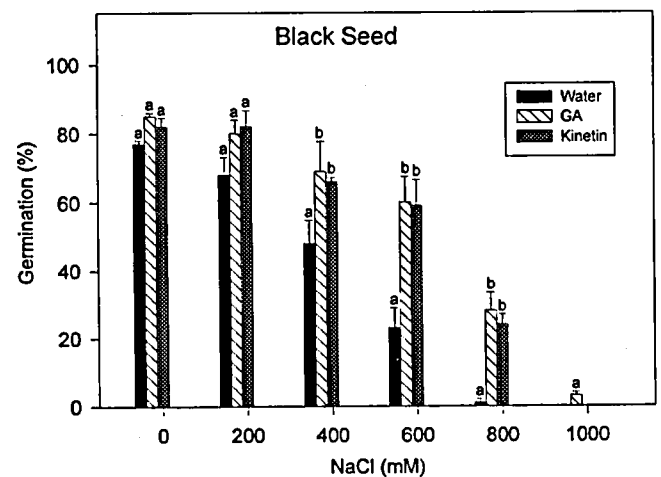
Seeds (brown and black) were germinated in an incubator at an alternating temperature regime of 15°–25°C, where the higher temperature coincided with the 12-h light period (Sylvania Cool White fluorescent lamps; 25  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; 400–

700 nm) and the lower temperature coincided with the 12-h dark period. This thermoperiod simulates the ambient conditions in the field and are optimal (unpublished data) for *A. indicum* germination. Concentrations of 3 mM GA, 0.5 mM kinetin, 1 mM proline and betaine and 0, 200, 400, 600, 800, and 1000 mM NaCl were used. Four replicates of 25 seeds each for each seed morph were used for each treatment. Seeds were considered to be germinated with the emergence of the radicle. Percent germination was recorded every alternate day for 20 d. The rate of germination was estimated by using a modified Timson index  $\bar{c} = \Sigma G/t$ , where  $G$  is percentage of seed germination at 2-d intervals, and  $t$  is total germination period (Khan and Ungar 1984). 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 was used to determine if there were significant treatment differences in germination veloc-



**Fig. 2** Final germination percentages of *Arthrocnemum indicum* brown seeds in 0, 200, 400, 600, 800, and 1000 mM NaCl, 3.0 mM GA, and 0.5 mM kinetin. Values at each NaCl concentration having the same letter are not significantly different ( $P > 0.05$ ).



**Fig. 4** Final germination percentages of *Arthrocnemum indicum* black seeds in 0, 200, 400, 600, 800, and 1000 mM NaCl, 3.0 mM GA, and 0.5 mM kinetin. Values at each NaCl concentration having the same letter are not significantly different ( $P > 0.05$ ).

**Table 1** *F*-ratios and Significance for the Results of a Two-Way Analysis of Variance of Germination Responses by Regulator/Osmotica and Salinity Treatments

Seed color	Dependent variable	Salinity	Regulator/ osmotica	Salinity × Regulator/ osmotica
Brown ...	Germination	12.8***	168.1***	4.9***
	Rate of germination	15.1***	201.7***	5.5***
Black ....	Germination	239.8***	29.2***	6.2***
	Rate of germination	314.8***	29.2***	4.1***

\*\*\* =  $P < 0.0001$ .

ity and final germination percentage. A Bonferroni test was carried out to determine if significant ( $P < 0.05$ ) differences occurred between individual treatments (SPSS 1994).

### Results

Increase in salinity inhibited the germination of brown seeds of *Arthrocnemum indicum*, but a few seeds did germinate at 1000 mM NaCl (fig. 1). Betaine enhanced the germination of *A. indicum* brown seeds in the nonsaline control. However, both betaine and proline failed to alleviate the effect of salinity on the germination of brown seeds. Bonferroni tests indicated that betaine and proline treatments were not significantly higher ( $P > 0.05$ ) than the control in any treatments. Gibberellic acid promoted seed germination both in nonsaline and saline treatments (fig. 2). Gibberellic acid significantly ( $P < 0.05$ ) enhanced germination at the lower concentrations of NaCl, but no significant ( $P > 0.05$ ) promotion was noted at salinities above 400 mM. Kinetin was effective in significantly ( $P < 0.05$ ) promoting germination only at 600 mM NaCl (fig. 2).

