

IMPROVING SEED GERMINATION OF *SALICORNIA RUBRA* (CHENOPODIACEAE) UNDER SALINE CONDITIONS USING GERMINATION-REGULATING CHEMICALS

M. Ajmal Khan^{1,2}, Bilquees Gul^{1,2}, and Darrell J. Weber^{1,3}

ABSTRACT.—*Salicornia rubra* Nels. (Chenopodiaceae) is a salt-tolerant annual species occurring in salt playas of the Great Basin desert in western United States. It forms pioneer communities on the most saline location of an inland salt playa at Goshen, Utah. Seeds of *Salicornia rubra* are capable of germinating at 1000 mM NaCl at 25–35°C alternating temperature regime. Dormancy-regulating chemicals were evaluated for their ability to alleviate the innate and salinity-enforced dormancy in seeds of *Salicornia rubra*. Betaine, GA3, kinetin, fusicoccin, ethephon, thiourea, proline, and nitrate had no effect in alleviating primary dormancy. Increases in NaCl concentration progressively inhibited germination of *Salicornia rubra* seeds. Ethephon, fusicoccin, GA3, kinetin, thiourea, and nitrate promoted germination under low saline conditions. At high salinity fusicoccin had no effect, whereas GA3, kinetin, and ethephon substantially alleviated salinity effects. Application of these dormancy-regulating compounds could be of practical value in seeding a saline area for restoration purposes, particularly under high saline conditions.

Key words: gibberellic acid, ethephon, fusicoccin, halophytes, kinetin, seed dormancy, *Salicornia rubra*, thiourea.

Salicornia rubra Nels. (Chenopodiaceae) is a salt-tolerant annual species occurring in salt playas of the Great Basin desert of western United States (Ungar 1965, 1974). *Salicornia rubra* is found in pure stands on the most saline location of an inland salt playa at Goshen, Utah, and is associated with *S. utahensis*, *Allerrolfea occidentalis*, and *Distichlis spicata* (Khan et al. 2000). *Salicornia rubra* is capable of germinating at 1000 mM NaCl at 25–35°C alternating temperature regime, but in a previous study a decrease in temperature inhibited germination (Khan et al. 2000). Most salt marsh and salt desert halophytes have physiological dormancy (Baskin and Baskin 1998). Dormancy, which is enforced by high salt concentrations, is probably controlled by the change in balance of endogenous growth regulators. If halophytes are used as restoration plants in areas of saline soils, it is important to understand conditions that can break seed dormancy. Germination under saline conditions is stimulated by applying dormancy-relieving compounds, which counteract the negative change in growth regulator balance in seeds when they are exposed to salt stress.

Dormancy-alleviating compounds like proline, betaine, fusicoccin, GA3, kinetin, nitrate,

thiourea, and ethephon are known to alleviate the effect of salinity on the germination of halophytes (Ungar 1977, 1982, 1984, Okusanya and Ungar 1984, Khan and Ungar 1985, 1997, 1998, Khan and Weber 1986, Khan and Rizvi 1994, Gul and Weber 1998, Khan et al. 1998). Nitrogenous compounds like nitrate and thiourea stimulate seed germination of halophytes (Mayer and Poljakoff-Mayber 1975). Thiourea alleviated high salt stress or high temperature-enforced germination inhibition (Esashi et al. 1979, Bewley and Black 1994, Gul and Weber 1998, Khan et al. 1998). Nitrate ions enhanced germination in controlled experiments when combined with other factors such as alternating temperatures, chilling, light, or plant growth regulators (Vincent and Roberts 1977, Bewley and Black 1994, Gul and Weber 1998). Compatible osmotica-like proline and betaine appeared to alleviate seed dormancy in many halophytes (Poljakoff-Mayber et al. 1994, Khan and Ungar 1997, Khan et al. 1998, Gul 1999). Both proline and betaine were effective in alleviating primary dormancy in *Kotzelskya virginica*, *Arthrocnemum indicum*, and *Zygophyllum simplex* (Poljakoff-Mayber et al. 1994, Khan and Ungar 1997, Khan et al. 1998), while salinity-induced dormancy was

¹Department of Botany and Range Science, Brigham Young University, Provo, UT 84602 USA.

