

## EFFECT OF GERMINATION REGULATING CHEMICALS IN ALLEVIATING SALINITY INDUCED GERMINATION INHIBITION OF *LIMONIUM STOCKSII* SEEDS

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### Abstract

Effect of different chemicals: kinetin (0.05 mM), ethephon (5  $\mu$ M), GA<sub>3</sub> (0.3 mM), proline (0.1 mM), betaine (0.1 mM), nitrate (10 mM) and thiourea (5 mM) was investigated in alleviating salinity induced seed dormancy from *Limonium stocksii*. Six salinity regimes (0-500 mM NaCl) were used in 12 h photoperiod and in complete darkness to study the effect of different germination regulating chemicals in inducing germination under saline condition. Only kinetin and ethephon successfully alleviated salinity enforced seed dormancy of *L. stocksii*. Kinetin was more successful than ethephon. Seed germination was substantially inhibited by salinity when germinated under complete darkness. Kinetin and ethephon appeared to be the only chemicals which improved seed germination in complete darkness. All other germination regulating chemicals had no effect at low salinity but inhibited germination at high salinity in both light and dark.

### Introduction

Seed dormancy is an adaptive mechanism to promote plant survival by distributing germination in both time and space (Lorenzo *et al.*, 1999). It is influenced by a large number of genes and environmental factors, especially by those which affect the growth potential of a seed (Korneel *et al.*, 2002). In case of halophytes, seed germination is usually prevented by physiological causes (Khan, 1996) induced by availability of less than optimal environmental conditions like water, light, oxygen, temperature and soil salinity (Corbineau & Come, 1995; Ungar, 1995). Exposure to osmotic stress causes plants to exhibit different morphological and developmental changes at the molecular, cellular and organismal levels (Yeo, 1998; Bohnert *et al.*, 1999; Hasegawa *et al.*, 2000) during the life cycle. These changes are due to the imbalance in growth regulators causing an increased level of endogenous ABA and other germination inhibitors and a decrease in endogenous growth promoters (Bewely & Black, 1994).

Kudret (1987) suggested that endogenous hormone level is affected by many environmental stresses however, external application of appropriate growth regulators optimize physical metabolic conditions for germination. The role of various germination regulating chemicals such as proline, betaine, gibberellin, kinetin, nitrate, thiourea and ethephon in reducing the inhibitory effects of salinity on germination is reported for several halophytes (Kudret, 1987; Bewely & Black, 1994; Plyler & Proseus, 1996; Gul & Weber, 1998; Khan & Ungar, 1997, 2000, 2001a,b,c, 2002). Different regulatory roles are suggested for these chemicals in breaking seed dormancy in halophytes. They are thought to alleviate salinity effects on the germination by 1) affecting gene expression and/or membrane function (Ho & Hagen, 1993); 2) substituting for light and temperature (Khan & Weber, 1986; Bewely & Black, 1994; Corbineau & Come, 1995; Sutcliffe & Whitehead, 1995); 3) acting as an osmoregulator or osmoprotectant of proteins in the cytoplasm (Poljakoff-Mayber *et al.*, 1994; Gorham, 1995); and 4) counteracting the effect

of reduced promoter (cytokinins and gibberellins) and increased inhibitor substances, such as abscisic acid in seeds under high salinity (Kabar & Baltepe, 1990). However, the ability of these chemicals to relieve seed dormancy and to stimulate germination varies with environmental factors such as temperature and light and from one species to another (Khan, 2003).

Seeds of *L. stocksii* are subjected to high salinity and temperature stress after dispersal. However, seeds germinate readily after monsoon rains under natural conditions. Salinity tolerance of seeds under laboratory conditions decreased sharply above 300 mM NaCl and only few seeds germinated at 500 mM (Zia & Khan, 2004). The inhibition of germination may be due to an imbalance of critical germination regulating chemicals. The present study was conducted to determine the role of germination regulating chemicals in alleviating salinity induced dormancy from seeds of *L. stocksii*.