Germination of black seeds was also inhibited by an increase in salinity (fig. 3), and no seeds germinated at 1000 mM NaCl in the water or compatible osmotica treatments. Compatible osmotica, proline and betaine, had no significant effect ( $P > 0.05$ ) on germination in any of the salinity treatments (fig. 3). Both GA and kinetin significantly ( $P < 0.05$ ) enhanced germination of black seeds at all salinity concentrations (fig. 4). A two-way ANOVA of rate and percent germination for salinity and regulators showed highly significant individual effects and their interaction for both seed types (table 1).

Rate of germination of black seeds decreased with an increase in salinity (table 2). GA and kinetin significantly ( $P < 0.05$ ) increased the germination rate in all salinity treatments in comparison to the control. Rate of germination of brown seeds decreased with an increase in salinity (table 3). Compatible osmotica, betaine and proline, had no effect on the rate of seed germination, while GA significantly ( $P < 0.05$ ) increased the rate of germination at all salinities, except at 600 mM NaCl.

### Discussion

The failure of recruitment of *Arthrocnemum indicum* through seeds could be the result of dormancy of seeds, and this dormancy could be either innate or regulated through inhibition of growth-promoting substances as a result of the high salinity of the soil and high salt content of seeds (Khan and Ungar 1996b). There is little information available on which halophytes can germinate at salinities higher than 800 mM NaCl and whether or not compatible osmotica and growth regulators can alleviate the inhibitory effects of these high salt concentrations (Ungar 1991). The present study indicates that increased salinity significantly decreased the germination percentages of both brown and black *A. indicum* seeds. Brown seeds appeared to be the more tolerant to salinity with some seeds germinating at 1000 mM NaCl. Similar results were determined with dimorphic seeds of a salt marsh species *Atriplex triangularis* in which brown seeds were less dormant than the black seeds (Khan and Ungar 1984). The more dormant black seeds in this species may provide a persistent seed bank under stressful salinity conditions, which germinate when salinity stress is alleviated during the periods of high precipitation (Ungar 1995).

GA and kinetin both partially alleviated the effect of salinity on germination of black and brown seeds in *A. indicum* (0–600 mM NaCl in brown seeds and 400–800 mM in black seeds). Seed germination of other species under saline conditions has similarly been reported to be significantly improved with the application of GA (Kabar and Baltepe 1989; Ungar 1991), kinetin (Khan and Ungar 1985, 1996a; Kabar and Baltepe 1989; Khan and Rizvi 1994), or a mixture of GA and kinetin (Ungar 1977; Tirmizi 1988; Kabar

**Table 2** Effect of Growth Regulators, Osmotica, and Salinity on the Rate of Germination (Timson index; max = 50) of Black *Arthrocnemum indicum* Seeds

NaCl (mM)	Water	Proline	Betaine	GA	Kinetin
0 ....	29.6 ± 0.5 <sup>ab</sup>	28.4 ± 3.6 <sup>ab</sup>	24.9 ± 0.7 <sup>a</sup>	33.5 ± 0.9 <sup>b</sup>	31.3 ± 1.8 <sup>ab</sup>
200 ....	24.6 ± 1.5 <sup>a</sup>	23.9 ± 2.9 <sup>a</sup>	25.6 ± 2.7 <sup>a</sup>	31.2 ± 1.4 <sup>b</sup>	30.6 ± 1.6 <sup>b</sup>
400 ....	15.9 ± 2.3 <sup>a</sup>	20.8 ± 0.8 <sup>ab</sup>	22.2 ± 0.9 <sup>ab</sup>	25.8 ± 3.2 <sup>b</sup>	23.5 ± 0.3 <sup>ab</sup>
600 ....	3.3 ± 1.7 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	18.6 ± 2.2 <sup>b</sup>	15.2 ± 2.4 <sup>b</sup>
800 ....	0.2 ± 0.2 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	3.4 ± 1.5 <sup>b</sup>	1.6 ± 0.9 <sup>b</sup>
1000 ....	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	0.9 ± 0.3 <sup>b</sup>	0 ± 0 <sup>a</sup>

Note. Values in each row with the same letter are not significantly different  $P > 0.05$ ; Bonferroni test.

