

ALLEVIATION OF SALINITY-ENFORCED SEED DORMANCY IN *ATRIPLEX PROSTRATA*

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Abstract

Atriplex prostrata (Chenopodiaceae) is a salt tolerant plant species that is widely distributed in inland and coastal salt marshes of the United States and Europe. *Atriplex prostrata* (Chenopodiaceae) seed germination decreased with an increase in salinity and few seeds germinated at 300 mM NaCl. Fusicoccin (5 mM), ethephon (10 mM), and nitrogenous compounds (nitrate 20 mM and thiourea 10 mM) were able to counteract the inhibition produced by salinity treatments. Thiourea was most effective in reversing the inhibitory effects of salinity on the germination of *A. prostrata* seeds.

Introduction

Plant growth regulators such as gibberellic acid (GA₃), abscisic acid (ABA), kinetin and ethylene are known to influence the dormancy status of seeds (Karssen, 1995). The effect of dormancy-regulating chemicals on the germination of halophytes has been studied mainly in relation to their response to salinity (Ungar, 1991). Changes in growth regulator balance that are induced by salt stress may be a mechanism that induces dormancy in seeds (Ungar, 1978, 1984). The application of GA₃ (Khan, 1991; Khan & Ungar, 1997), kinetin (Khan & Ungar, 1985, 1997) fusicoccin (FC) (Ismail, 1990; Gul & Weber, 1998), and ethylene (Kepczynski & Karssen, 1985; Kepczynski, 1986; Ismail, 1990) were shown to alleviate salinity-enforced dormancy. Nitrogenous compounds such as nitrate and thiourea are also reported to promote germination (Bewley & Black, 1994).

Kinetin is reported to promote germination of *A. prostrata* seeds at all salinities and application of GA₃ was somewhat stimulatory (Ungar, 1978). Seeds of *A. prostrata* could be inhibited by the presence of phenolic compounds but their effects, except for that of salicylic acid, were completely alleviated by the application of GA₃ and kinetin (Khan & Ungar, 1986). The present investigation is designed to determine if the application of dormancy-relieving compounds could alleviate the innate and enforced dormancies of *Atriplex prostrata* seeds under saline conditions.

Materials and Methods

Seeds of *Atriplex prostrata* were collected from approximately 100 plants in a 0.1 hectare area, during August 1995, from a brine spill location in Athens County, Ohio, U.S.A. (39°20' N and 81°57' W) and stored at 4 °C. Germination studies were started in September 1995. Seeds were surface sterilized using the fungicide Phygon and germination carried out in 50 x 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 to prevent loss of water by evaporation. Four replicates of 25 seeds every were used for every treatment and seeds considered germinated when the radicle emerged.

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Seeds were germinated in an incubator at a day/night temperature regime of 12h- 25 °C and 12h-5 °C, with a 12-h light period (Sylvania cool white fluorescent lamps, 25 μ mol.m⁻². s⁻¹, 400 - 700 nm). Concentrations of 20 mM nitrate, 10 mM Thiourea 10 mM ethephon, 5 μ M fusicoccin, and 0, 100, 200, and 300 mM NaCl were used. Percent germination was recorded every alternate day for 20 days. Rate of germination was estimated using a modified Timson index of germination velocity = $\Sigma G/t$, where G is the percentage of seed germination at 2-days' intervals, and t is total germination period (Khan & Ungar, 1984). The maximum value possible using this index with our data was 50 (*i.e.* 1000/20) and the higher the value, the more rapid was the rate of germination.

Germination data were arcsine transformed before statistical analysis. The analysis of variance (ANOVA) was used to determine, if significant differences were present among means. When significant differences were found among means, a Bonferroni post-hoc test was carried out to determine if significant ($P < 0.05$) differences occurred between individual treatments (SPSS, 1996).

Results

A one-way ANOVA of the germination responses at each salinity level revealed that most of the dormancy relieving compounds significantly increased seed germination at 200 mM NaCl ($F = 23.4$, $P < 0.001$) and 300 mM NaCl ($F = 38.3$, $P < 0.0001$). Germination of seeds was inhibited with the addition of greater than 100 mM NaCl and only 28 % of the seeds germinated in the 300 mM NaCl treatment compared to 79 % in the distilled water controls (Fig. 1). All of the dormancy relieving compounds at least partially alleviated the inhibitory effect of salinity on germination in 300 mM NaCl (Fig. 1). However, thiourea was the most effective in promoting germination in 300 mM NaCl, increasing it from 18 % in the controls to about 60 % in thiourea treatments. Fusicoccin stimulated 45 % of the seeds to germinate in the 300 mM NaCl treatment (Fig. 1).

