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EFFECT OF GERMINATION REGULATING CHEMICALS ON SEED GERMINATION OF *HALOGETON GLOMERATUS* FOR ALLEVIATION OF SALINITY STRESS

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Abstract

Halogeton glomeratus (Bieb.) C.A. Mey (Chenopodiaceae) is an annual forb that is widely distributed in mixed desert shrub, salt desert shrub and pinyon-juniper communities of northern Utah. *Halogeton glomeratus* seeds gave only 78% germination in distilled water. Fusicoccin (FC) had some effects in partially alleviating innate dormancy while other chemicals had no effect. Seed germination was inhibited with the increases in salinity, still 24% of the seeds germinated at 900 mM NaCl treatment. The application of germination regulating chemicals like fusicoccin, proline and nitrate almost completely alleviated the salinity effect on seed germination. Kinetin, GA_3 and thiourea were partially successful, while ethephon and betaine had little effect. The rate of germination was not significantly different from the control under non-saline condition for all germination regulating chemicals. At low salinity, thiourea, nitrate and fusicoccin were effective, while at moderate salinity, all of them except betaine and ethephon improved the rate of germination. At highest salinity, proline, FC, GA_3, and nitrate alleviated the rate of germination.

Introduction

Halogeton glomeratus (Bieb.) C.A. Mey is a succulent annual forb with a tap root (Khan *et al*., 2001) and poses a serious threat to grazing animals, especially to sheep due to rich oxalate contents (Welsh *et al*., 1987). *H. glomeratus* is adapted to alkaline soils and semiarid environments and its seeds contain about 25% oil with 85% un-saturated fatty acids making it a first rate edible oil (Weber *et al*., 2001).

Most salt marsh and salt desert halophytes have physiological dormancy (Baskin & Baskin, 1998). Dormancy alleviating compounds like proline, betaine, fusicoccin, GA_3, kinetin, nitrate, thiourea and ethephon are known to alleviate the effect of salinity on the germination of halophytes (Yaniv *et al*., 1995; Pyler & Proseus, 1996; Gul & Weber, 1998; Khan *et al*., 1998; Khan & Ungar, 1998). Germination regulating chemicals have no effect on the innate dormancy of Great Basin halophytes like *Allerolfea occidentalis* (Gul & Weber, 1998; Gul *et al*., 2000) and *Sarcobatus vermiculatus* (Gul *et al*., 2001), whereas most of the above-mentioned chemicals have some effect on releasing innate dormancy as in the case of *Suaeda moquinii*, *Kochia scoparia*, *Salicornia utahensis*, and *Triglochin maritima* (Khan & Ungar, 2001c; Gul & Khan, 2003). Fusicoccin alleviated the innate dormancy of most of the Great Basin species studied, while proline, betaine, kinetin, ethephon and thiourea alleviated salinity effect in about 50% of the studied species.

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The aim of this study was to document that germination regulating chemicals like growth regulators, nitrogenous compounds and osmotica have a role in regulating the innate and salinity-enforced dormancy in *Halogeton glomeratus*. Khan & Gul (2006) presented some hypotheses on the control of seed dormancy by various germination regulating chemicals and of these, the following hypotheses were tested: 1. Germination percentage and rate of decline with increasing salinity, (2) Nitrogenous compounds like thiourea and nitrate alleviate salinity effects, (3) Plant growth regulators like gibberellin, kinetin, ethylene and fusicoccin could overcome innate as well salinity enforced dormancy and (4) osmotica such as betaine and proline could alleviate innate and salinity enforced dormancy in the seeds of *Halogeton glomeratus*.

Materials and Methods

Seeds of *Halogeton glomeratus* were collected during the fall from a salt flat located 2.5 miles northwest of Faust, Utah, USA. Seeds were collected randomly from the entire population to get an adequate representation of genetic diversity. Germination studies were started in the late fall. Seeds were surface sterilized with the fungicide Phygon which had no effect on seed germination. Seeds had 79% germination in distilled water in a viability test. Germination was 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 as an added precaution against loss of water by evaporation. Four replicates of 25 seeds each were used for each treatment. Seeds were considered to be germinated with the emergence of the radicle.