**Table 3** Effect of Growth Regulators, Osmotica, and Salinity on the Rate of Germination (Timson index, max = 50) of Brown *Arthrocnemum indicum* Seeds

NaCl (mM)	Water	Proline	Betaine	GA	Kinetin
0 ....	27.3 ± 1.5 <sup>ab</sup>	27.4 ± 3.6 <sup>ab</sup>	31.9 ± 1.4 <sup>ab</sup>	35.8 ± 0.6 <sup>b</sup>	25.6 ± 1.3 <sup>a</sup>
200 ....	22.0 ± 2.7 <sup>a</sup>	22.4 ± 1.6 <sup>a</sup>	25.1 ± 1.9 <sup>a</sup>	30.7 ± 0.5 <sup>b</sup>	21.8 ± 0.5 <sup>a</sup>
400 ....	23.3 ± 1.3 <sup>a</sup>	16.9 ± 1.7 <sup>b</sup>	15.7 ± 1.6 <sup>b</sup>	28.9 ± 1.8 <sup>b</sup>	15.0 ± 1.3 <sup>b</sup>
600 ....	11.9 ± 1.8 <sup>a</sup>	8.6 ± 0.8 <sup>a</sup>	15.8 ± 2.1 <sup>a</sup>	15.4 ± 1.6 <sup>a</sup>	24.6 ± 2.4 <sup>b</sup>
800 ....	1.5 ± 1.1 <sup>a</sup>	4.2 ± 2.7 <sup>a</sup>	3.0 ± 0.4 <sup>a</sup>	6.2 ± 2.0 <sup>b</sup>	4.1 ± 1.2 <sup>a</sup>
1000 ....	1.0 ± 0.7 <sup>a</sup>	0 ± 0 <sup>a</sup>	0 ± 0 <sup>a</sup>	3.7 ± 0.6 <sup>b</sup>	0.3 ± 0.3 <sup>a</sup>

Note. Values in each row with the same letter are not significantly different  $P > 0.05$ ; Bonferroni test.

and Baltepe 1989). Khan and Ungar (1996a) reported that GA and kinetin were very effective in alleviating innate as well as salinity-induced dormancy in *Zygophyllum simplex* seeds. At 125 mM NaCl, a low concentration of kinetin (0.05 mM) and a high concentration of GA (3 mM) were effective in alleviating seed dormancy.

Effects of NaCl on seed germination are both ionic and osmotic (Ungar 1991; Khan and Ungar 1996c). Compatible osmotica like proline and glycinebetaine, which increase the tolerance level of plants to high levels of salinity in their tissues by acting as an osmoregulator in the cytoplasm or as an osmoprotectant of proteins (Schobert 1977; Gorham 1995), may be of significance as compatible solutes in seeds. Although Poljakoff-Mayber et al. (1994) found low levels of proline and a significant amount of betaine in dry seeds of *Kosteletzkya virginica*, external application of proline and betaine to *K. virginica* seeds in both the control and saline media had no effect on seed germination. Khan and Ungar (1996a) reported that both proline and betaine alleviated the innate dormancy of *Z. simplex* seeds, but neither was effective at high salinities. Proline application substantially promoted seed germination from 12% to 60%, while betaine promoted germination from 12% to 70% in distilled water treatments. At low salinity (25 mM NaCl), 0.1 mM proline was more effective than 1 mM betaine in alleviating inhibition of germination induced by salinity (from 10% to 50%). Higher concentrations of betaine (1 mM) only increased germination from 10% to 25%. Both proline and betaine failed to show any stimula-

tory effect on germination of *Z. simplex* at higher salinity concentrations. Our results with *A. indicum* are also in agreement with those of Poljakoff-Mayber et al. (1994) for *K. virginica*, in that proline and betaine failed to alleviate either innate dormancy or dormancy induced by salt in both the brown and black seeds.

*Arthrocnemum indicum* produces a large number of seeds at the end of fall and beginning of winter. After dispersal, seeds remain in the seed bank until the next monsoon rains reduce the ambient temperature and soil salinity and also provide much needed moisture for germination (Gul and Khan, unpublished data). However, few seeds germinate under natural conditions. Dormancy of seeds induced by salt may be caused by an inhibition of growth promoter activity. Our data indicate that the growth regulators, kinetin and GA, are capable of directly stimulating germination of seeds at higher salinities. It is possible that under hypersaline conditions there is a significant decrease in the endogenous content of growth-promoting substances in these seeds. Further investigations are necessary to determine the changes in the content of endogenous growth regulators and compatible osmotica under salt stress to explain the inability of halophyte seeds to germinate at high salinities.

