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EFFECT OF COMPATIBLE OSMOTICA AND PLANT GROWTH REGULATORS IN ALLEVIATING SALINITY STRESS ON THE SEED GERMINATION OF *ALLENROLFEA OCCIDENTALIS*

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Abstract

Allenrolfea occidentalis, a perennial halophytic shrub in the family Chenopodiaceae is widely distributed in the inland salt marshes and salt playas of western United States. Experiments were conducted to determine the influence of compatible osmotica (betaine and proline) and growth regulators (gibberellic acid and kinetin) in alleviating salinity stress on the seed germination of *A. occidentalis*. Seed germination decreased with an increase in salinity stress and no seed germinated at 800 mM NaCl. Compatible osmotica and growth regulators were able to counteract the inhibitory effect of salinity. Betaine (0.1 and 1.0 mM) and proline (0.1 and 1.0 mM) completely alleviated the salinity-enforced dormancy at all treatments. Gibberellic acid (0.3 and 3.0 mM) and kinetin (0.05 and 0.5 mM) significantly ($p<0.0001$) improved the seed germination inhibited by NaCl but the gibberellic acid alleviated more than kinetin. At higher salinity, 800 mM NaCl low concentration of kinetin (0.05 mM) and gibberellic acid (0.3 mM) were more effective.

Introduction

Allenrolfea occidentalis (S. Wats.) Kuntze (iodine bush) is a stem succulent shrub distributed in the salt marshes and playas of Great Basin, California and south west Texas deserts (Flowers, 1934, Ungar, 1974) with soil NaCl concentration up to 1025 mM (Gul &Weber, 1999). It is also a highly drought tolerant plant (Young *et al.*, 1995). Seeds borne in utricles are often enclosed by winged, inflated or hair bracts. Seed of *A. occidentalis* could germinate in up to 800 mM NaCl and germination improved at warmer (25-35^{o}C) thermoperiod (Gul & Weber, 1998b) and the effect of salinity could be alleviated by external application of fusicoccin, ethephon, nitrate and thiourea (Gul & Weber, 1998).

Many plants in saline or dry habitats are known to accumulate organic solutes such as glycine-betaine, proline, polyols and others (Poljakoff-Mayber *et al.*, 1994; Khan & Gul, 2006). It is assumed that under stress conditions these substances serve as compatible cytoplasmic solutes that compensate osmotically for external osmolarity or for ions sequestered in the vacuole (Gorham, 1995). Poljakoff-Mayber *et al.*, (1994) reported that dry seeds of *Kosteletzkya virginica* contain a significant amount of betaine and proline. Betaine content decreased during germination in the presence of NaCl, while proline content increased, suggesting a possible role of proline during germination. They also found that both proline and betaine (10 mM) were ineffective in alleviating the effect of salinity stress on germination or in breaking innate dormancy. Proline and betaine (0.1 mM) alleviated the innate dormancy in *Zygophyllum simplex* and in *Arthrocnemum*

indicum (Khan & Gul, 2006) but did not improve germination under saline conditions. Proline was effective in alleviating salinity effect on the seed germination of *Halogeton glomeratus*, *Salicornia utahensis* and *Triglochin maritima* but failed in *Atriplex rosea*, *Salicornia rubra*, *Sarcobatus vermiculatus* and *Salsola iberica* (Gul *et al.*, 2000; Khan *et al.*, 2002, 2004; Khan & Gul, 2006). Betaine did not alleviate salinity effects on seed germination of Great Basin species (Khan & Gul, 2006).

Gibberellic acid (GA_3) and kinetin substantially alleviated both innate and salinity induced dormancy in *Salicornia pacifica var utahensis* (Khan & Weber 1986) and in *Ceratoides lanata* seeds (Khan *et al.*, 2004). Growth regulators like GA_3 and kinetin are reported to alleviate the inhibitory effect of salinity on germination (Khan & Weber 1986; Ismail, 1990; Khan *et al.*, 2002, 2004; Khan & Gul, 2006).

The purpose of this study was to determine the effect of external application of compatible osmotica such as proline and betaine and growth regulators such as GA_3 and kinetin on the germination inhibited by high salinity.