²Present address: Department of Botany, University of Karachi, Karachi-75270, Pakistan.

³Corresponding author.

partially alleviated in *Allenrolfea occidentalis* seeds (Gul and Weber 1998). Fusicoccin markedly stimulates germination, growth, and several physiological processes (Marre 1979, Ballio and Scalorbi 1981) and acts like cytokinins and gibberellins to affect seed germination (Marre 1979). Fusicoccin completely reversed the effect of salinity on the germination of *Zygophyllum qatarensis*, *Z. simplex*, and *Allenrolfea occidentalis* (Ismail 1990, Gul and Weber 1998). Ethylene is able to break dormancy of seeds of several species and appears to have a regulatory role in determining germination (Esashi and Leopold 1969, Gul and Weber 1998). Ethylene is formed soon after the start of imbibition and is perhaps capable of stimulating germination (Toole et al. 1964, Esashi and Leopold 1969, Ketring and Morgan 1969). Salt-enforced dormancy in *Allenrolfea occidentalis*, *Atriplex griffithii*, and *Atriplex prostrata* was partially alleviated by application of ethephon (ethylene compound; Gul and Weber 1998). The role of gibberellic acid and kinetin in breaking dormancy is well known (Bewley and Black 1985, Kabar and Beltepe 1990, Ungar 1991). GA3 and kinetin have been found to alleviate dormancy induced by salinity in a number of halophytes (Ungar 1977, Khan and Ungar 1985, 1998, Khan and Weber 1986, Kabar 1987, Khan and Rizvi 1994).

Maintaining salt playa areas generally requires seed germination of the halophytes. Often this involves breaking dormancy in the presence of high salinity. It is of interest to evaluate the relationship between dormancy-regulating compounds and salinity in maintaining the salt playa or restoring saline areas with halophytes. The present study was designed to determine the role of dormancy-regulating chemicals in alleviating the innate and salinity-enforced dormancy in *Salicornia rubra*.

METHODS

During fall 1994 we collected seeds of *Salicornia rubra* from salt flats situated at Goshen, Utah, USA. Seeds were separated from the inflorescence and stored at 4°C. They were surface sterilized using the fungicide Phygon (2,3 dichloro-1,4-naphthoquinone). Germination was carried out in 50 × 9-mm tight-fitting plastic petri dishes with 5 mL of test solution. As an added precaution against loss of water by evaporation, we placed each dish in a 10-cm-

diameter plastic petri dish. Four replicates of 25 seeds each were used for each treatment. When the radicle emerged (2 mm), we considered seeds to be germinated.

Seeds were germinated in a growth chamber at an alternating temperature regime of 25–35°C, where the higher temperature coincided with the 12-hour light period (cool white fluorescent lamps, 110 μmol photons m⁻² · s⁻¹, 400–750 nm) and the lower temperature coincided with the 12-hour dark period. Nitrate concentrations of 20 mM, thiourea concentration of 10 mM, ethephon concentration of 10 mM, fusicoccin concentration of 5 μM, gibberellic acid concentration of 3 mM, kinetin concentration of 0.05 mM, and NaCl concentration of 0, 300 (–1.38 MPa), 600 (–2.76 MPa), and 900 (–4.14 MPa) mM were used. Percent germination was recorded every alternate day for 20 days. We estimated the rate of germination by using a modified Timson index of germination velocity = $\Sigma G/t$, where G is percentage of seed germination at 2-day 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. We used an ANOVA analysis to determine if significant differences were present among means and a Bonferroni test to determine if significant ($P < 0.05$) differences occurred between individual treatments (SPSS 1996).

