

Short communication

Effect of sea salt and L-ascorbic acid on the seed germination of halophytes

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Abstract

Effects of L-ascorbic acid and sea salt solutions on the seed germination of *Atriplex stocksii*, *Arthrocnemum macrostachyum*, *Haloxylon stocksii*, *Suaeda fruticosa*, *Desmostachya bipinnata* and *Aeluropus lagopoides* were studied. Increasing concentration of sea salt inhibited seed germination of all species with comparatively more adverse effect on *A. stocksii* and *S. fruticosa* than others. Pretreatment of seeds with L-ascorbic acid alleviated the sea salt effects only in these species, i.e. *A. stocksii* and *S. fruticosa*, where as *A. macrostachyum*, *H. stocksii*, *D. bipinnata* and *A. lagopoides* remained unaffected. This indicates the variability of metabolic responses to salinity effects during seed germination.

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Halophytes are exposed to great deal of variations in temperature and salinity and their success is dependent on maintaining viability and their ability to germinate readily when temperature and salinity stress are reduced (Khan and Gul, 2005). It appears that seawater inhibits germination more in comparison to NaCl (De Villiers et al., 1994; Houle et al., 2001) however, the actual mechanism(s) of salinity effect is still unclear (Tester and Bacic, 2005; Vinocur and Altman, 2005). Salinity-induced oxidative stress could be a reason for germination inhibition (Amor et al., 2005). Application of L-ascorbic acid may help in improving germination by neutralizing the excessive superoxide radicals or singlet oxygen. The primary purpose of this investigation was to test the hypothesis that L-ascorbic acid

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pretreatment can completely or partially alleviate sea salt effects on seed germination of halophytes.

Mature inflorescence of *A. stocksii*, *A. macrostachyum*, *S. fruticosa*, *H. stocksii*, *D. bipinnata* and *A. lagopoides* were collected from saline areas of Karachi coast, during March 2003. Seeds were separated from inflorescence, cleaned, surface sterilized using 10% Clorox solution and dry-stored at room temperature. Germination experiments were started on June 2003 using 50-mm dia. tight fitting Petri-plates with 5 ml of test solution. Seeds of the halophytes were pretreated with L-ascorbic acid (0, 20, 40 and 60 mM) for 3 h and air dried before immersing in various sea salt concentrations (0, 10, 20, 30 and 40 dS m⁻¹). Four replicates of 25 seeds each were taken and seeds were considered to be germinated with radical emergence. Germination experiments were carried out in a programmed incubator (Percival) at dark/light thermoperiod of 20/30 °C with a 12-h photoperiod (25 μmol m⁻² s⁻¹, 400–700 nm Sylvania cool white florescent lamps). Percent germination was recorded after every 48 h for 20 d. Rate of germination was estimated by using modified Timson's index of germination velocity (Khan and Ungar, 1984). 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 (SPSS, 2001).

Final germination and rate of germination decreased linearly ($P < 0.001$) with an increase in sea salt concentrations for all six halophytes. Maximum germination was observed in non-saline controls (Fig. 1). Similar results were reported by De Villiers et al. (1994), Houle et al. (2001) and Zia and Khan (2002). Germination failure under saline conditions has been attributed to lowering of soil water potential and/or ionic toxicity (Katembe et al., 1998; Bajji et al., 2002), but recently this effect was also ascribed to oxidative stress induced by salinity (Amor et al., 2005; Demiral and Turkan, 2005).

Effects of L-ascorbic acid pretreatments on seed germination of six halophytic species were found to be species-specific. L-ascorbic acid pretreatments alleviated the inhibitory effect of sea salt on seed germination of *Atriplex stocksii*, ($F = 18.3$, $p < 0.001$) more than other species where both mean final germination and rate of germination were significantly ($F = 95.5$, $P < 0.001$) improved by L-ascorbic acid pretreatments at all salinity levels (Fig. 2). L-ascorbic acid had little effect at low salinity in *S. fruticosa*; however, it partially alleviated salinity effect ($F = 244.7$, $P < 0.001$) at concentrations higher than 20 dS m⁻¹ (Fig. 3, Table 1). While, in *A. macrostachyum*, *H. stocksii*, *D. bipinnata* and *A. lagopoides*, L-ascorbic acid completely failed in improving seed germination (data not shown) under saline conditions. At germination level reactive oxygen species (ROS) are generated mainly during depletion of food reserves and oxidative phosphorylation (Crowe and Crowe, 1992) but their quantitative level is controlled by seed's protective antioxidant system. However, it has been reported that seeds contain various antioxidants in small amounts and compounds like ascorbic acid are not present (Gidrol et al., 1994; Asada, 1997). Thus under stress situations such as salinity, this protective antioxidant system becomes inefficient and may lead to germination failure and even seed death. An artificial increase in cellular level of an antioxidant such as ascorbic acid should theoretically be beneficial in improving stress tolerance at germination level. While in certain cases seed germination was enhanced by oxidizing germination inhibitor(s) through oxidants such as H₂O₂, O⁻², OH⁻ and free radicals under low stresses (Ogawa and Iwabuchi, 2001). This scattered available information suggests that the reason for variable germination responses may be