## Materials and Methods

Seeds of *Limonium stocksii* were collected from a salt flat at the upper end of Maura creek near Hawks Bay, Karachi (24°45'-25°N and 66°45'-67°E). Seeds were separated from the inflorescence, surface sterilized using sodium hypochlorite (0.52 %) for one minute followed by thorough rinsing with distilled water and air-drying. Germination was carried out in 50 mm diam. tight fitting plastic Petri dishes with 5 mL of test solution. Four replicates of 25 seeds each were used for each treatment. Seeds were considered to be germinated with the emergence of radicle.

Seeds were germinated in an incubator at a day/night temperature of 30/20 °C in incubators (Percival Scientific, USA) with a 12-h photoperiod (Sylvania cool white fluorescent lamps, irradiance of 25  $\mu\text{mol. m}^{-2} \text{s}^{-1}$ , 400-700 nm) and in complete darkness in six salinity concentrations (0, 100, 200, 300, 400 and 500 mM NaCl). Dormancy relieving compounds: kinetin (0.05 mM), ethephon (5  $\mu\text{M}$ ), GA<sub>3</sub> (0.3 mM), proline (0.1 mM), betaine (0.1 mM), nitrate (10 mM) and thiourea (5 mM) were used. The concentrations used were based on optimal level promoting seed germination in most other species. Percent germination was recorded alternately for 20 days. Similarly seeds were germinated in complete darkness by placing petri-plates in black plastic bags for 20 days and the germination was recorded after 20 days.

Rate of germination was estimated by using modified Timson's index of germination velocity,  $\Sigma G/t$ , where G is percentage of seed germinated at 2-d intervals, and t is total germination period (Khan & Ungar, 1997). The maximum value possible for our data using this index was 50 (*i.e.* 1000/20). The higher the value the more rapid the germination. Germination data was arcsine transformed before statistical analysis using SPSS for Windows release 11 (SPSS, 2002). The 2-way and 3-way ANOVA were used to determine significant effects of various factors and their interaction on rate and percentage germination. A Bonferroni test was used to determine significant differences between means.

## Results

Three way ANOVA of percentage germination of *L. stocksii* indicated significant ( $P < 0.0001$ ) effect of salinity, light, chemicals and their interactions (Table 1). Best seed germination was obtained in distilled water control and in 100 mM NaCl under 12-h photoperiod. Further increase in salinity gradually decreased seed germination and only a few seeds could germinate in 500 mM NaCl (Fig. 1).

**Table 1. Results of three-way analysis of variance of final germination by salinity (SAL), chemicals (CHEM) and light/dark (LD) conditions. (The F values are highly significant at  $P < 0.0001$  level).**

Source	Sum of squares	df	F
Salinity (SAL)	387319.21	5	573.87***
Chemical (CHEM)	54753.63	7	57.95***
Light/Dark (LD)	68373.38	1	506.52***
SAL * CHEM	28835.12	35	6.10***
SAL * LD	33555.88	5	49.72***
CHEM * LD	4100.96	7	4.74***
SAL * CHEM * LD	40723.79	35	8.62***

**Table 2. Effect of different germination regulating chemicals (thiourea, nitrate, proline, betaine, gibberellic acid, kinetin, and ethephon) on rate of germination of *Limonium stocksii* seeds in different salinity (0, 100, 200, 400 and 500 mM NaCl) treatments. (Different letters in superscript represent significant ( $P < 0.05$ ) differences within each salinity treatment, Bonferroni test).**

