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# Salinity inhibits seed germination of perennial halophytes *Limonium stocksii* and *Suaeda fruticosa* by reducing water uptake and ascorbate dependent antioxidant system

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ABSTRACT

Information about production and detoxification of reactive oxygen species during seed germination of halophytes under saline conditions is scanty. We therefore studied levels of common oxidative stress markers, antioxidant substances and antioxidant enzyme activities in germinating seeds of two subtropical coastal halophytes *Limonium stocksii* and *Suaeda fruticosa* under various NaCl (0, 200 and 400 mM) treatments. Mature seeds of both species lacked reduced ascorbate (AsA) in dry state. However, glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX) and glutathione reductase (GR) were detected in dry seeds of both species. Higher and rapid germination was noted in *L. stocksii* seeds compared to *S. fruticosa* in distilled water. Ascorbate (AsA) was detected in water imbibed seeds of both species, along with increase in GSH levels and SOD, APX and GR activities during germination in distilled water. Germination and hydration of the seeds of both species decreased with increases in NaCl concentration. CAT and GPX activities were higher while APX, AsA and GSH decreased in salt stressed seeds compared to water-imbibed seeds. Thus, it appears that the salinity inhibits seed germination of *L. stocksii* and *S. fruticosa* by reducing water uptake and compromising ascorbate dependent antioxidant system.



## 1. Introduction

Seed germination (*sensu stricto*) includes all those physiological, cellular, biochemical and molecular events which are necessary for the protrusion of embryonic axis (Nonogaki et al., 2010; Bewley et al., 2013). The reactivation of metabolism upon water uptake especially the oxidative phosphorylation and a gradual transition of damaged mitochondrial membranes from gel to liquid-crystalline state result in an oxidative burst through the production of excessive reactive oxygen species (ROS) before the resumptions of regular mitochondrial activity (Crowe and Crowe, 1992; Tommasi et al., 2001; Noctor et al., 2007; Nonogaki et al., 2010). Plasma membrane bound NADPH oxidases, peroxisomes and glyoxisomes are other sources of ROS during seed germination (Huang et al., 1983; Lamb and Dixon, 1997; Grant and Loake, 2000). Higher concentrations of ROS damage cellular lipids, proteins and nucleic acids (Gill and Tuteja, 2010), therefore germination of seeds could only be achieved if ROS production is properly managed (De Gara et al., 1997; Tommasi et al., 2001; Khan et al., 2006; Sekmen et al., 2012). Tightly regulated levels of ROS constitute an "oxidative window" under optimum conditions which is essential for seed germination (Bailly, 2004; Bailly et al., 2008; El-Maarouf-Bouteau and Bailly, 2008). This is achieved through seeds antioxidant defense system which consists of both enzymatic and non-enzymatic antioxidants (Tommasi et al., 2001; De Tullio and Arrigoni, 2003). Such controlled accumulation of ROS is reported to facilitate seed germination through cell wall loosening (Müller et al., 2009), signaling (El-Maarouf-Bouteau and Bailly, 2008) and/or decreasing abscisic acid levels (Wang et al., 1995, 1998). However, little is known about such systems in salt stressed seeds more so of halophytes (Khan et al., 2006; Bogdanović et al., 2008; Kranner and Seal, 2013).

*Abbreviations:* APX, ascorbate peroxidase; AsA, ascorbate; CAT, catalase; DHA, dehydroascorbate; FW, fresh weight; GPX, guaiacol peroxidase; GR, glutathione reductase; GSH, glutathione reduced; GSSG, glutathione oxidized; $H_2O_2$, hydrogen peroxide; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase; $W_f$, relative increase in fresh weight of seeds.

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Halophyte seeds can germinate at salinity levels where seeds of all other species including crops do not have any chance (Vallejo AJ et al., 2010; Gul et al., 2013; Kranner and Seal, 2013). This seed germination under highly saline conditions may be attributed to the efficient regulation of ROS production (Khan et al., 2006). To test this hypothesis we studied the levels of hydrogen peroxide ($H_2O_2$; a common ROS), malondialdehyde (MDA; a common oxidative stress marker), concentrations of common antioxidant substances and activities of some antioxidant enzymes during seed germination "*sensu stricto*" of two subtropical halophytes *Suaeda fruticosa* (Amaranthaceae) and *Limonium stocksii* (Plumbaginaceae) with and without NaCl treatments.

## 2. Materials and methods

### 2.1. Test species and seed collection site

*S. fruticosa* Forssk. is a leaf succulent halophyte from family Amaranthaceae, commonly found in both coastal and inland saline habitats of Pakistan, while *L. stocksii* (Boiss.) Kuntze (Plumbaginaceae) is a salt secreting plant from family Plumbaginaceae, which is found in coastal salt flats of Pakistan (Khan and Qaiser, 2006). Seed-bearing inflorescences of both species were collected from a coastal salt-flat near Hawks bay, Karachi, Pakistan (24°52′21.87″ N, 66°51′24.58″ E). Seeds were separated from inflorescence, surface sterilized by using 1% sodium hypochlorite for 1 min (no effect on germination of either species when treated for 1 min but extended application may improve seed coat permeability; Zia and Khan, 2007; Hameed et al., 2009), rinsed with distilled water and air-dried. Seeds were then stored at room temperature in clear plastic Petri-plates until use.