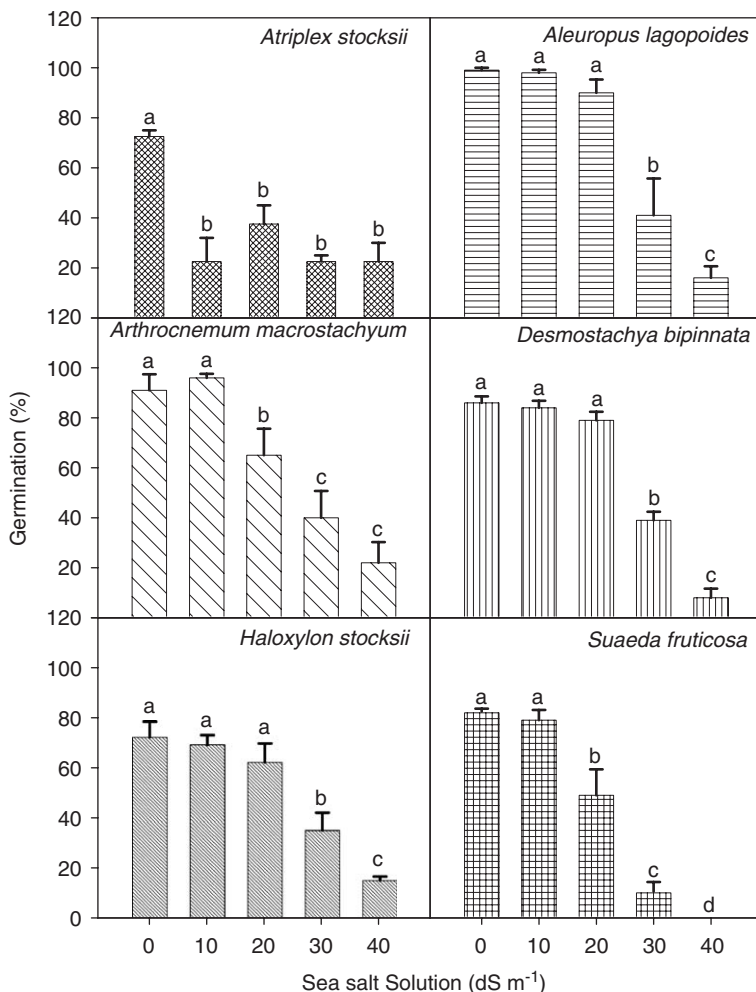


Fig. 1. Mean (\pm S.E.) final germination percentages of *Atriplex stocksii*, *Aleuropus lagopoides*, *Arthrocnemum macrostachyum*, *Desmostachya bipinnata*, *Haloxylon stocksii*, *Suaeda fruticosa*, seeds in 0, 10, 20, 30, and 40 dS m⁻¹ sea salt at thermoperiods of 20–30 °C. Values for each salinity concentration having the same letter are not significantly different ($P > 0.05$).

related to their seed structure and chemical composition. Enzyme(s) and/or accessory antioxidants facilitating ascorbic acid in scavenging ROS might be absent in species that did not respond to L-ascorbic acid pretreatments. Similarly genes coding for the synthesis of enzymes essential for ascorbic acid activities might require a differential stress level to be activated, thus, in *Suaeda fruticosa* seed germination was promoted by additional supplies of ascorbic acid at higher salinities only, unlike *Atriplex stocksii*, where ascorbic acid pretreatments promoted germination at all salinity levels. Detailed studies on components of antioxidant defense systems of seeds of these species might provide an appropriate reason for this.