RESULTS

In distilled water, 66% of the seeds germinated. Proline, betaine, GA3, kinetin, thiourea, nitrate, fusicoccin, and ethephon had no effect in alleviating primary dormancy of the seeds (Table 1). Fusicoccin treatment increased the germination rate and produced up to 80% germination compared to 66% in distilled water ($P > 0.05$; Table 2).

Increase in NaCl concentration in the medium progressively inhibited germination of *Salicornia rubra* seeds. A maximum of 66% germination was obtained in distilled water, and no seed germinated at 900 mM NaCl (Table 1). Inclusion of ethephon partially alleviated ($P < 0.01$) salinity-enforced germination inhibition (Table 1). Fusicoccin alleviated

TABLE 1. Percentage of *Salicornia rubra* seeds that germinated under various salinities and germination-regulating chemicals. Values in the same column (i.e., each dormancy-regulating chemical) followed by the same letter are not significantly different ($P > 0.05$) from each other (Bonferroni test).

NaCl (mM)	Germination-regulating chemicals								
	Water	Proline	Betaine	GA3	Kinetin	Thiourea	Nitrate	Fusicoccin	Ethephon
0	66 ± 4.1 ^a	62 ± 6.1 ^a	52 ± 3.9 ^a	63 ± 2.3 ^a	62 ± 4.0 ^a	74 ± 6.0 ^a	65 ± 4.0 ^a	80 ± 4.0 ^a	72 ± 5.9 ^a
300	22 ± 2.1 ^b	30 ± 3.1 ^b	25 ± 1.9 ^b	28 ± 2.4 ^b	41 ± 3.6 ^b	60 ± 3.9 ^b	36 ± 3.2 ^b	38 ± 3.0 ^b	40 ± 7.1 ^b
600	4 ± 1.9 ^c	10 ± 3.2 ^c	5 ± 0.9 ^c	34 ± 6.4 ^c	33 ± 6.4 ^c	8 ± 2.0 ^c	13 ± 4.0 ^c	17 ± 5.6 ^c	16 ± 2.8 ^c
900	0 ± 0 ^d	4 ± 0.8 ^d	3 ± 0.8 ^c	8 ± 4.1 ^c	8 ± 4.1 ^d	7 ± 0.9 ^c	5 ± 2.7 ^d	0 ± 0 ^d	14 ± 1.0 ^c

TABLE 2. Rate of seed germination of *Salicornia rubra* seeds under various salinities and germination-regulating chemicals. Values in the same column (i.e., each dormancy-regulating chemical) followed by the same letter are not significantly different ($P > 0.05$) from each other (Bonferroni test).

NaCl (mM)	Germination-regulating chemicals								
	Water	Proline	Betaine	GA3	Kinetin	Thiourea	Nitrate	Fusicoccin	Ethephon
0	27 ± 2.2 ^a	24 ± 3.5 ^a	29 ± 1.3 ^a	29 ± 1.52 ^a	32 ± 2.8 ^a	28 ± 1.8 ^a	36 ± 2.7 ^a	33 ± 3.0 ^a	29 ± 1.9 ^a
300	13 ± 2.3 ^b	12 ± 0.7 ^b	17 ± 0.9 ^b	18 ± 1.4 ^b	25 ± 1.9 ^b	17 ± 5.9 ^b	16 ± 1.2 ^b	18 ± 5.3 ^b	10 ± 1.1 ^b
600	5 ± 1.4 ^c	3 ± 0.5 ^c	15 ± 3.4 ^b	15 ± 3.4 ^b	4 ± 0.7 ^c	6 ± 2.0 ^c	8 ± 2.4 ^c	8 ± 1.3 ^c	1.9 ± 0.7 ^c
900	2 ± 0.7 ^d	2 ± 0.5 ^d	4 ± 1.8 ^c	4 ± 1.5 ^c	3 ± 0.3 ^c	2 ± 1.2 ^d	0 ± 0 ^d	7 ± 0.6 ^c	0 ± 0 ^d

($P < 0.01$) seed germination at low NaCl treatment (Table 1). Gibberellic acid and kinetin both were ineffective in promoting germination in nonsaline treatments, while both compounds significantly ($P < 0.01$) alleviated salinity effects on germination (Table 1). Nitrogenous compounds (thiourea and nitrate) promoted germination under low saline conditions. At 300 mM NaCl treatment, application of thiourea substantially alleviated germination from 22% in control to about 60%. In the presence of nitrate and thiourea, some germination was reported at 900 mM NaCl (Table 1). Proline and betaine did not alter germination under saline and nonsaline conditions (Table 1). At low salinity (300 mM NaCl) all the chemicals promoted the rate of germination as compared to high salinity (Table 2).