Seeds were germinated in a growth chamber at an alternating temperature regime of 25-35^{o}C, where the higher temperature coincided with the 12-hr light period (Sylvania cool white fluorescent lamps, 25 (µM. $m^{-2}.s^{-1}$, 400 - 750 nM) and the lower temperature coincided with the 12-hr dark period (Khan *et al.*, 2001). Nitrate concentrations of 20 mM, Thiourea concentration of 10 mM, Ethephon concentration of 10 mM, proline concentration of 1 mM, betaine concentrations of 1 mM, Fusicoccin concentration of 5 µM, Gibberellic acid concentration of 3 mM, Kinetin concentration of 0.05 mM and NaCl concentration of 0, 300, 600 and 900 mM were used (Khan & Gul, 2006). Percent germination was recorded every alternate day for 20 days. The rate of germination was estimated by using a modified Timson's index of germination velocity = $\sum G/t$, where G is percentage of seed germination at 2-days intervals, and t is total germination period (Khan & Ungar, 1998). The maximum value possible using this index with our data was 50 (i.e., 1000/20). Higher the values indicate more rapid rate of germination.

Germination data were transformed (arcsine) before statistical analysis. An ANOVA analysis was used to determine if significant differences were present among means. A Bonferroni test was carried out to determine if significant ($p<0.05$) differences occurred between individual treatments (Anon., 1996).

Results

A two-way ANOVA of the percentage germination showed a significant effect of salinity ($F = 81.5$, $p<0.0001$), germination regulating chemicals ($F = 51.4$, $p<0.0001$) and their interaction ($F = 3.97$, $p<0.0001$). A maximum of 79% seeds germinated in distilled water. Increase in NaCl concentration in the medium progressively inhibited the

germination of *H. glomeratus* seeds and still 28% of the seeds germinated at 900 mM NaCl (Fig. 1). Inclusion of ethephon had little effect on the seed germination both in non-saline and saline conditions (Fig. 1). Fusicoccin, GA_3 and kinetin significantly ($p<0.01$) alleviated seed germination (Fig. 1). Fusicoccin alleviated both innate and salinity enforced dormancy in *H. glomeratus* seeds. There was complete alleviation at low to moderate salinity and partial alleviation at the highest salinity (Fig. 1). Gibberellic acid and kinetin had little effect under non-saline conditions, however, they substantially alleviated salinity effects at all concentrations (Fig. 1).

Glycine-betaine was ineffective in promoting germination in both non-saline and saline treatments (Fig. 2). Proline was ineffective in affecting germination under non-saline and low saline conditions but substantially alleviated highly salinity effects on seed germination (Fig. 2). Nitrate treatment resulted in more than 90% germination from control to up to 600 mM NaCl (Fig. 2). About 75% seeds germinated in 900 mM NaCl when treated with kinetin compared to 24% in non-treated salinity control (Fig. 2). Thiourea had little effect under non-saline conditions and completely alleviated the effect 300 mM NaCl and partially alleviated high salinity concentrations (Fig. 2).

A two-way ANOVA of the rate of germination showed a significant effect of salinity ($F = 78.77$, $p<0.0001$), germination regulating chemicals ($F = 53.73$, $p<0.0001$), and their interaction ($F = 4.43$, $p<0.0001$). Rate of germination was not significantly different from control under non-saline condition for all germination regulating chemicals (Table 1). At low salinity (300 mM NaCl) only thiourea, nitrate and fusicoccin promoted the rate of germination (Table 1). At moderate salinity (600 mM NaCl) only betaine and ethephon failed to improve the rate of germination while at highest salinity only proline, GA_3, and nitrate alleviated the salinity effect on the rate of germination (Table 1).