NaCl (mM)	Control	Kinetin	Ethephon	GA <sub>3</sub>	Proline	Betaine	Nitrate	Thiourea
0	49.30 <sup>a</sup> ±0.17	49.70 <sup>a</sup> ±0.19	49.45 <sup>a</sup> ±0.55	50.00 <sup>a</sup> ±0.00	49.45 <sup>a</sup> ±0.55	49.50 <sup>a</sup> ±0.50	46.70 <sup>a</sup> ±1.74	49.45 <sup>a</sup> ±0.55
100	46.30 <sup>a</sup> ±0.52	49.55 <sup>a</sup> ±0.22	48.90 <sup>a</sup> ±0.58	37.30 <sup>a</sup> ±12.43	40.70 <sup>a</sup> ±6.71	46.60 <sup>a</sup> ±2.00	37.25 <sup>a</sup> ±5.27	43.95 <sup>a</sup> ±2.50
200	37.90 <sup>a</sup> ±4.47	47.10 <sup>ab</sup> ±1.29	41.70 <sup>a</sup> ±6.25	0.75 <sup>b</sup> ±0.43	15.15 <sup>ad</sup> ±6.79	11.85 <sup>b</sup> ±4.99	16.95 <sup>ab</sup> ±5.36	29.90 <sup>a</sup> ±6.23
300	15.65 <sup>a</sup> ±5.30	38.45 <sup>b</sup> ±4.97	30.00 <sup>b</sup> ±6.08	0.30 <sup>a</sup> ±0.30	2.45 <sup>a</sup> ±2.07	8.55 <sup>a</sup> ±5.14	1.85 <sup>a</sup> ±0.82	6.75 <sup>a</sup> ±2.72
400	1.50 <sup>a</sup> ±0.58	17.60 <sup>b</sup> ±4.50	3.20 <sup>a</sup> ±1.38	0.00 <sup>a</sup> ±0.00	2.30 <sup>a</sup> ±1.41	3.05 <sup>a</sup> ±1.25	0.35 <sup>a</sup> ±0.35	2.05 <sup>a</sup> ±0.87
500	0.60 <sup>a</sup> ±0.36	3.10 <sup>ab</sup> ±1.23	0.00 <sup>ab</sup> ±0.00	0.00 <sup>ab</sup> ±0.00	0.25 <sup>a</sup> ±0.25	0.60 <sup>a</sup> ±0.60	0.20 <sup>a</sup> ±0.20	1.30 <sup>a</sup> ±0.90

**Table 3. Results of two-way analysis of variance of rate of germination by salinity (SAL) and chemicals (CHEM). (The F values are significant at  $P < 0.0001$ ).**

Source	Sum of squares	Df	F
Salinity (SAL)	66829.57	5	266.98***
Chemical (CHEM)	7138.56	7	20.37***
SAL * CHEM	7625.55	35	4.35***

Application of kinetin and ethephon successfully alleviated inhibitory effects of salinity from seeds of *L. stocksii* (Fig. 1). Seed germination improved from 42 to 92 % at 300 mM, from 4 to 56 % at 400 mM and from 3 to 11 % at 500 mM NaCl with kinetin. Ethephon enhanced seed germination in 300 mM NaCl and germination improved two-folds from 43 to 83 % (Fig. 1). Seed germination in complete darkness showed an interaction with salinity (Table 1). All seeds germinated in non-saline condition under complete darkness but germination was highly inhibited in saline solutions (Fig. 1). Kinetin partially alleviated salinity effects only at low levels, whereas ethephon could not affect germination in complete darkness (Fig. 1).