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#### Literature Cited

- Boucaud J, IA Ungar 1973 The role of hormones in controlling the mechanically induced dormancy of *Suaeda* spp. *Physiol Plant* 29: 97-102.
- Bozcuk S 1981 Effects of kinetin and salinity on germination of tomato, barley and cotton seeds. *Ann Bot* 48:81-84.
- Gorham J 1995 Mechanisms of salt tolerance of halophytes. Pages 207-223 in R Chouk-Allah, CV Malcolm, A Hamdy, eds. *Halophytes and biosaline agriculture*. Marcel Dekker Inc., New York.
- Gul B 1993 Demography, ecophysiology and seed bank dynamics of *Arthrocnemum indicum*. MS thesis, University of Karachi, Karachi, Pakistan.
- Ismail AMA 1990 Germination ecophysiology in population of *Zygophyllum qatarenses* Hadidi from contrasting habitats. *J Arid Environ* 18:185-194.
- Kabar K 1987 Alleviation of salinity stress by plant growth regulators on seed germination. *J Plant Physiol* 28:179-183.
- Kabar K, S Baltepe 1989 Effect of kinetin and gibberellic acid in overcoming high temperature and salinity (NaCl) stresses on the germination of barley and lettuce seeds. *Phyton* 30:65-74.
- Karim A, SA Qadir 1979 Phytosociological studies of the coastal swamps of Karachi. *Bangladesh J Bot* 8:20-29.
- Khan MA 1991 Studies on germination of *Cressa cretica*. *Pakistan J Weed Sci Res* 4:89-98.
- Khan MA, Y Rizvi 1994 Effect of salinity, temperature, and growth regulators on the germination and early seedling growth of *Atriplex griffithii* var. *stocksii*. *Can J Bot* 72:475-479.
- Khan MA, N Sankhla, DJ Weber, ED McArthur 1987 Seed ger-

- mination characteristics of *Chrysothamnus nauseosus* ssp *viridulus* (Asteraceae, Asteraceae). *Great Basin Nat* 47:220–226.
- Khan MA, IA Ungar 1984 The effect of salinity and temperature on the germination of polymorphic seeds and growth of *Atriplex triangularis* Willd. *Am J Bot* 71:481–489.
- 1985 The role of hormones in regulating the germination of polymorphic seeds and early seedling growth of *Atriplex triangularis* Willd. under saline conditions. *Physiol Plant* 63:109–113.
- 1996a Alleviation of seed dormancy in the desert forb *Zygophyllum simplex* L. from Pakistan. *Ann Bot* 80:395–400.
- 1996b Comparative study of chloride, calcium, magnesium, potassium and sodium content of seeds in temperate and tropical halophytes. *J Plant Nutr* 19:517–525.
- 1996c Germination responses of the subtropical annual halophyte *Zygophyllum simplex*. *Seed Sci Technol* 25:83–91.
- Khan MA, DJ Weber 1986 Factors influencing seed germination in *Salicornia pacifica* var. *utahensis*. *Am J Bot* 73:1163–1167.
- Okusanya OT, IA Ungar 1983 The effects of time of seed production on the germination response of *Spergularia marina*. *Physiol Plant* 59:335–342.
- Plyler DB, TE Proseus 1996 A comparison of the seed dormancy characteristics of *Spartina patens* and *Spartina alterniflora* (Poaceae). *Am J Bot* 83:11–14.
- Poljakoff-Mayber A, GF Somers, E Werker, JL Gallagher 1994 Seeds of *Kosteletzkya virginica* (Malvaceae): their structure, germination, and salt tolerance. II. Germination and salt tolerance. *Am J Bot* 81:54–59.
- Schobert B 1977 Is there an osmotic regulatory mechanism in algae and higher plants? *J Theor Biol* 68:17–26.
- SPSS 1994 SPSS: SPSS 6.1 for windows update. SPSS, Chicago.
- Tirmizi SAS 1988 Factors influencing germination and dormancy of *Honkenya peploides* (L.) Ehrh. I. Improvement of germination. *Pak J Sci Ind Res* 31:842–847.
- Ungar IA 1977 Salinity, temperature and growth regulator effects on seed germination of *Salicornia europaea* L. *Aquatic Bot* 3:329–335.
- 1982 Germination ecology of halophytes. Pages 143–154 in DN Sen, KS Rajpurohit, eds. *Contributions to the ecology of halophytes*. Junk, The Hague.
- 1984 Alleviation of seed dormancy in *Spergularia marina*. *Bot Gaz* 145:33–36.
- 1991 *Ecophysiology of vascular halophytes*. CRC Press, Boca Raton, Fla.
- 1995 Seed bank ecology of halophytes. Pages 65–79 in MA Khan and IA Ungar, eds. *Biology of salt tolerant plants*. University of Karachi, Karachi.
- Ungar IA, P Binet 1975 Factors influencing seed dormancy in *Spergularia media* (L.) C. Presl. *Aquatic Bot* 1:45–55.
- Yaniv Z, N Lisker, F Corbineau 1995 Germination potential of *Sinapsis alba* seeds collected in Israel. *J Arid Environ* 29:293–303.