Discussion

Halogeton glomeratus seeds showed 79% germination in non-saline control and the innate dormancy in these seeds were only alleviated by fusicoccin. Seed germination in *H. glomeratus* was inhibited with an increase in salinity and about 24% of the seeds germinated at 900 mM NaCl. Fusicoccin, GA_3, kinetin, nitrate and thiourea partially to completely alleviated the inhibitory effects of salinity on the germination whereas betaine and ethephon had no effect.

Compatible osmotica like proline and betaine are reported to partially alleviate innate dormancy in *Zygophyllum simplex, Atriplex stocksii, A. prostrata, Halopyrum mucronatum* and *Arthrocnemum indicum* (Khan & Ungar, 1997; Khan *et al*., 1998; Khan & Ungar, 2000; Khan *et al*., 2003). However, proline and betaine did not relieve salinity-induced dormancy in *Arthrocnemum indicum* (Khan *et al.*, 1998), *Kosteletzkya virginica* (Poljakoff-Mayber *et al*., 1994), *Halopyrum mucronatum* (Khan & Ungar, 2001c), *Salicornia rubra* (Khan *et al*., 2002) and *Salicornia utahensis* (Gul & Khan, 2003). Both proline and betaine alleviated the innate dormancy of *Zygophyllum simplex* seeds but neither was effective at high salinities (Khan & Ungar, 1997) while Gul & Weber (1998) reported that proline and betaine both alleviated high salinity effects in *Allenrolfea occidentalis*. Our results with *H. glomeratus* showed that proline was very effective in alleviating salinity induced dormancy while betaine had no effect.