Materials and Methods

Seeds of *A. occidentalis* were collected during the Fall 1995 from a salt playa located at 1 km East of Goshen, Northwestern Utah. Seeds were randomly collected from the whole population to get the adequate representation of genetic diversity of the population. The flowering spikes and seeds were stripped as the seeds matured and the inflorescence dried. The seeds were air dried and threshed by hand through screens. A small fanning mill was used to separate the seeds from chaff. Seeds were stored in sealed plastic jars at 4°C. Seeds were surface sterilized with fungicide Phygon (phygon had no effect on seed germination). Seeds showed 100% germination in distilled water in a viability test before germination. Four 25-seed replicates were placed directly in 50x9-mm (Gelman No. 7232) tight-fitting plastic Petri dishes and submerged in 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. Seeds were considered to have germinated with the emergence of a radicle. Each experiment was conducted at least two times.

Seeds were germinated in a chamber at an alternating temperature regime of 25-35ºC, where the higher temperature coincided with the 12 hr light period (Sylvania cool white fluorescent light, 110 μmol photons.m^{-2}.s^{-1}, 400-700 nm) and the lower temperature coincided with the 12 hr dark period. GA concentrations of 0.3 and 3 mM, kinetin concentrations of 0.05 and 0.5 mM, proline and betaine concentrations of 0.1 and 1 mM and NaCl (0, 200, 400, 600 and 800 mM) solutions were used to enhance germination in salinity treatments. The preliminary experiments showed that the concentrations that have been used are the optimal concentrations of these chemicals. 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 $=\Sigma G/t$, where G is the percentage of seed germination at 2-days intervals and t is the total germination period (Khan & Ungar, 1984). The maximum value possible using this index with our data was 1000/20 = 50. The higher the value, the more rapid the rate of germination.

Germination data were transformed (arcsine) before statistical analysis in order to ensure homogeneity of variance before analysis. These data were analyzed using SPSS, V.11.0 (Anon., 2001). A two-way ANOVA analysis was used to determine if significant differences were present among means. A Bonferroni test was used to determine whether difference between individual treatments were significant ($p<0.05$).

Table 1. Results of two-way analysis of variance of characteristics by Kinetin (K), Salinity (S), GA_3 (G), Proline (P) and Betaine (B) treatments.

Dependent variable	Independent variable		
	K	**S**	**KxS**
Germination %	22.8***	26.2***	4.3***
Germination (velocity)	21.9***	43.1***	2.5***
	G	**S**	**GxS**
Germination %	2.1***	20.2***	1.7***
Germination (velocity)	10.2***	51.5***	1.3***
	P	**S**	**PxS**
Germination %	5.4***	14.7***	1.3***
Germination (velocity)	5.4***	53.7***	2.9**
	B	**S**	**BxS**
Germination %	43.8***	19.2***	17.5***
Germination (velocity)	140.2***	83.1***	25.2***

Note: Numbers represent F-values: *** = $p < 0.0001$

Table 2. Index of germination velocity of *Allenrolfea occidentalis* seeds at various salinity, growth regulator and osmotica treatments (Mean ± standard error).

Salinity (mM)	Water	Proline (mM)		Betaine (mM)		GA (mM)		Kinetin (mM)	
		0.1	**1.0**	**0.1**	**1.0**	**0.03**	**0.3**	**0.05**	**0.5**
0	48.1^{a}	45.4^{a}	42.7^{a}	47.5^{a}	47.0^{a}	48.9^{a}	46.7^{a}	47.8^{a}	43.3^{a}
	±9.3	±0.6	±1.8	±0.5	±1.0	±0.7	±0.6	±1.1	±1.9
200	38.2^{a}	40.5^{a}	34.3^{a}	47.8^{a}	45.8^{a}	48.7^{a}	42.3^{a}	47.3^{a}	41.2^{a}
	±2.3	±1.4	±2.8	±0.9	±0.3	±1.4	±1.2	±1.5	±2.3
400	20.6^{b}	33.7^{a}	34.5^{a}	46.6^{a}	39.8^{a}	43.9^{a}	44.0^{a}	41.2^{a}	26.4^{b}
	±1.6	±1.2	±1.2	±0.3	±1.4	±2.7	±1.1	±1.6	±4.7
600	9.7^{bc}	27.7^{ab}	18.3^{b}	46.2^{a}	26.3^{b}	37.3^{ab}	33.6^{a}	35.2^{a}	26.2^{b}
	±2.1	±2.1	±1.1	±1.2	±2.3	±4.1	±2.1	±2.7	±1.5
800	0.7^{c}	17.4^{b}	19.5^{b}	37.0^{a}	21.6^{b}	26.6^{b}	18.2^{c}	19.0^{b}	18.5^{b}
	±0.4	±2.4	±3.7	±1.4	±1.2	±2.5	±1.6	±1.4	±2.7

Values in column having the same letter are not significantly different at $p > 0.05$, Bonferroni test.