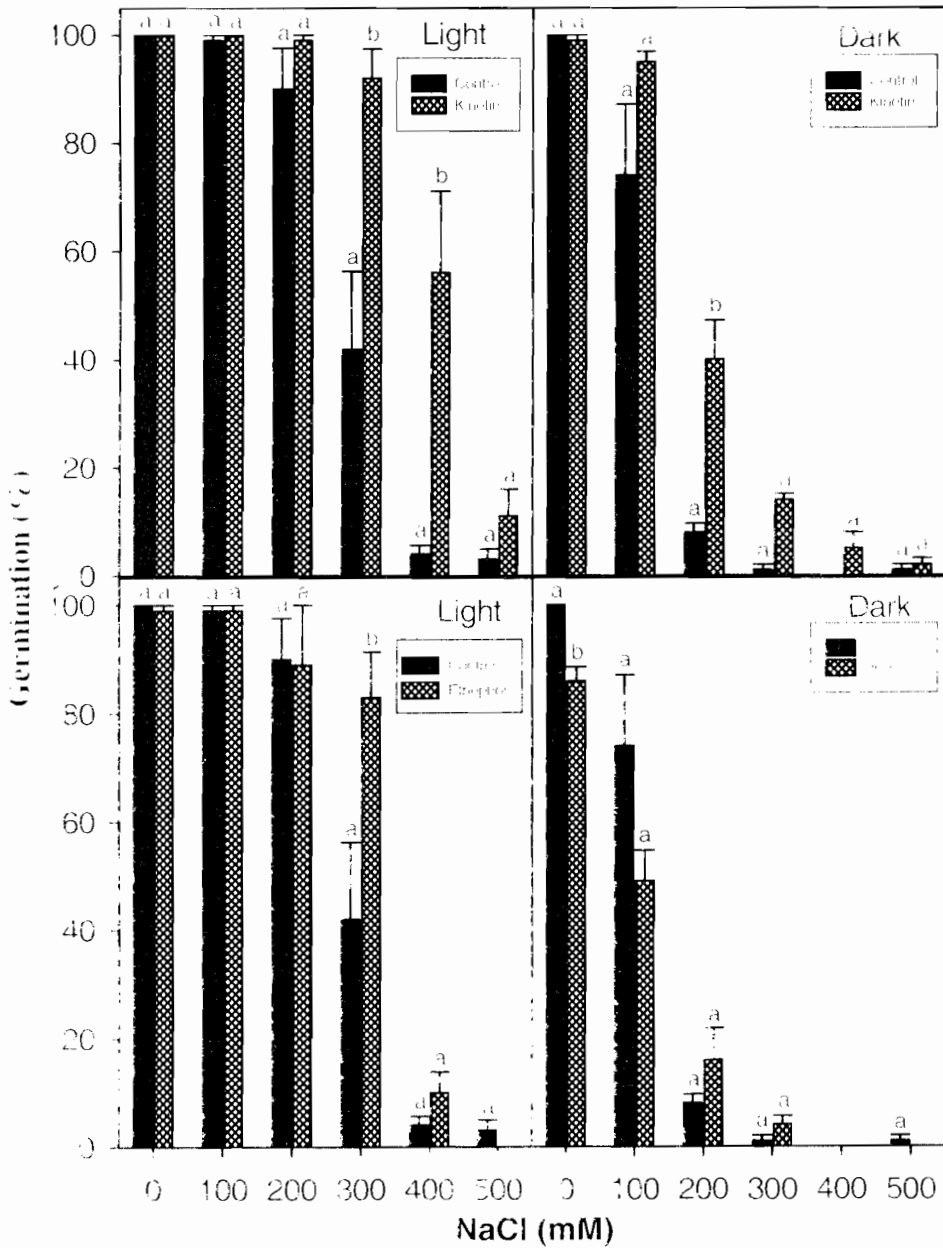


Fig. 1. Effect of kinetin and ethephon in different light and dark conditions on seed germination of *Laminium stocksii*. Mean within each salinity treatment having different letters are significantly different from one another ( $P > 0.05$ , Bonferroni test).

Rate of germination in kinetin and ethephon was higher than non-saline control (Table 2). The 2-way ANOVA of rate of germination indicated significant effects of salinity, chemicals and their interactions (Table 3). Addition of other plant growth regulators used in saline solution could not alleviate salinity induced dormancy from the seeds of *L. stocksii* in both light and dark (Fig. 2 & 3).

## Discussion

Sub-tropical halophytes usually face limitations in the availability of good quality water due to low rainfall and high temperature (above 40°C). The only available water is brackish or seawater. These abiotic stresses (temperature, light and salinity) result in a less than optimal individual plant performance in terms of germination, growth and reproduction (Ungar, 1995). Halophytes have evolved several mechanisms to survive these conditions. Recruitment in the field is usually through vegetative propagation by stolons and rhizomes. Recruitment through seeds is noticed only after monsoon rains when soil salinity is low. Seeds of some local halophytes have the ability to withstand high saline periods in the soil and remain viable (Khan, 2003).

Application of kinetin in saline condition reduced the stress response of *L. stocksii* to a considerable extent and caused the alleviation of salinity enforced dormancy. Kinetin may be a limiting factor under salinity stress for seed germination of *L. stocksii*. Khan & Ungar (2002) also reported 0.05 mM kinetin to be effective in alleviating innate as well as salinity induced dormancy in *Zygophyllum simplex* seeds. Seed germination of other species under saline conditions has also been reported to improve significantly with the application of kinetin (Khan & Ungar, 1985; Khan & Rizvi, 1994; Khan *et al.*, 1998).