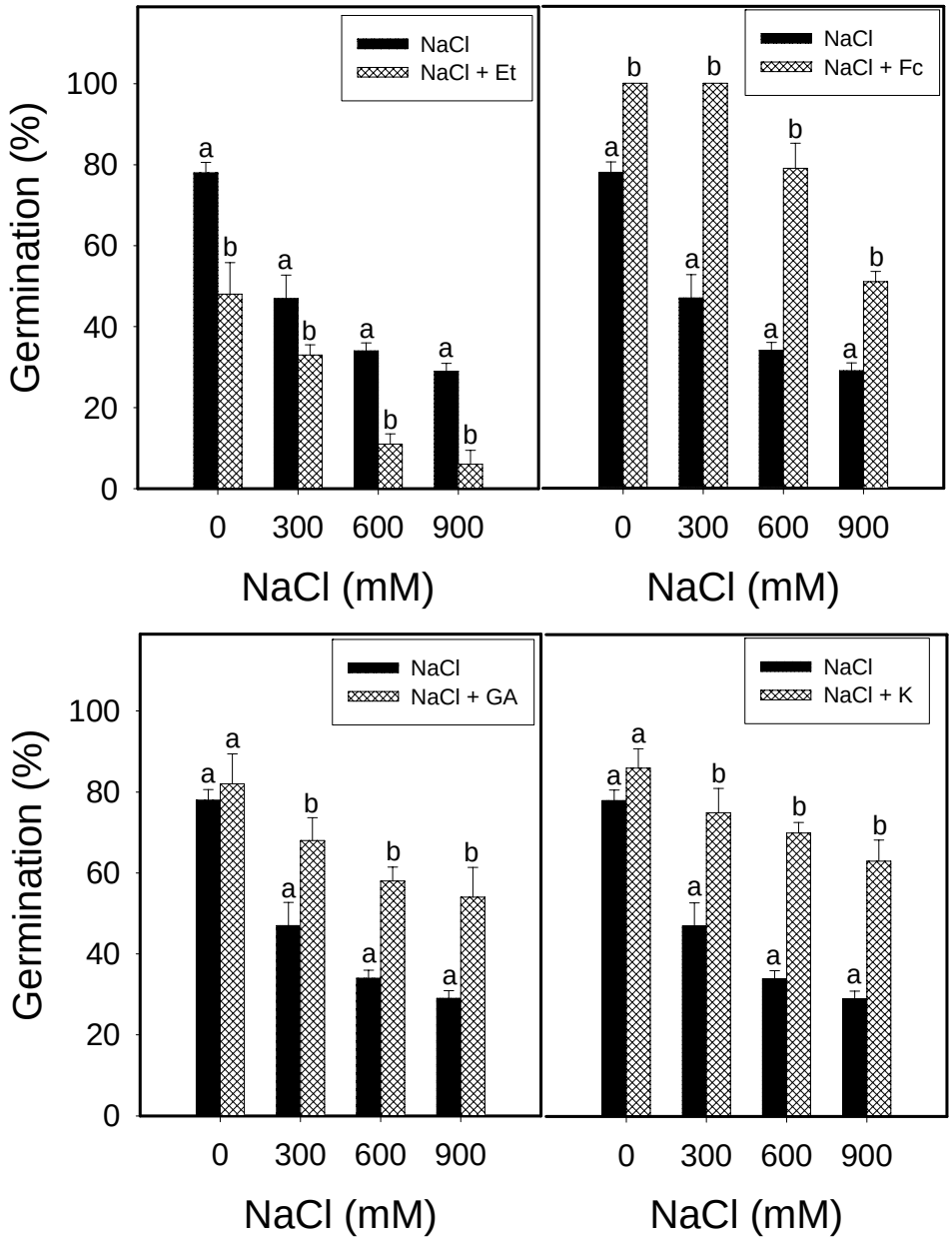

Fig. 1. Percent germination of *Halogeton glomeratus* seeds in NaCl, ethephon, fusicoccin, gibberellic acid and kinetin. Value for dormancy regulating chemicals having the same letter are not significantly different from the control (Bonferroni test).