A one-way ANOVA for each salinity level revealed that dormancy relieving compounds significantly increased the rate of seed germination at 200 mM NaCl ($F = 23.4$, $P < 0.001$) and 300 mM NaCl ($F = 18.8$, $P < 0.0001$). There was no significant difference in the rate of germination by the application of dormancy relieving compounds in the non-saline and 100 mM NaCl treatments (Fig. 2). At 200 mM NaCl, both thiourea and nitrate significantly alleviated the inhibitory effects of salinity on the rate of germination while at 300 mM NaCl all of the dormancy relieving compounds significantly increased the rate of germination (Fig. 2).

Discussion

Atriplex prostrata grows in a brine-contaminated site in southeastern Ohio, where soil specific conductance varied from 10-40 dS. m⁻¹. Seeds of *A. prostrata* do not require stratification and readily germinate in distilled water under laboratory conditions (Khan & Ungar, 1984). However, our data indicate that increases in medium salinity enforced dormancy in seeds and the level of dormancy increased with an increase in salt concentration. A low night (5 °C) and high day (25 °C) temperature regime may partially prevent the induction of dormancy under saline conditions (Khan & Ungar, 1984). Application of GA₃ had a small stimulatory effect, whereas, kinetin substantially alleviated salinity induced dormancy in *A. prostrata* seeds (Khan & Ungar, 1985).