Ethephon is known to affect seed germination by releasing ethylene, which reverses the inhibiting effects of PEG and ABA (Kepezyński, 1986). It is also known to break embryo or seed coat imposed dormancy (Satchell & Whitehead, 1995; Kepezyński & Kepezyńska, 1997). There are a number of reports on dormancy breaking ability of ethylene (Kepezyński, 1986; Kepezyński & Kepezyńska, 1997). These reports raise the possibility that the production of ethylene may contribute to the breaking of dormancy in imbibed seeds (Bewley & Black, 1994; Khan & Ungar, 2000). Application of ethephon partially alleviated salinity induced dormancy from the light exposed seeds of *L. stocksii*. Scholbeck & Epley (1981) demonstrated that ethylene action is dependent on availability of light. Partial alleviation of salinity induced dormancy was also obtained for *Zygophyllum simplex*, *Sporobolus arabicus* and *Atriplex griffithii* (Khan & Ungar, 1998, 2000, 2001b, 2002). Ethylene is known to lower the water potential of the germinating seeds causing the removal of dormancy. However, partial alleviation of dormancy indicates that under saline condition water availability is not the only constrain on the seed but an interaction of various abiotic factors is responsible for this inhibition.

Baskin & Baskin (1998) suggested that most salt desert and salt marsh halophytes have some form of physiological dormancy, which is usually alleviated by the application of GA. Giberellic acid is reported to be one of the potent plant growth regulators to alleviate salinity effect on the germination of dicotyledonous halophytes (Khan *et al.*, 1998; Gu *et al.*, 2000; Khan & Ungar, 2001a,b,c). However, GA failed to alleviate salinity enforced dormancy in seeds of *L. stocksii*. Similar results were obtained for *Azusa umbricata* (Mehrun-Nisa, 2003), *Sporobolus arabicus* (Khan & Ungar, 2001b) and *Eragrostis maritima* (Khan & Ungar, 2001a).