Results

A two-way ANOVA of percent and rate of germination indicated a significant ($p<0.0001$) main effect of salinity, proline, GA_3, kinetin and betaine and their interactions (Table 1). The inhibition of germination caused by NaCl was completely alleviated by betaine. Betaine applied at either 0.1 or 1 mM broke the salt induced dormancy of *Allenrolfea occidentalis* seeds at all salinity treatments (Fig. 1). However at higher salinity treatments 1 mM betaine concentrations was less effective in alleviating the salt induced dormancy of seeds. Rate of germination of seeds decreased with an increase in salinity (Table 2).

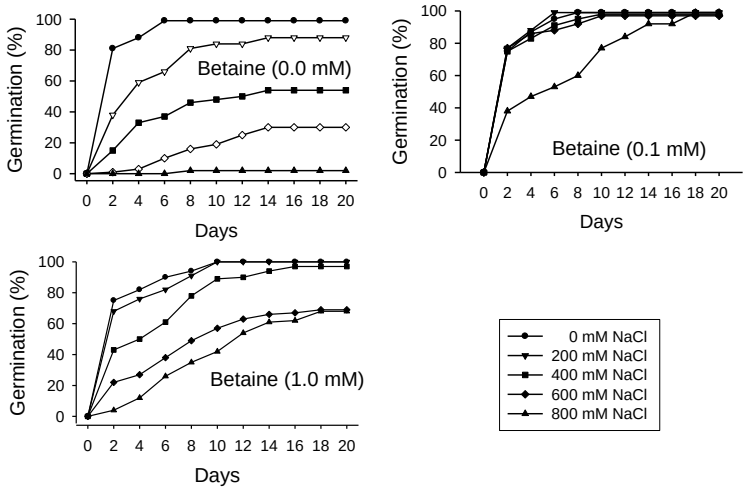

Fig. 1. Germination percent of *Allenrolfea occidentalis* seeds in 0, 200, 400, 600 and 800 mM NaCl and 0, 0.1 and 1.0 mM betaine.

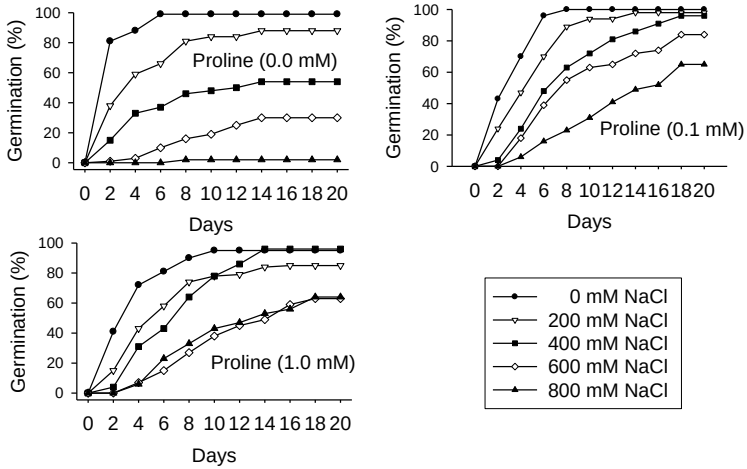

Fig. 2. Germination percent of *Allenrolfea occidentalis* seeds in 0, 200, 400, 600 and 800 mM NaCl and 0, 0.1 and 1.0 mM proline.