DISCUSSION

Salicornia rubra grows in the most saline region of inland salt playas, but seed germination in natural conditions takes place during the early spring when spring rains and runoff from mountains considerably reduce playa salinity. Salinity progressively increases during late spring and early summer, and few seeds germinate during these periods. Seeds

of *S. rubra* showed a partial dormancy (66% germination) in nonsaline control under laboratory conditions. This dormancy could not be alleviated with the application of proline, betaine, gibberellic acid, kinetin, ethylene, thiourea, and nitrate, while fusicoccin had some promoting effect. Seed germination in *S. rubra* was inhibited with an increase in salinity, and no seed germinated at 900 mM NaCl. Fusicoccin, ethephon, GA3, kinetin, nitrate, and thiourea partially alleviated the inhibitory effects of salinity on germination whereas proline or betaine showed little effect.

Compatible osmotic-like proline and betaine are reported to partially alleviate innate dormancy in *Zygophyllum simplex*, *Atriplex griffithii*, *Halopyrum mucronatum*, and *Arthrocnemum indicum* (Khan and Ungar 1997, Khan et al. 1998, Khan and Ungar unpublished data). Khan et al. (1998) showed that proline and betaine did not relieve salinity-induced dormancy in *Arthrocnemum indicum*. Similar results are reported for *Kosteletzkya virginica* and *Halopyrum mucronatum* (Poljakoff-Mayber et al. 1994). Both proline and betaine alleviated the innate dormancy of *Zygophyllum simplex* seeds, but neither was effective at high salinities (Khan and Ungar 1996). Gul (1998), however, reported that proline and

betaine both alleviated high salinity effects in *Allenrolfea occidentalis*. Our results with *S. rubra* showed that proline and betaine failed to alleviate both innate and salinity-induced dormancy.

Seed dormancy enforced by low salinity was partially alleviated by fusicoccin in *S. rubra*, and there was no significant effect on high salinity treatments. This alleviation may be due to the stimulation of ATPase during the early phases of germination to facilitate proton extrusion and K⁺ uptake (Marre 1979, Stout 1988). A stimulation of germination by fusicoccin in various kinds of seeds has been observed (Lado et al. 1974, Ismail 1990, Gul and Weber 1998). Fusicoccin has the ability to remove the inhibitory effect of abscisic acid on germination of normal seeds and on embryo growth of decoated seeds (Lado et al. 1975). It is more likely that abscisic acid production due to salinity stress could be counteracted by fusicoccin and thus alleviate the inhibitory effect of salinity.

Dormancy in seeds of numerous species is reported to be relieved by applying ethephon (Ketring 1977, Bewley and Black 1985), which reverses the inhibitory effect of abscisic acid and osmotic stress (Karssen 1976, Schonbeck and Egley 1981). Applying ethephon to *S. rubra* seeds stimulated germination under nonsaline and saline conditions.

Thiourea and nitrate both stimulated the germination of *S. rubra* seeds under saline conditions. The alleviating effect of thiourea on osmoinhibition gradually decreases with an increase in salinity. *Salicornia rubra* showed a full recovery from the low salinity effects and partial alleviation of the inhibitory effect of high salinities. GA3 and kinetin both alleviated the salinity effect on the germination of *S. rubra* seeds. GA3 and kinetin are known to alleviate the salinity effect in some halophytic seeds (Khan and Ungar 1996, 2000, Khan et al. 1998), while it was ineffective in other halophytes such as *Suaeda fruticosa* and *Haloxylon recurvum* (Khan and Ungar unpublished data).