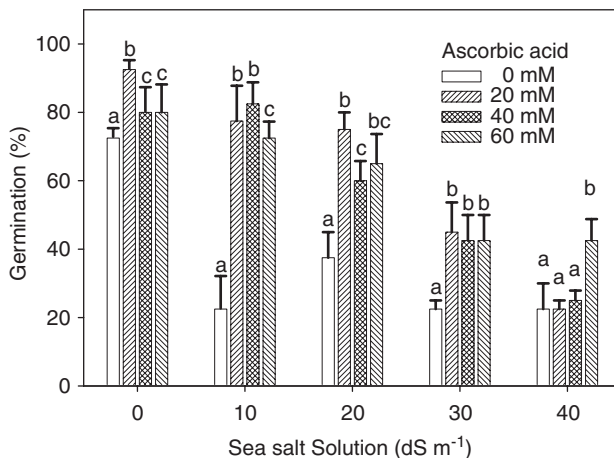


Fig. 2. Mean (\pm S.E.) final germination percentages of seeds of *A. stocksii*, in 0, 10, 20, 30 and 40 dS m⁻¹ sea salt solutions at 0, 20, 40 and 60 mM Ascorbic acid pretreatments. Values for each salinity concentration having the same letter are not significantly different ($P > 0.05$).

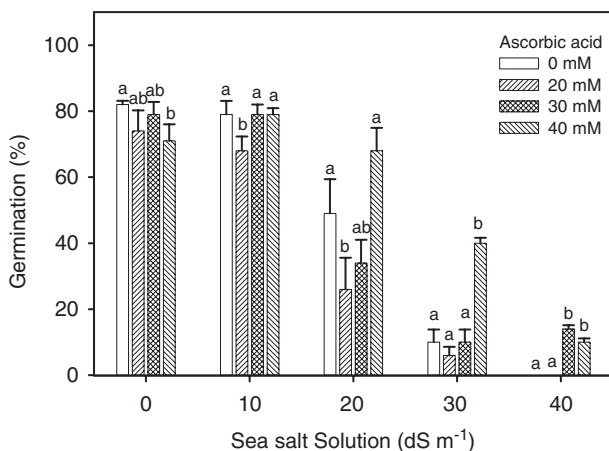


Fig. 3. Mean (\pm S.E.) final germination percentages of seeds of *S. fruticosa* in 0, 10, 20, 30 and 40 dS m⁻¹ sea salt solutions at 0, 20, 40 and 60 mM ascorbic acid pretreatments. Values for each salinity concentration having the same letter are not significantly different ($P > 0.05$).

Sea salt solution inhibited seed germination of all species. Pretreatment of seeds with antioxidant (L-ascorbic acid) alleviated sea salt effects on germination in some species while in others there was no effect. This perhaps indicates that a complete set of antioxidant defense system, rather than a single antioxidant, is responsible for protection in stressed plant (Foyer et al., 1994). Thus attempts to increase stress tolerance by simply increasing the concentration of single antioxidants in plants may not always be successful. These findings, however, could be useful in cultivating selected cash-crops under saline conditions by providing them opportunity to germinate under highly saline conditions.

Table 1

Effect of L-ascorbic acid pretreatments on rate of germination (mean \pm S.E.) of *A. stocksii* and *S. fruticosa* seeds, in 0, 10, 20, 30 and 40 dS m⁻¹ sea salt solutions

Species	Sea salt (dS m ⁻¹)	Ascorbic acid pretreatments (mM)			
		0	20	40	60
<i>Atriplex stocksii</i>	0	34 \pm 1.6 ^a	45 \pm 1.5 ^a	36 \pm 3.5 ^a	36 \pm 4.4 ^a
	10	10 \pm 4.7 ^b	37 \pm 4.9 ^{ab}	40 \pm 2.5 ^a	35 \pm 2.6 ^a
	20	18 \pm 3.6 ^{bc}	35 \pm 3.7 ^b	26 \pm 3.0 ^b	30 \pm 3.8 ^a
	30	8 \pm 2.1 ^b	19 \pm 2.9 ^c	17 \pm 4.2 ^c	19 \pm 3.5 ^b
	40	8 \pm 2.9 ^b	8 \pm 0.9 ^d	11 \pm 1.3 ^c	18 \pm 2.9 ^b
<i>Suaeda fruticosa</i>	0	36 \pm 0.8 ^a	31 \pm 3.0 ^a	34 \pm 1.7 ^a	30 \pm 1.6 ^a
	10	33 \pm 1.7 ^a	27 \pm 1.2 ^a	30 \pm 1.0 ^a	32 \pm 0.5 ^a
	20	17 \pm 3.6 ^b	9 \pm 3.6 ^b	11 \pm 2.5 ^b	24 \pm 2.5 ^b
	30	2 \pm 0.8 ^c	1 \pm 0.3 ^c	2 \pm 0.8 ^c	10 \pm 0.3 ^c
	40	0 \pm 0.0 ^d	0 \pm 0.0 ^d	2 \pm 0.2 ^c	2 \pm 0.2 ^d

Values for each L-ascorbic acid pretreatment having the same letter are not significantly different ($P > 0.05$).

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