Proline treatments applied at either 0.1 and 1 mM markedly alleviated the inhibitive effect of the high concentration of salt on the germination and as a result of this treatment, the seeds germinated as if they were at the lower levels of the NaCl. The proline treatment not only increased the percentage germination of the seeds under saline conditions but also shortened the time required for germination (Fig. 2). Salt induced dormancy of *A. occidentalis* seeds was significantly alleviated by both concentrations of proline used. In the 800 mM NaCl concentration proline (0.1 and 1.0 mM) significantly ($p<0.05$) increased the percentage germination in comparison to the non treated control. At higher salinities proline alleviated seed dormancy up to 63% (Fig. 2). The rate of germination significantly increased ($p<0.05$) with proline application in control and at all salinity treatments (Table 2).

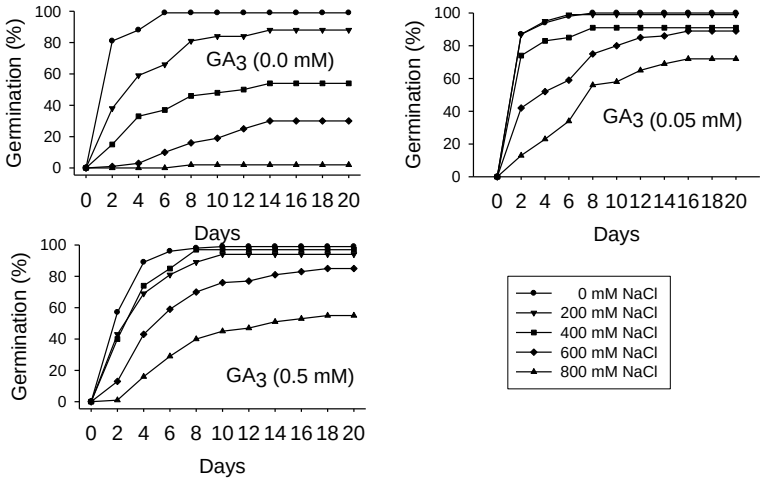

Fig. 3. Germination percent of *Allenrolfea occidentalis* seeds in 0, 200, 400, 600 and 800 mM NaCl and 0, 0.3 and 3.0 mM GA_3.

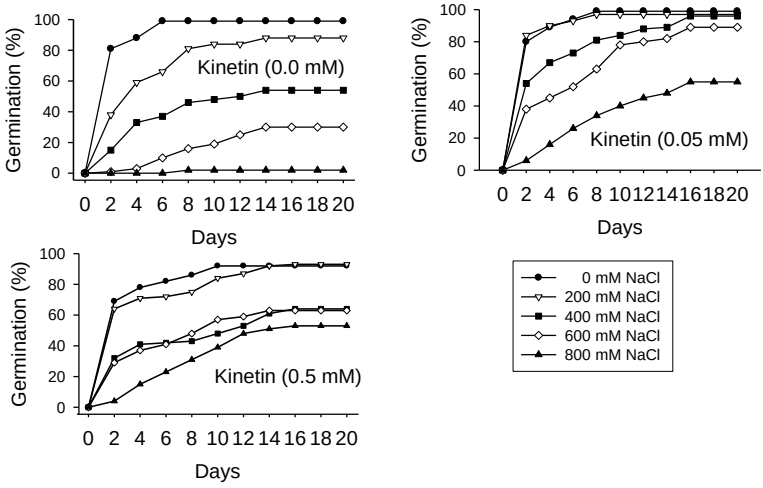

Fig. 4. Germination percent of *Allenrolfea occidentalis* seeds in 0, 200, 400, 600 and 800 mM NaCl and 0, 0.05 and 0.5 mM kinetin.

GA_3 substantially alleviated the germination inhibited by salinity (Fig. 3). Lower concentration of GA_3 (0.3 mM) was more effective in comparison to 3 mM GA_3. The rate of germination decreased with an increase in salinity (Table 2). Addition of 0.3 mM GA_3 significantly ($p<0.05$) promoted the rate of germination at all salinity treatments.

Kinetin substantially alleviated the salinity enforced germination inhibition (Fig. 4). Low concentration of kinetin (0.05 mM) almost completely alleviated salinity effect at low concentrations (200-600 mM NaCl). The rate of germination significantly increased ($p<0.05$) with kinetin application in controls and in the lowest salinity treatments (Table 2).