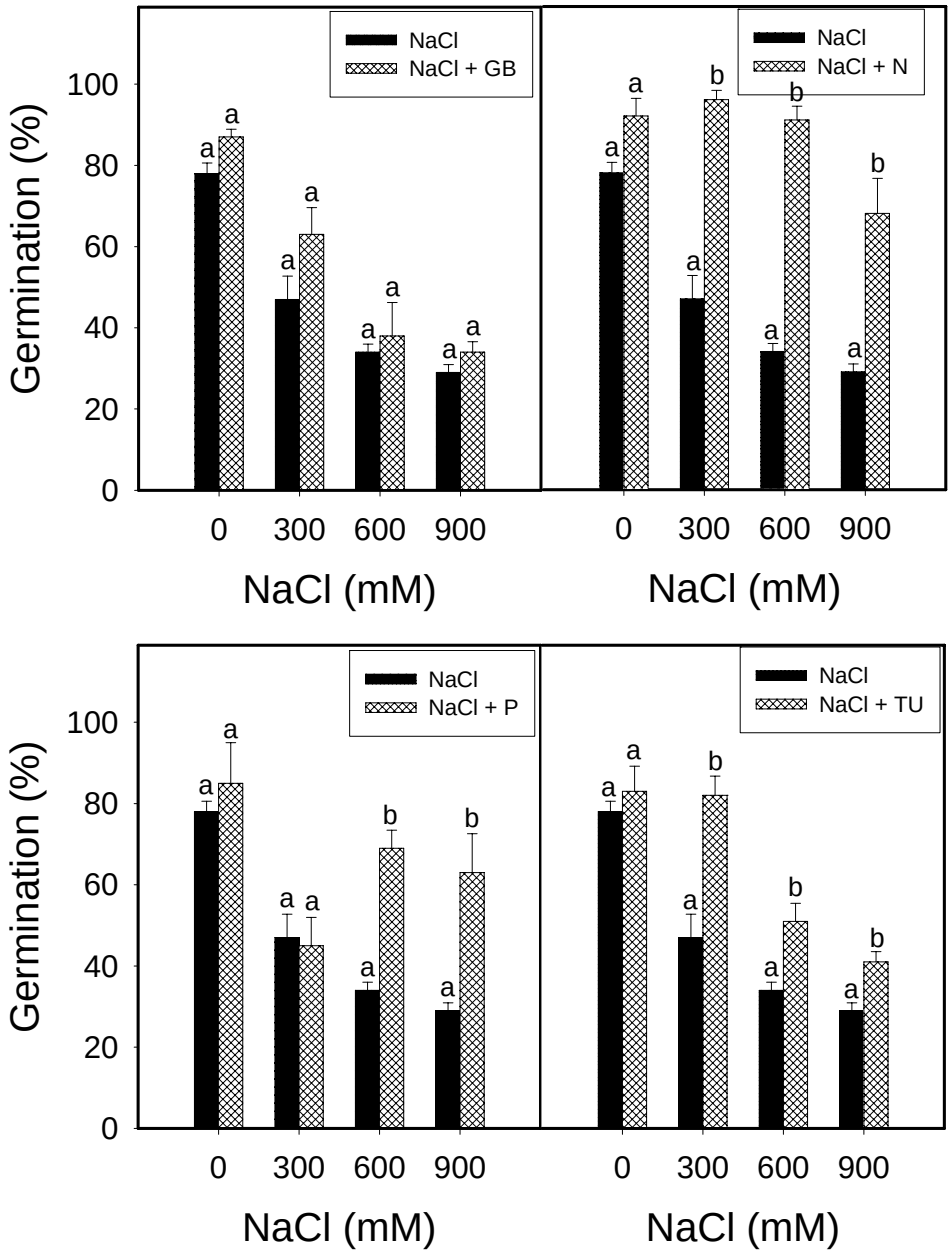

Fig. 2. Percent germination of *Halogeton glomeratus* seeds in NaCl, glycinebetaine (GB), nitrate (N), proline (P) and thiourea (TU). Value for dormancy regulating chemicals having the same letter are not significantly different from the control (Bonferroni test).

Table 1. Rate of seed germination of *Halogeton glomeratus* under various salinities and germination regulating chemicals.

NaCl (mM)	Germination regulating chemicals								
	Water	Proline	Betaine	GA_3	Kinetin	Thiourea	Nitrate	Fusicoccin	Ethephon
0	39±1.1[a]	42±4.9[a]	45±0.9[a]	43±3.7[a]	46±2.5[a]	48±3.2[a]	51±2.1[a]	54±0.2[a]	31±3.9[a]
300	23±3.2[b]	32±3.1[b]	33±3.4[b]	37±2.9[b]	41±3.0[b]	47±2.4[a]	52±1.6[a]	55±0.1[a]	24±1.2[b]
600	16±1.1[c]	35±2.6[b]	20±4.1[c]	32±1.8[b]	39±1.4[b]	31±1.7[b]	51±1.7[a]	44±3.1[b]	13±1.3[c]
900	14±0.9[c]	33±4.7[b]	19±0.9[c]	30±3.8[b]	35±2.6[c]	25±0.7[c]	40±4.3[b]	31±1.2[c]	11±1.6[c]

Values in rows at each dormancy regulating chemicals having the same letter are not significantly different ($p>0.05$) from control. Bonferroni test.

Fusicoccin was most effective in alleviating both innate and salinity induced dormancy in *H. glomeratus*. A stimulation of germination by FC in various kinds of seeds has been observed (Ismail, 1990; Gul & Weber, 1998; Gul & Khan, 2003; Khan *et al*., 2002, 2004). FC has the ability to remove the inhibitory effect of ABA on germination (Lado *et al.,* 1975). Fusicoccin may act as gibberellic acid to counter the effects of ABA produced due to salinity effects. This alleviation may be due to the stimulation of ATPase production to facilitate proton extrusion and K^+ uptake (Marre, 1979; Stout, 1988).