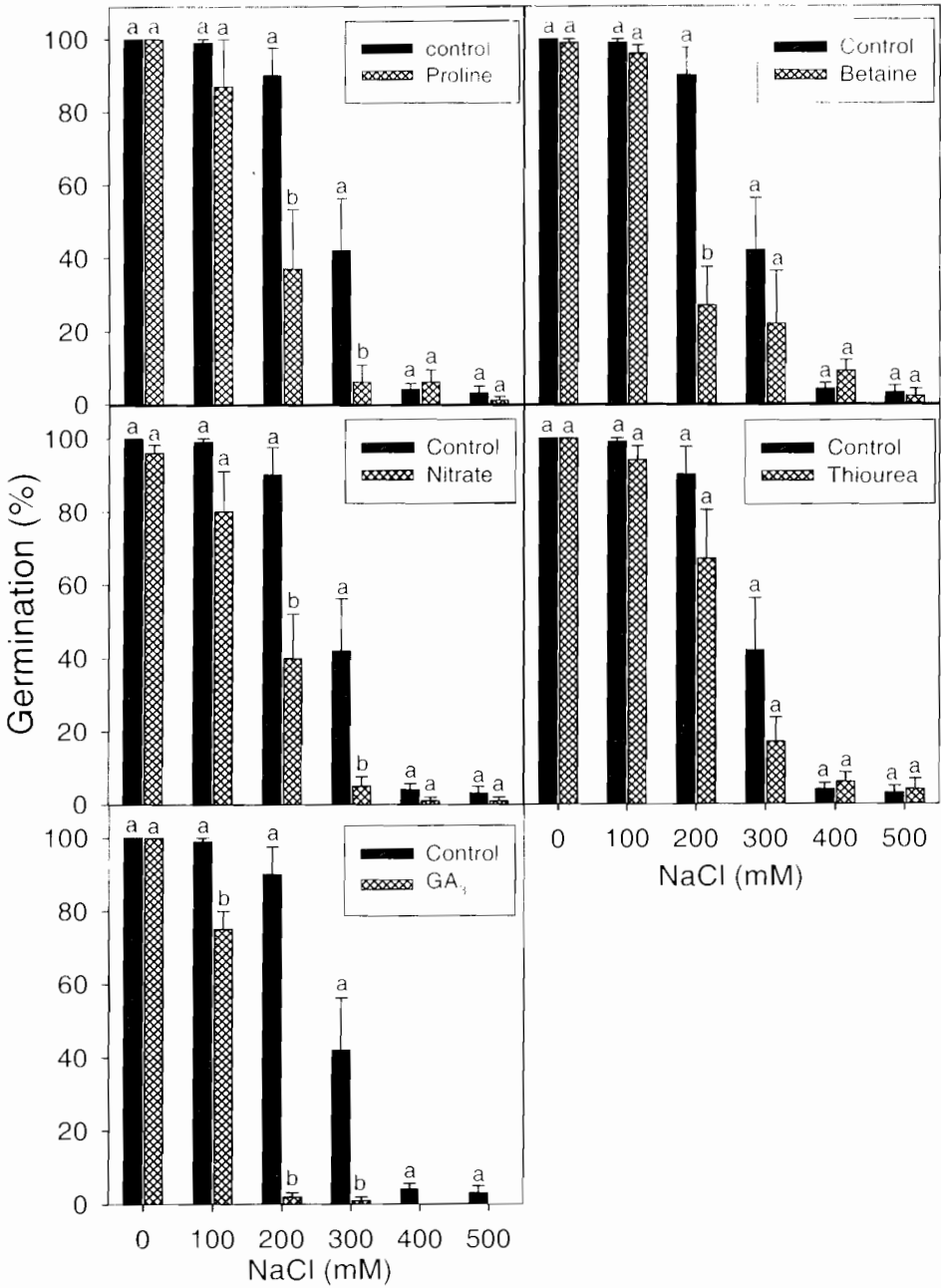


Fig. 2. Effect of different germination regulating chemicals on the seed germination of *L. stocksii* under 12-h photoperiod in different NaCl salinities. (Means within each salinity treatment having different letters are significantly different from one another ( $P < 0.05$ ), Bonferroni test).