Discussion

Dormancy in seeds of halophytes is a significant factor in the ecophysiology of salt marsh species. It permits seeds to remain viable in the soil during periods when the environment is not suitable for germination (Ungar, 1991). Betaine and proline are compatible osmotica, both completely alleviated the effect of salinity on germination of *A. occidentalis* seeds. Effect of NaCl on seed germination are both ionic and osmotic (Ungar, 1991). Compatible osmotica such as proline and betaine which acclimatize plants to high levels of salinity in their tissues may be of significance as compatible solutes in seeds. Poljakoff-Mayber *et al.,* (1994) studied the proline and betaine level in *Kosteletzkya virginica* seeds during the process of germination. They found low concentration of proline and betaine level in *Kosteletzkya virginica* seeds during the processes of germination. However when seeds were germinated in a saline media the proline content increased while their betaine concentration decreased. They suggested that proline could be the compatible osmotica for germinating seeds. They did not find any external application effect of proline and betaine in both control and saline medium on seed germination. Khan *et al.,* (2004) reported that both proline and betaine alleviated the innate dormancy of the *Ceratoides lanata* seeds but failed to improve salinity enforced dormancy. Our results showed that low (0.1 mM) concentration of proline and betaine was more effective in alleviating salinity induced dormancy in *A. occidentalis* seeds. Application of proline (0.1 mM) substantially promoted seed germination from 2 to 70% at 800 mM NaCl, while betaine (0.1 mM) completely overcame the effect of salinity and promoted germination 99% at 800 mM NaCl. This indicates that low concentrations of proline and betaine were more effective to alleviate salt induced dormancy in contrary to previous reports (Poljakoff-Mayber *et al.,* 1994). Proline and GB have been shown to provide tolerance to environmental stress *via* preserving osmotic balance and stabilizing the quaternary structures of complex proteins, membranes and many functional units like oxygen evolving PS-II complex (Rajasekaran *et al.,* 1997; Thakur & Sharma, 2005; Demiral & Turkan, 2006). Therefore external application of these compounds may help seeds to negotiate high saline stress. Proline was effective in alleviating salinity effect on the seed germination of *Halogeton glomeratus, Salicornia utahensis* and *Triglochin maritima* but failed in *Atriplex rosea, Salicornia rubra, Sarcobatus vermiculatus* and *Salsola iberica* (Gul *et al.*, 2000; Khan *et al.,* 2002, 2004; Khan & Gul, 2006) while betains was ineffective in these species. Compatible osmotica like proline and glycinebetaine, which increase the tolerance level of plants to high levels of salinity in their tissues by acting as an osmoregulator in the cytoplasm or as an osmoprotectant of proteins (Schobert, 1977; Gorham, 1995), may be of significance as compatible solutes in seeds.

Gibberellic acid and kinetin were found to be effective in alleviating salt induced dormancy in *A. occidentalis* seeds. In the high salinity treatments the effect of GA_3 was greater than kinetin. GA_3 and kinetin both partially alleviated the effect of salinity on germination of *A. occidentalis* seeds. The GA_3 are known to alleviate salinity effect in some halophytic seeds (Li *et al.,* 2005) while it was ineffective in other halophytes like *Suaeda fruticosa* and *Haloxylon recurvum* (Khan & Gul, 2006), *Sarcobatus vermiculatus, Ceratoides lanata* and *H. glomeratus* (Khan & Gul, 2006). Kinetin is also a more potent growth regulator known to alleviate salinity effects in a number of halophytes (Khan *et al.,* 2002; 2004; Li *et al.,* 2005).

Allenrolfea occidentalis produces abundant seeds at the end of fall and beginning of winter. After dispersal, seeds remain in the seed bank until the next spring when rains provide much needed moisture for germination. However not all of the seeds germinate.

Dormancy of the seeds could be either innate or salt induced. Seeds prevented from germination by salt may be due to inhibition of growth regulators or interference of salinity with metabolic activity. Our data indicate that the compatible ocmotica and growth regulators overcame the ionic stress in seeds and stimulated germination of *A. occidentalis* seeds at higher salinities. It is possible that under hypersaline conditions there is a significant decrease in the production of growth regulators and compatible osmotica in these seeds and exogenous applications of osmotica and growth regulators compensated for this deficit and ameliorated the inhibitory effect of NaCl on germination. Further investigations are necessary to determine the changes in the content of endogenous osmotica and growth regulators under salt stress to explain the inhibition in germination of halophyte seeds at high salt stress.

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