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

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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, Allenrolfea 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 *Kotzeltskya virginica, Arthrocnemum indicum,* and *Zygophyllum simplex* (Poljakoff-Mayber et al. 1994, Khan and Ungar 1997, Khan et al. 1998), while salinity-induced dormancy was

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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 $m^{-2} \cdot s^{-1}$, 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 (–1.38MPa), 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 = $\sum G/t$, 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).

	Germination-regulating chemicals								
NaCl (mM)	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^b	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).

	Germination-regulating chemicals								
NaCl (mM)	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 osmotica-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.

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