Salicornia rubra produces numerous seeds at the end of autumn and beginning of winter. Most of the seeds (65%) readily germinate if proper conditions are provided. Seeds in their natural environment are prevented from germinating due to very cold temperatures. Seeds begin germinating very early during spring, but germination decreases with increased

salinity. For restoration of saline areas, seeds of *S. Rubra* could be scattered prior to spring rains. In highly saline soil areas, treatment of the seeds with GA3 or kinetin prior to scattering would increase the germination rate.

LITERATURE CITED

- BALLIO, A., AND D. SCALORBI. 1981. Fusicoccin structure-activity relationships: *in vitro* binding to microsomal preparations of maize coleoptiles. *Plant Physiology* 52:476-481.
- BASKIN, J.M., AND C.C. BASKIN. 1998. Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, New York.
- BEWLEY, J.D., AND M. BLACK. 1985. Physiology and biochemistry of seeds. Springer-Verlag, Berlin.
- _____. 1994. Seeds: physiology of development and germination. Plenum Press, New York.
- ESASHI, Y., AND A.C. LEOPOLD. 1969. Dormancy regulation in subterranean clover seeds by ethylene. *Plant Physiology* 44:1470-1472.
- ESASHI, Y., Y. OHARA, M. OKAZAKI, AND K. HISHINUMA. 1979. Control of cocklebur seed germination by nitrogenous compounds: nitrite, nitrate, hydroxylamine, thiourea, azide, and cyanide. *Plant and Cell Physiology* 20:349-361.
- GUL, B. 1999. Ecophysiology and population biology of the perennial halophytic shrub *Allenrolfea occidentalis* (S. Wats.) Kuntze (Chenopodiaceae) growing in a salt playa near Goshen, northwestern, Utah, USA. In: E. Durant McArthur, W. Kent Ostler, Carl L. Wambolt, editors, Proceedings: shrubland ecotones, 12-14 August 1998, Ephraim, UT. RMRS-P-000, USDA, Forest Service, Rocky Mountain Research Station, Ogden, UT.
- GUL, B., AND D.J. WEBER. 1998. Effect of dormancy compounds on the seed germination of non-dormant *Allenrolfea occidentalis* under salinity stress. *Annals of Botany* 82:555-560.
- ISMAIL, A.M.A. 1990. Germination ecophysiology in population of *Zygophyllum qatarenses* Hadidi from contrasting habitats. *Journal of Arid Environments* 18: 185-194.
- KABAR, K. 1987. Alleviation of salinity stress by plant growth regulators on seed germination. *Journal of Plant Physiology* 128:179-183.
- KABAR, K., AND S. BELTEPE. 1990. 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.
- KARSEN, C.M. 1976. Two sites of hormonal action during germination of *Chenopodium album* seeds. *Physiologia Plantarum* 36:264-270.
- KETRING, D.L. 1977. Ethylene and seed germination. Pages 157-178 in A.A. Khan, editor, The physiology and biochemistry of seed dormancy and germination. North Holland Publishing Co., Amsterdam.
- KETRING, D.L., AND P.W. MORGAN. 1969. Ethylene as a component of the emanations from germinating peanut seeds and its effect on dormant Virginia-type seeds. *Plant Physiology* 44:326-330.
- KHAN, M.A., B. GUL, AND D.J. WEBER. 2000. Germination responses of *Salicornia rubra* to temperature and salinity. *Journal of Arid Environments* 45:207-214.

