

Alleviation of salinity-induced dormancy in perennial grasses

S. GULZAR* and M.A. KHAN**

Department of Botany, University of Karachi, Karachi-75270, Pakistan

Abstract

All seeds of *Aeluropus lagopoides* and *Urochondra setulosa* germinated under non-saline conditions except for *Sporobolus ioclados* which showed only 40 % germination. Increase in salinity substantially inhibited germination and few seeds germinated at 400 mM NaCl. Germination at 200 mM NaCl was alleviated in *U. setulosa* by the application of gibberellic acid and fusicoccin, in *A. lagopoides* by thiourea, betaine, kinetin, fusicoccin and ethephon, and in *S. ioclados* by gibberellin and ethephon. High salinity (400 mM NaCl) induced germination inhibition was alleviated by proline, kinetin, fusicoccin and ethephon only in *A. lagopoides*.

Additional key words: *Aeluropus lagopoides*, betaine, ethephon, fusicoccin, halophytic grasses, kinetin, nitrate, proline, *Sporobolus ioclados*, thiourea, *Urochondra setulosa*.

Seed germination of halophytic grasses is usually prevented by physiological causes induced by availability of less than optimal environmental factors like water, oxygen, light, temperature, and soil salinity (Ungar 1995). These conditions usually cause the imbalance of growth regulators resulting in increased levels of ABA and other germination inhibitors, and a decrease in endogenous growth promoters (Bewley and Black 1994). Germination regulating chemicals like ethephon, fusicoccin, nitrate, thiourea, gibberellic acid (GA₃), kinetin, proline, and betaine are reported to release dormancy imposed by such factors (Khan and Ungar 2001abc). However, the effect of these chemicals varies from one species to another (Bewley and Black 1994, Ismail 1990, Gul *et al.* 2000, Khan and Ungar 2001abc).

Grasses (*Aeluropus lagopoides*, *Sporobolus ioclados* and *Urochondra setulosa*) are highly salt tolerant at germination and some seeds germinated in up to 500 mM NaCl (Gulzar and Khan 2001, Gulzar *et al.* 2001, and unpublished data) at 20/30 °C. The present study was carried out to study the effects of germination regulating chemicals in alleviating the salinity-induced dormancy of three perennial grasses.

Inflorescence of *Urochondra setulosa* were collected from Sands pit along the Arabian Sea, *Aeluropus lagopoides*

and *Sporobolus ioclados* from their populations at the Karachi University campus. Seeds were separated from inflorescence, cleaned and dry stored at room temperature after surface sterilization with 0.85 % Clorox (sodium hypochlorite) for 1 min. NaCl concentrations of 0, 100, 200, and 400 mM (Gulzar and Khan 2001, Gulzar *et al.* 2001) and thiourea (10 mM), nitrate (20 mM), proline (0.1 mM), betaine (0.1 mM), gibberellin (3 mM), kinetin (0.05 mM) fusicoccin (5 µM) and ethephon (10 mM) were used. Germination was tested in a programmed incubator (*Percival*, Boone, USA) at (dark/light) temperature 20/30 °C (the optimum for all species studied) with a 12-h photoperiod (*Sylvania* cool white fluorescent lamps, irradiance of 25 µmol m⁻² s⁻¹, 400-700 nm). Twenty five seeds each were germinated in folded *Whatman No.1* filter paper placed in 2.5 cm × 18 cm glass test tubes with 5 cm³ of test solution. The tubes were sealed using parafilm. Four test tubes with 25 seeds each were used for each treatment. A seed was considered to have germinated at the emergence of the radicle (Bewley and Black 1994). Germination data (after 20 d) were arcsine transformed and were analyzed using SPSS, version 9.0. The differences among means were examined using a three-way ANOVA and Bonferroni post-hoc test was used to test for significant differences between individual treatments.

Received 18 July 2001, accepted 5 October 2001.

Abbreviations: ABA - abscisic acid; DW - distilled water; FC - fusicoccin; GA₃ - gibberellic acid.

*Current address: Department of Botany, Government Superior Science College, Shah Faisal Colony, Karachi-75230, Pakistan.

**Corresponding author; fax: (+92) 21 9243976, e-mail: ajmal@botany.ku.edu.pk

Three way ANOVA of percentage germination indicated significant ($P < 0.05$) main effects of species, salinity, dormancy relieving compounds, and their interactions. All *A. lagopoides* seed germinated in non-saline control and the germination decreased with the increase in salinity and few seeds germinated at 400 mM NaCl (Table 1). Inclusion of thiourea, proline, betaine, kinetin and fusicoccin partially alleviated the effect of salinity, whereas ethephon completely reversed the inhibition caused by NaCl (Table 1). Nitrate and gibberellic acid had no effect on germination (Table 1).

Table 1. Effect of growth regulators (thiourea, nitrate, proline, betaine, gibberellic acid, kinetin, fusicoccin and ethephon) on the germination of *Aeluropus lagopoides* seeds in different salinity (0, 100, 200 and 400 mM NaCl) treatments. Different letters in superscript represent significant ($P < 0.05$) differences between salinity treatments, Bonferroni test.