### 2.2. Seed characteristics

Fresh weight (FW) of 100 freshly collected seeds was recorded and moisture content was determined by drying them in a forced-draft oven at 105 °C for 48 h.

### 2.3. Seed germination experiments

Germination experiments were carried out in programmed incubators (Percival, USA) with 30/20 °C day/night temperature regimes and 12-h photoperiod (Philips fluorescent lamps, 25 μmol $m^{-2}$ $s^{-1}$, 400–700 nm). Tight fitting Petri-plates (50 mm Φ) with clear lids were used. There were three NaCl concentrations (0, 200 and 400 mM) with four replicates of 25 seeds each. Percent germination (embryo protrusion from seeds; Bewley and Black, 1994) was recorded after every 48 h for a period of 20 days. Rate of germination was calculated using a modified Timson index of germination velocity i.e. $\Sigma G/t$, where $G$ is germination percentage after every 48 h and $t$ is the total germination period (Khan and Ungar, 1984). Maximum value possible for this index is 50 for our data (i.e., 1000/20). The higher the value, the more rapid is the germination. After completion of germination experiment, all un-germinated seeds from various NaCl concentrations were transferred to distilled water for another 20 days to study recovery of germination. Germination recovery percentage was calculated by the following formula: $[(a-b)/(c-b)\times 100]$, where '*a*' is the number of seeds germinated after transfer to distilled water, '*b*' is the number of seeds germinated in saline solution, and '*c*' is the total number of seeds.

### 2.4. Seed hydration experiments

Seeds were immersed in 0, 200 and 400 mM NaCl and relative increase in fresh weight of seeds ($W_r$) was calculated following Song et al. (2005) after 18 and 23 h (time required for embryo protrusion in distilled water) for *L. stocksii* and *S. fruticosa* seeds respectively.

### 2.5. Biochemical analyses

Biochemical analyses given below were carried out in seeds at different stages like dry condition, early imbibition, maximal imbibition and embryo protrusion in distilled water and NaCl solutions in *L. stocksii* (0, 3, 12 and 18 h) and *S. fruticosa* (0, 3, 17 and 23 h).

#### 2.5.1. Hydrogen peroxide and malondialdehyde

Seeds were ground fine using liquid nitrogen in ice-chilled mortar and pestle and homogenized in 1% ice-cold trichloroacetic acid. Homogenate was then centrifuged at 12,000 × *g* for 20 min at 4 °C. Supernatant was used for the estimation of hydrogen peroxide ($H_2O_2$; Loreto and Velikova, 2001) and malondialdehyde (MDA; Heath and Packer, 1968).

### 2.6. Antioxidant substances

Contents of ascorbate (AsA) and dehydroascorbate (DHA) in TCA extracts were determined following the method of Law et al. (1983). While, reduced (GSH) and oxidized (GSSG) glutathione contents were determined according to the method of Anderson (1985).

### 2.7. Antioxidant enzymes

Extraction and assays of different antioxidant enzymes superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), guaiacol peroxidase (GPX, EC 1.11.17), ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2) were done using the methods described in Hameed et al. (2012).

### 2.8. Statistical analyses

Germination data were arcsine transformed before statistical analyses to ensure homogeneity of variance. Analyses of variance (ANOVA) were used to test whether the treatments had significant effect on germination and other parameters. Significant differences among individual means were determined using a Bonferroni test (SPSS version 11.0 for windows, 2001).

## 3. Results

### 3.1. Seed characteristics

Fresh weight (FW) of 100 *S. fruticosa* seeds was 37.35 ± 0.35 mg and for *L. stocksii* was 29.25 ± 0.95 mg. Moisture contents of freshly collected seeds of *S. fruticosa* and *L. stocksii* were 2.42 ± 0.14 and 1.99 ± 0.21%, respectively.

### 3.2. Seed germination

Seeds of *L. stocksii* (97%) and *S. fruticosa* (81%) readily germinated in distilled water (Fig. 1A). Rate of germination was 47 in *L. stocksii* and 30 in *S. fruticosa* (Fig. 1B). Seed germination of both species significantly decreased with an increase in NaCl concentration (Table 1, Fig. 1A). Most un-germinated seeds of both species from saline treatments showed recovery of germination, after transfer to distilled water (Fig. 1C).

### 3.3. Seed hydration

Embryo extension in seeds of *L. stocksii* began after 18 h of imbibition when an increase in 96% of fresh weight ($W_r$) was attained (Fig. 2). However, only 72% increase was recorded in seeds imbibed