- KHAN, M.A., AND Y. RIZVI. 1994. Effect of salinity, temperature, and growth regulators on the germination and early seedling growth of *Atriplex griffithii* var. *stocksii*. Canadian Journal of Botany 72:475-479.
- KHAN, M.A., AND I.A. UNGAR. 1985. The role of hormones in regulating the germination of polymorphic seeds and early seedling growth of *Atriplex triangularis* Willd. under saline conditions. Physiologia Plantarum 63:109-113.
- _____. 1996. Influence of salinity and temperature on the germination of *Haloxylon recurvum*. Annals of Botany 78:547-551.
- _____. 1997. Alleviation of seed dormancy in the desert forb *Zygophyllum simplex* L. from Pakistan. Annals of Botany 80:395-400.
- _____. 1998. Seed germination and dormancy of *Polygonum aviculare* L. as influenced by salinity, temperature, and gibberellic acid. Seed Science and Technology 26:107-117.
- _____. 2000. Alleviation of salinity-enforced dormancy in *Atriplex griffithii* Moq. var. *stocksii* Boiss. Seed Science and Technology 28:29-37.
- KHAN, M.A., I.A. UNGAR, AND B. GUL. 1998. Action of osmotica and growth regulators in alleviating the effect of salinity on the germination of dimorphic seeds of *Arthrocnemum indicum* L. International Journal of Plant Science 159:313-317.
- KHAN, M.A., AND D.J. WEBER. 1986. Factors influencing seed germination in *Salicornia pacifica* var. *utahensis*. American Journal of Botany 73:1163-1167.
- LADO, P., F. RASI-CALDOGNO, AND R. COLOMBO. 1974. Promoting effect of fusicoccin on seed germination. Physiologia Plantarum 31:149-152.
- _____. 1975. Promoting effect of fusicoccin on seed germination. Physiologia Plantarum 34:359-364.
- MARRE, E. 1979. Fusicoccin: a tool in plant physiology. Annual Review of Plant Physiology 30:273-278.
- MAYER, A.M., AND A. POLJAKOFF-MAYBER. 1975. Germination of seeds. Pergamon Press, New York.
- OKUSANYA, O.T., AND I.A. UNGAR. 1984. The effects of time of seed production on the germination response of *Spergularia marina*. Physiologia Plantarum 59:335-342.
- POLJAKOFF-MAYBER, A., G.G. SOMERS, E. WERKER, AND J.L. GALLAGHER. 1994. Seeds of *Kosteletzkya virginica* (Malvaceae): their structure, germination and salt tolerance, II. Germination and salt tolerance. American Journal of Botany 81:54-59.
- SCHONBECK, M.W., AND G.H. EGLEY. 1981. Phase-sequence of redroot pigweed seed germination responses to ethylene and other stimuli. Plant Physiology 68:175-179.
- SPSS, INC. 1996. SPSS: SPSS 7.0 for Windows update. SPSS Inc., USA.
- STOUT, R.G. 1988. Fusicoccin activity and building in *Ara-bidopsis thaliana*. Plant Physiology 88:999-1001.
- TOOLE, V.K., W.K. BAILEY, AND E.H. TOOLE. 1964. Factors influencing dormancy of peanut seeds. Plant Physiology 39:822-832.
- UNGAR, I.A. 1965. An ecological study of the vegetation of Big Salt Marsh, Stafford County, Kansas. University of Kansas Science Bulletin 46:1-98.
- _____. 1974. Inland halophytes of the United States. Pages 235-305 in R. Reimold and W. Queen, editors, Ecology of halophytes. Academic Press, New York.
- _____. 1977. Salinity, temperature, and growth regulator effects on seed germination of *Salicornia europaea* L. Aquatic Botany 3:329-335.
- _____. 1982. Germination ecology of halophytes. Pages 143-154 in D.N. Sen and K.S. Rajpurohit, editors, Contribution to the ecology of halophytes. Junk, The Hague, Netherlands.
- _____. 1984. Alleviation of seed dormancy in *Spergularia marina*. Botanical Gazette 145:33-36.
- _____. 1991. Ecophysiology of vascular halophytes. CRC Press, Boca Raton, FL.
- VINCENT, E.M., AND E.H. ROBERTS. 1977. The interaction of light, nitrate, and alternating temperature in promoting germination of dormant seeds of common weed species. Seed Science and Technology 5:659-670.

Received 6 March 2000
Accepted 1 November 2000