Effect of salinity on the germination of *H. glomeratus* seeds was not affected by the application of ethephon. Ethylene is reported to have variable response in alleviating salinity effects on the germination of halophytes. Ethylene alleviated salinity stress on the germination of *Zygophyllum simplex* (Khan & Ungar, 1997), *Arthrocnemum macrostachyum* (Khan *et al*., 1998), *Allenrolfea occidentalis* (Gul & Weber, 1998), *Atriplex prostrata* (Khan *et al*., 2003), *Aeluropus lagopoides* and *Sporobolus ioclados* (Gulzar & Khan, 2002), *Ceratoides lanata* (Khan *et al*., 2004) and *Suaeda salsa* (Li *et al*., 2005). However, seeds of many halophytes like *Atriplex griffithii, Salicornia utahensis, Sarcobatus vermiculatus, Sporobolus arabicus, Triglochin maritima,* and *Zygophyllum qatarensis* do not respond to ethylene application (Ismail, 1990; Khan & Ungar, 2000, 2001ab; Gul *et al*., 2001; Gul & Khan, 2003). Dormancy in seeds of numerous species is reported to be relieved by the application of ethephon (Ketring, 1977; Bewley & Black, 1994) and reverse the inhibitory effect of abscisic acid and osmotic stress (Karssen, 1976; Schonbeck & Egley, 1981).

Thiourea and nitrate both stimulated the germination of *H. glomeratus* seeds under saline conditions. The alleviating effect of thiourea on osmoinhibition gradually decreases with an increase in salinity, however, nitrate completely alleviated salinity effects on germination and partially alleviated at highest concentration. Gul & Weber (1998) reported that nitrate and thiourea almost completely alleviated the seed germination of *Allenrolfea occidentalis* at all salinities. A substantial promotion was also reported in *Triglochin maritima, Salicornia utahensis, Sporobolus arabicus* and *Aeluropus lagopoides* (Khan & Ungar, 2001a; Gul & Khan, 2003; Gulzar & Khan, 2002). This effect is quite varied since some species showed some effect at all concentrations while others only at low concentration and still other do not response to either nitrate or thiourea treatments (Khan & Gul, 2006). Some nitrogenous compounds such as nitric oxide, nitrate, nitrite and thiourea are known to stimulate the germination of seeds (Yoshiyama *et al*., 1996; Bethke *et al*., 2004; Li *et al*., 2005). Thiourea is also known to break dormancy and overcome the negative effect of temperature on seed germination (Esashi *et al*., 1979; Aldosaro *et al*., 1981).

The GA_3 are known to alleviate salinity effect in some halophytic seeds (Khan & Ungar, 1998; Khan *et al*., 1998; Li *et al*., 2005) while it was ineffective in other halophytes like *Suaeda fruticosa* and *Haloxylon recurvum* (Khan & Gul, 2006), *Sarcobatus vermiculatus* (Gul *et al*., 2001), *Ceratoides lanata* (Khan *et al*., 2004). Seed

germination of *H. glomeratus* was partially alleviated by the application of GA_3 and kinetin. Kinetin is also a more potent growth regulator known to alleviate salinity effects in a number of halophytes (Gul & Khan, 2003; Khan *et al*., 2002; 2004, Li *et al*., 2005).

Halogeton glomeratus is a plant which occupies a relatively drier part of the Great Basin Desert and very highly tolerant to salinity at germination stage. The effect of salinity on germination is regulated through the change in plant germination regulating chemicals (Khan & Gul, 2006). Application of proline, nitrate, fusicoccin, GA_3, and kinetin substantially alleviated the salinity effects on germination while thiourea, betaine and ethylene have little effect. Inhibition of germination may be associated with the decrease in nitrogen supply and decrease in growth regulators. *Halogeton glomeratus* has a potential of being the source of seeds that could yield high quality edible oil with about 85% un-saturation (Weber *et al*., 2001). This plant could be grown under saline conditions and the initial recruitment could be enhanced if the seeds are treated with Fusicoccin, GA_3, nitrate, kinetin or proline.

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