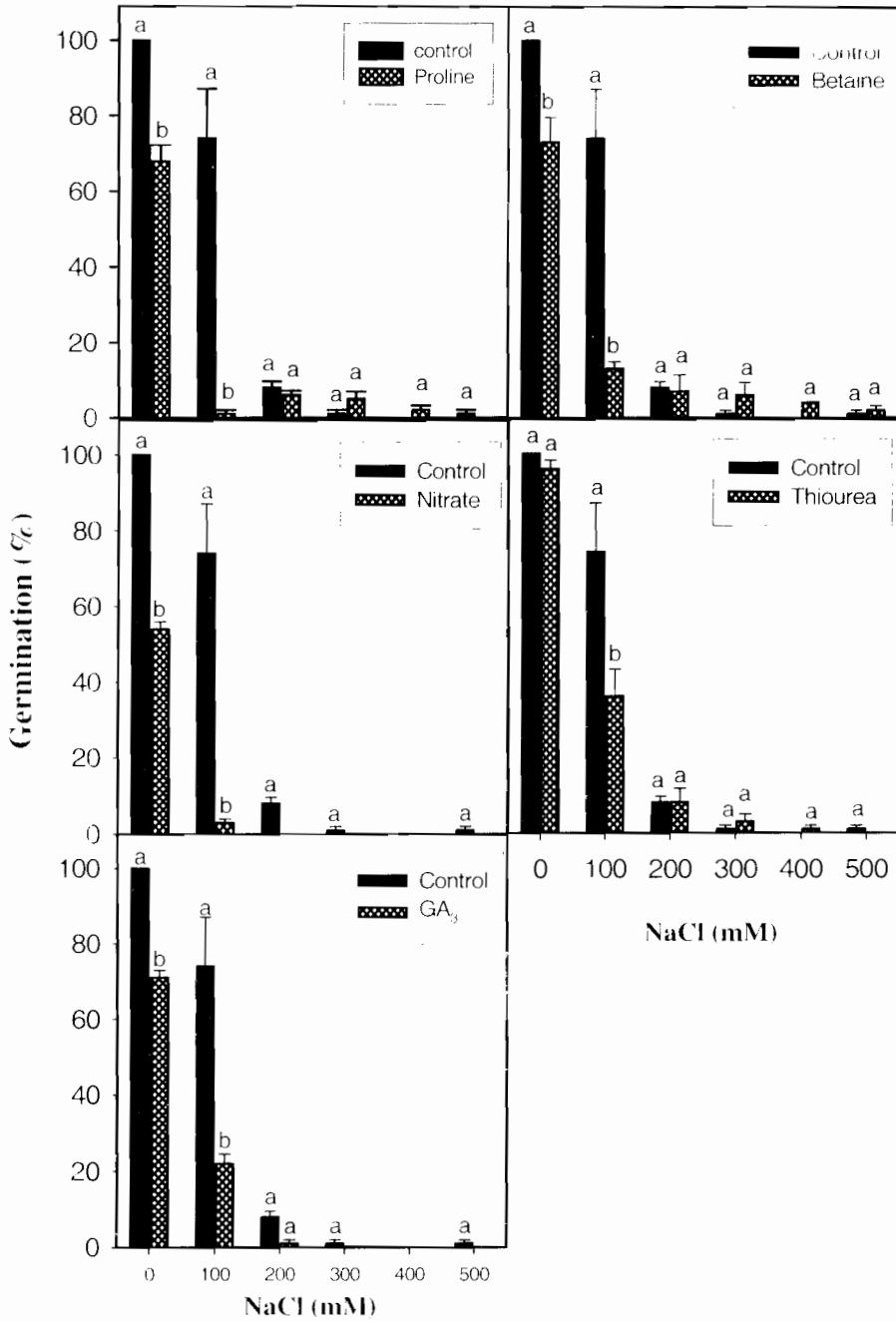


Fig. 3. Effect of different germination regulating chemicals on seed germination of *L. stockii* under complete darkness in different NaCl salinities. (Mean with in each salinity treatment having different letters are significantly different from one another (P<0.05), Bonferroni test).

Compatible osmotica like proline and betaine have some effects in alleviating dormancy (Khan *et al.*, 1998). In *L. stocksii* both the chemicals failed to alleviate salinity-enforced dormancy. Similar results were obtained by Gulzar & Khan (2002) in seeds of *A. lagopoides*, *Urochondra setulosa* and *Sporobolus ioclados*. Potjakoň Mayber *et al.* (1994) found that there were low levels of proline and significant amounts of betaine in dry seeds of *Kosteletzkya virginica*, which may alleviate the inhibitory effects by acting as an osmoregulator or osmoprotectant of proteins in the cytoplasm. However, external application of proline and betaine had no effect on seed germination. Proline and betaine failed to revert high salinity induced dormancy in the seeds of *Zygophyllum simplex* (Khan & Ungar, 1997), *Arthrocnemum macrostachyum* and *Salicornia rubra* (Khan *et al.*, 1998) while dormancy was alleviated in seeds of *Atriplex stocksii* (Khan & Ungar, 2000).

The promotion of seed germination by Nitrogenous compounds such as thiourea, nitrite and nitrate has been reported (Bewely & Black, 1994). They are known to counteract the effect of reduced promoter (cytokinins and gibberellins) and increased inhibitor substances, such as abscisic acid, in seeds when they are exposed to high salt stress (Kabar & Baltepe, 1990). Treatment with thiourea was highly effective in alleviating the inhibition of germination by salinity or high temperature (Gul & Weber, 1998). Nitrogenous compounds had no effect on the germination of *L. stocksii*. Similar results were obtained for *S. imbricata* (Mehrun-Nisa, 2004), *S. ioclados* and *U. setulosa* (Gulzar & Khan, 2002).

*Limonium stocksii* is a perennial woody shrub distributed in coastal areas of Karachi. It produces large number of seeds twice a year, which become a part of the seed bank where they face high temperature and salinity stress. Seeds maintain viability and germinate soon after monsoon rains. Laboratory experiments related to seed germination of *L. stocksii* indicated that seeds germinate as soon as salinity stress is relieved. A wide range of plant growth regulating substances was used to examine their effect on salinity induced dormant seeds. Seed germination of *L. stocksii* was partially alleviated by the application of kinetin and ethephon while other chemicals remained ineffective. This complete or partial failure of dormancy reversal is reported for many subtropical halophytes. Failure to respond to growth regulators in subtropical halophytes could be due to the loss of viability or osmotic and ionic effects of salinity. However, in *L. stocksii* it appears that seeds are prevented from germination due to osmotic effects only without any change to its metabolism, therefore germination promoting chemicals had little effect. This study indicates the need to examine the differential effects of various germination regulating chemicals to understand better the germination inhibition mechanisms under saline conditions.

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