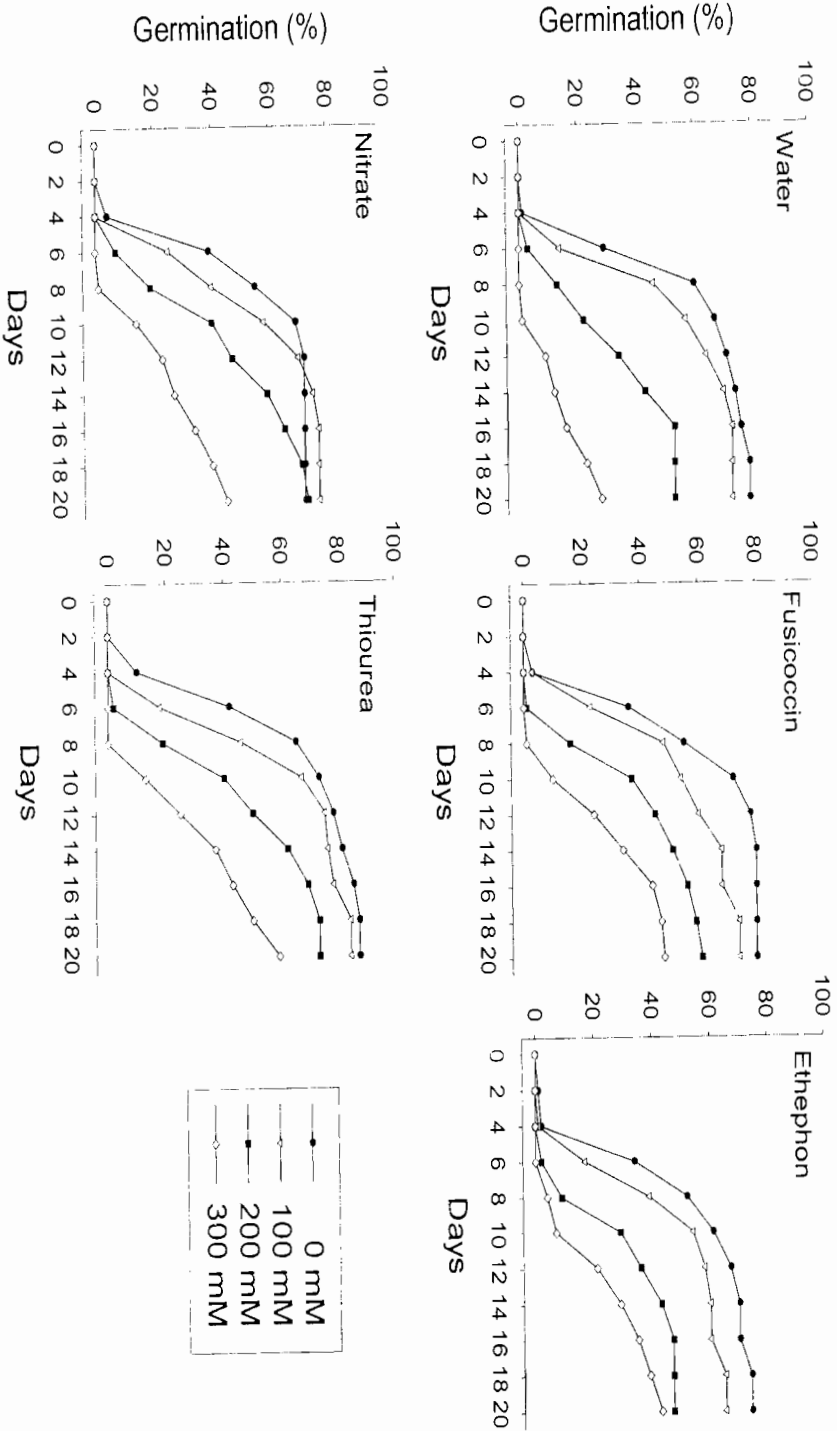


Fig. 1. Final germination percent \pm S.E. of *Arriples prostrata* seeds treated with 20 mM nitrate, 10 mM thiourea, 10 mM ethephon, 5 μ M fusicoccin and 0, 100, 200, and 300 mM NaCl.

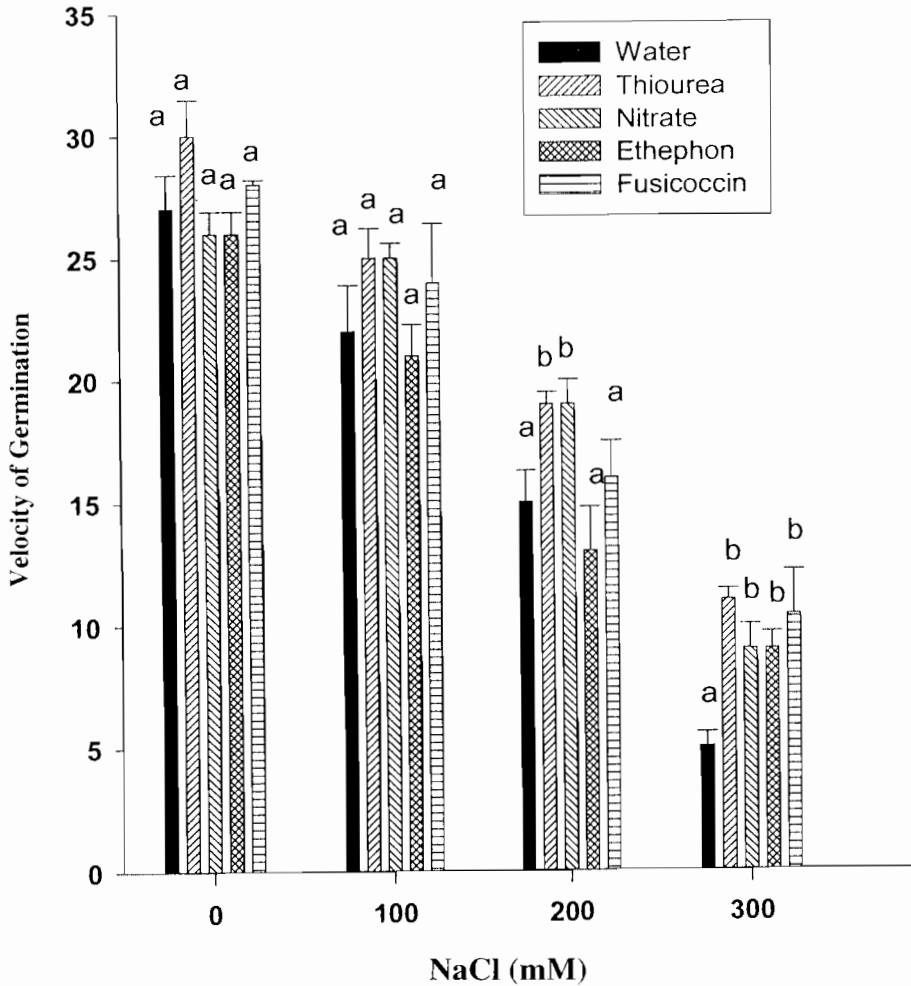


Fig. 2. Timson Index of germination velocity (max. = 50) of *Atriplex prostrata* seeds treated with 20 mM nitrate, 10 mM thiourea, 10 mM ethepon, 5 μM fusicoccin and 0, 100, 200, and 300 mM NaCl. Bars with the same letter for each dormancy-relieving compound at a salinity are not significantly different ($P < 0.05$) from one another.