NaCl [mM]	DW	Thiourea	Nitrate	Proline	Betaine	GA ₃	Kinetin	FC	Ethephon
0	100	100	100	100	100	100	100	100	100
100	94 ± 4.76a	100 ± 0a	97 ± 1.91a	91 ± 3.42a	81 ± 5.00a	92 ± 4.90a	90 ± 6.63a	100 ± 0a	100 ± 0a
200	59 ± 8.70b	93 ± 7.00a	66 ± 7.57b	61 ± 10.4b	33 ± 7.90b	70 ± 6.22b	99 ± 1.00a	82 ± 11.5ab	100 ± 0a
400	6 ± 2.58c	20 ± 10.5b	12 ± 8.49c	47 ± 7.55b	6 ± 2.58c	9 ± 4.43c	30 ± 10.6b	47 ± 20.0b	94 ± 3.46a

Table 2. Effect of growth regulators (thiourea, nitrate, proline, betaine, gibberellic acid, kinetin, fusicoccin and ethephon) on the germination of *Sporobolus ioclados* seeds in different salinity (0, 100, 200 and 400 mM NaCl) treatments. Different letters in superscript represent significant ($P < 0.05$) differences between salinity treatments, Bonferroni test.

NaCl [mM]	DW	Thiourea	Nitrate	Proline	Betaine	GA ₃	Kinetin	FC	Ethephon
0	28 ± 12.3a	40 ± 8.33a	36 ± 13.0a	20 ± 8.64a	8 ± 1.63a	35 ± 4.12a	28 ± 10.6a	27 ± 8.23a	35 ± 10.1a
100	14 ± 4.16a	21 ± 6.81ab	19 ± 5.26a	8 ± 1.63a	6 ± 2.58a	28 ± 4.32a	17 ± 5.26a	4 ± 1.63a	36 ± 4.32a
200	7 ± 2.52a	7 ± 1.91b	5 ± 1.91a	3 ± 1.91a	4 ± 1.63a	21 ± 6.61ab	11 ± 3.00a	2 ± 1.15a	28 ± 6.32a
400	2 ± 1.15a	5 ± 2.52b	7 ± 4.43a	1 ± 1.00a	0 ± 0a	5 ± 2.52b	4 ± 1.63a	0 ± 0a	10 ± 3.46a

Table 3. Effect of growth regulators (thiourea, nitrate, proline, betaine, gibberellic acid, kinetin, fusicoccin and ethephon) on the germination of *Urochondra setulosa* seeds in different salinity (0, 100, 200 and 400 mM NaCl) treatments. Different letters in superscript represent significant ($P < 0.05$) differences between salinity treatments, Bonferroni test.

NaCl [mM]	DW	Thiourea	Nitrate	Proline	Betaine	GA ₃	Kinetin	FC	Ethephon
0	98 ± 2.00a	95 ± 5.00a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a	100 ± 0a
100	69 ± 17.16a	61 ± 12.37b	91 ± 3.42a	69 ± 3.42b	56 ± 5.89b	87 ± 7.55a	66 ± 13.7b	77 ± 9.00ab	94 ± 6.00a
200	22 ± 6.63b	10 ± 2.58c	30 ± 4.76b	24 ± 7.48c	19 ± 3.00c	45 ± 3.00b	14 ± 6.00c	55 ± 8.23b	26 ± 2.58b
400	3 ± 1.00b	0 ± 0c	1 ± 1.00c	3 ± 1.91c	2 ± 2.00d	1 ± 1.00c	4 ± 1.63c	7 ± 1.91c	4 ± 1.63c

Gibberellic acid had no effect on *A. lagopoides* but alleviated effects of salinity on the germination of *S. ioclados* and *U. setulosa*. Gibberellic acid was ineffective in breaking the seed dormancy of some salt marsh grass species (Amen *et al.* 1970, Khan and Ungar 2001ab) whereas, application of GA₃ had alleviated salinity induced seed dormancy in *Halopyrum mucronatum* (Khan and Ungar 2001c). Kinetin is reported to alleviate salinity effect on the germination of several grass halophytes (Khan and Ungar 2001abc), however, not of *Sporobolus arabicus* (Khan and Ungar

2001b). Kinetin was able to partially alleviate salinity effects on the germination of *A. lagopoides* and had no effect on the seeds of *S. ioclados* and *U. setulosa*. Proline and betaine did not stimulate germination of any grass seed studied. Both proline and betaine alleviated the effects of low salinity and innate dormancy of *Zygophyllum simplex* and *Atriplex griffithii* seeds but neither was effective at high salinities (Khan and Ungar 1997, 2000).

Thiourea significantly alleviated the inhibitory effects of salinity in the seeds of *A. lagopoides*, while nitrate had

no effect. Thiourea could stimulate seed germination by alleviating the negative effects of salinity on germination (Esashi *et al.* 1979, Gul *et al.* 2000). The inhibitory effect of salinity on *H. mucronatum* seeds was partially alleviated by nitrate and thiourea (Khan and Ungar 2001c). Khan and Ungar (1997) determined that both thiourea and nitrate significantly improved germination of *Z. simplex* seeds at low salinities.

Seed dormancy enforced by salinity was partially alleviated by FC in *A. lagopoides* and *U. setulosa*, and this may be due to stimulation of ATPase production and the ATPase rapidly increases during the early phases of germination to facilitate proton extrusion and K⁺ uptake (Stout 1998). A stimulation of germination by FC in various kinds of seeds has been observed (Ismail 1990, Gul *et al.* 2000, Khan and Ungar 2000, 2001abc). Application of ethephon relieves dormancy in seeds of

several species (Ketring 1977, Bewley and Black 1994, Khan and Ungar 2001abc) and reverses the inhibitory effect of abscisic acid and osmotic stress (Karsen 1976, Schönbeck and Egley 1981). The presence of ethephon completely reversed the effect of salinity on the seed germination of *A. lagopoides* but had little effect on other grasses.

Seeds from the *A. lagopoides*, *S. ioclados* and *U. setulosa* exhibited physiological differences in germination response. Differences in the physiological requirements of different grass species may provide alternative temporal and spatial conditions for germination and recruitment in its stressful and unpredictable coastal environment. However, the biochemical and physiological basis of sensitivity of various chemicals during germination needs to be studied in detail.

References

Amen, R.D., Carter, G.E., Kelly, R.J.: The nature of seed dormancy and germination in the salt marsh grass *Distichlis spicata*. - *New Phytol.* **69**: 1005-1013, 1970.

Bewley, J.D., Black, M.: *Seeds: Physiology of Development and Germination*. - Plenum Press, London 1994.

Esashi, Y., Ohara, Y., Okazaki, M., Hishinuma, K.: Control of cocklebur seed germination by nitrogenous compounds: Nitrite, nitrate, hydroxylamine, thiourea, azide, and cyanide. - *Plant Cell Physiol.* **20**: 349-361, 1979.

Gul, B., Khan, M.A., Weber, D.J.: Alleviation of salinity and dark-enforced dormancy in *Allenrolfea occidentalis* seed under various thermoperiods. - *Aust. J. Bot.* **48**: 745-752, 2000.

Gulzar, S., Khan, M.A.: Seed germination of a halophytic grass *Aeluropus lagopoides*. - *Ann. Bot.* **87**: 319-324, 2001.

Gulzar, S., Khan, M.A., Ungar, I.A.: Effect of temperature and salinity on the germination of *Urochondra setulosa*. - *Seed Sci. Technol.* **29**: 21-29, 2001.

Ismail, A.M.A.: Germination ecophysiology in population of *Zygophyllum qatarense* Hadidi from contrasting habitats. - *J. arid Environ.* **18**: 185-194, 1990.

Karssen, C.M.: Two sites of hormonal action during germination of *Chenopodium album* seeds. - *Physiol. Plant.* **36**: 264-270, 1976.

Ketring, D.L.: Ethylene and seed germination. - In: Khan, A.A. (ed.): *The Physiology and Biochemistry of Seed Dormancy and Germination*. Pp. 157-178. North-Holland Publishing Company, Amsterdam 1977.

Khan, M.A., Ungar, I.A.: Alleviation of seed dormancy in the desert forb *Zygophyllum simplex* L. from Pakistan. - *Ann. Bot.* **80**: 395-400, 1997.

Khan, M.A., Ungar, I.A.: Alleviation of salinity enforced dormancy in *Atriplex griffithii* Moq. var. *stocksii* Boiss. - *Seed Sci. Technol.* **28**: 29-37, 2000.

Khan, M.A., Ungar, I.A.: Effect of dormancy regulating chemicals on the germination of *Triglochin maritima*. - *Biol. Plant.* **44**: 301-303, 2001a.

Khan, M.A., Ungar, I.A.: Role of dormancy regulating chemicals in release of innate and salinity-induced dormancy in *Sporobolus arabicus* Boiss. - *Seed Sci. Technol.* **29**: 299-306, 2001b.

Khan, M.A., Ungar, I.A.: Alleviation of salinity stress and the response to temperature in two seed morphs of *Halopyrum mucronatum* (Poaceae). - *Aust. J. Bot.* **49**: in press, 2001c.

Schönbeck, M.W., Egley, G.H.: Phase-sequence of red-root pigweed germination responses to ethylene and other stimuli. - *Plant Physiol.* **68**: 175-179, 1981.

Stout, R.G.: Fusicoccin activity and building in *Arabidopsis thaliana*. - *Plant Physiol.* **88**: 999-1001, 1998.

Ungar, I.A.: Seed germination and seed-bank ecology of halophytes. - In: Kigel, J., Galili, G. (ed.): *Seed Development and Seed Germination*. Pp. 599-628. Marcel Dekker, New York 1995.