Application of ethepon relieved dormancy in a number of species (Bewley & Black, 1994) and reverses the inhibitory effects of ABA and osmotic stress (Karssen, 1976, Schonbeck & Egley, 1981). Seed germination is promoted by ethylene (Abeles & Lonski, 1969; Adkins & Ross, 1981; Corbineau & Come, 1995) and it may act by stimulating the germination of non dormant seeds or by breaking dormancy in seeds that exhibit an embryo dormancy (Ketring & Morgan, 1969; Egley & Dale, 1970; Whitehead & Nelson, 1992; Sutcliffe & Whitehead, 1995). However, seeds of some species are not stimulated to germinate by ethylene and in other cases its promotive effects are not substantial (Ismail, 1982). We determined that ethepon significantly alleviated the

inhibitory effect of salinity on germination of *A. prostrata* seeds. Ethylene also was found to alleviate the salinity effect on germination in *Allenrolfea occidentalis* (Gul & Weber, 1998), *Zygophyllum simplex*, *Arthrocnemum indicum*, but no such stimulation of germination was reported for *Atriplex griffithii*, *Sporobolus arabicus*, *Suaeda fruticosa*, and *Haloxylon recurvum* (Khan & Ungar, 2000, 2001, unpublished data).

Fusicoccin significantly promoted germination of *A. prostrata* seeds in the 300 mM NaCl treatment. Seed dormancy enforced by salinity is alleviated by FC in *A. prostrata*, and this may be due to the stimulation of ATPase production, which rapidly increases during the early phases of germination to facilitate proton extrusion and K⁺ uptake (Marre, 1979). Stimulation of germination by FC has been demonstrated (Ismail, 1990; Gul & Weber, 1998). The FC has ability to remove the inhibitory effect of ABA on germination of normal seeds and promotes embryonic growth of deoated seeds (Lado *et al.*, 1975). It is possible that an increase in ABA production because of salinity stress is counteracted by FC, alleviating the inhibitory effects of salinity. The FC strongly influences a number of important physiological processes, in particular those of cell enlargement, potassium uptake, and stomatal opening (Marre, 1979).

Both thiourea and nitrate stimulated the germination of *A. prostrata* seeds. Others have reported the stimulatory action of nitrogenous compounds on seed germination (Bewley & Black, 1994). However, we determined that the dormancy alleviating effect of thiourea on salt stress gradually decreased in seeds of *A. prostrata* with an increase in salinity. Khan & Ungar (unpublished data) reported that nitrate and thiourea promoted the germination of *Zygophyllum simplex* seeds at the lowest (25 mM NaCl) salinity but did not promote germination at higher (125 mM NaCl) salinities. However, seed germination of *Allenrolfea occidentalis* and *Halopyrum mucronatum* is promoted by thiourea in NaCl treatments (Noor & Khan, 1995; Gul & Weber, 1998). Some nitrogenous compounds such as nitrate, nitrite and thiourea are known to stimulate the germination of seeds (Esashi *et al.*, 1979; Aldosaro *et al.*, 1981, Yoshiyama *et al.*, 1996). Thiourea counteracts the effects of ABA and the reduced level of cytokinins in plant tissues (Kabar & Baltepe, 1989). These adverse hormonal changes occur when plant tissues are subjected to water stress induced by drought, salinity or high temperatures. We showed that thiourea can alleviate the inhibitory effects of salinity on *A. prostrata* seed germination and it is also reported to break dormancy and overcome the negative effects of temperature on seed germination (Esashi *et al.*, 1979; Aldosaro *et al.*, 1981).

Dormancy in seeds of *A. prostrata*, which is enforced by high salt concentrations, is probably controlled by the change in balance of endogenous growth regulators. Germination under saline conditions is stimulated by the application of dormancy relieving compounds, which counteract the negative change in growth regulator balance in seeds when they are exposed to salt stress.

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