Environmental and Experimental Botany 107 (2014) 32–38

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

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

Abdul Hameed [a], Aysha Rasheed [a], Bilquees Gul [a], M. Ajmal Khan [b,*]

[a] Institute of Sustainable Halophyte Utilization (ISHU), University of Karachi, Karachi 75270, Pakistan
[b] Department of International Affairs, College of Arts and Sciences, Qatar University, PO Box 2713, Doha, Qatar

ARTICLE INFO

Article history:
Received 15 November 2013
Received in revised form 11 March 2014
Accepted 17 April 2014

Keywords:
Antioxidant
Germination
Halophyte
Oxidative stress
Reactive oxygen species

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.

* Corresponding author. Tel.: +974 4403 4952; fax: +974 4403 4931.
E-mail addresses: ahameed@uok.edu.pk (A. Hameed), halophyte_aysha@yahoo.com (A. Rasheed), bilqueesgul@uok.edu.pk (B. Gul), ajmal.khan@qu.edu.qa, ajmal.khan@qu.edu.qa (M.A. Khan).

http://dx.doi.org/10.1016/j.envexpbot.2014.04.005
0098-8472/

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

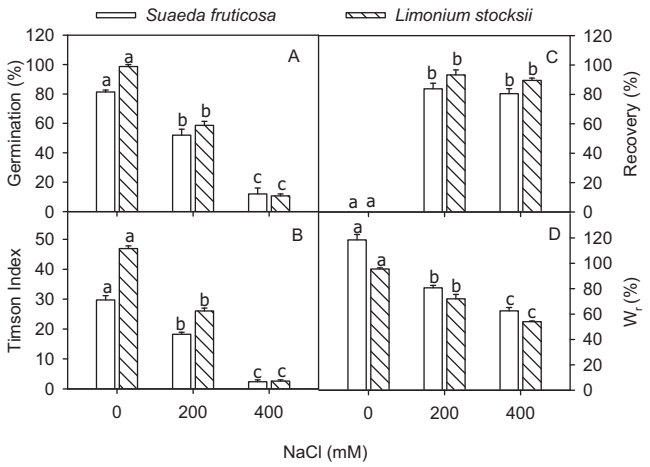

Fig. 1. Final germination, rate of germination (Timson index), recovery of germination and relative water uptake (W_r) of *S. fruticosa* and *L. stocksii* seeds in different NaCl concentrations. Bars represent mean ± standard error. Different Bonferroni letters ($P < 0.05$) indicate significant differences among the individual means for each species.

Table 1
ANOVA results for the effect of salt stress on final germination percentage (FG), rate of germination (RG) and relative water uptake (W_r) of *S. fruticosa* and *L. stocksii* seeds. (Numbers representing *F-values* are significantly different at $P < 0.0001$).

Species	Parameters		
	FG	RG	W_r
Suaeda fruticosa	107.57	190.36	210.42
Limonium stocksii	546.00	775.81	381.79

in 200 mM NaCl and only 54% in 400 mM NaCl (Table 1; Fig. 1D). Seed embryo extension in *S. fruticosa* started after 23 h of imbibition in distilled water with W_r value of about 118%, which was decreased to about 81% in 200 mM NaCl and 63% in 400 mM (Fig. 1D and 2).

3.4. Hydrogen peroxide and malondialdehyde

A two-way ANOVA indicated a significant effect of salinity, time and their interaction on endogenous H_2O_2 in both species (Table 2). Endogenous hydrogen peroxide (H_2O_2) increased during

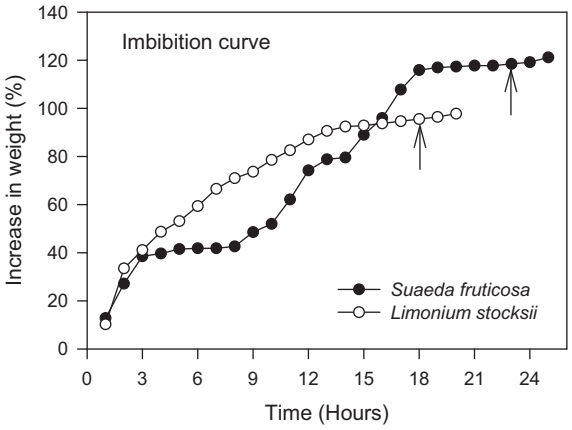

Fig. 2. Imbibition curves of *S. fruticosa* and *L. stocksii* seeds in distilled water. Arrow marks indicate start of radicle protrusion. Data is given as mean of different replicates ($n = 4$).

seed germination with ~1.5 fold higher values in *S. fruticosa* in distilled water (Fig. 3A and B). H_2O_2 content further increased with increases in NaCl concentration in both test species.

Malondialdehyde (MDA) content in water-imbibed seeds of *S. fruticosa* did not change with time (Fig. 3C). However, MDA content in water imbibed seeds of *L. stocksii* significantly increased after 3 h compared to dry seeds and no subsequent change was recorded (Fig. 3D). Malondialdehyde content increased with increases in salinity in both species in comparison to distilled water (Fig. 3C and D).

3.5. Antioxidant substances

A two-way ANOVA indicated significant ($P < 0.001$) effects of salinity, time and their interaction on endogenous ascorbate (AsA) contents in both species (Table 2). Ascorbate was not detected in dry seeds of both species however it was detected 17 h after water imbibition in *S. fruticosa* and 12 h in *L. stocksii* AsA (Fig. 4A and C). Ascorbate content was significantly ($P < 0.05$) lower in 200 mM NaCl than control while absent in 400 mM NaCl in seeds of both species (Fig. 4A and C). Dehydroascorbate (DHA) was present in dry seeds of both species, which increased further with imbibition (Fig. 4B and D). Contents of DHA decreased upon NaCl exposure in both species.

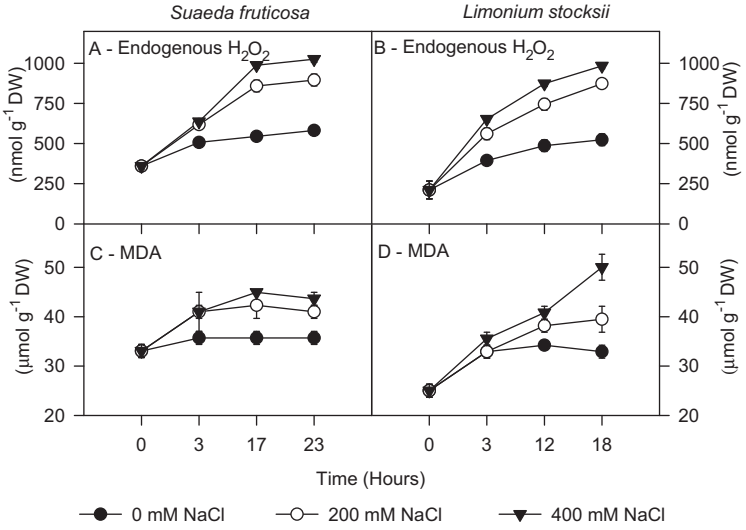

Fig. 3. Endogenous H_2O_2 and MDA contents in germinating seeds of *S. fruticosa* and *L. stocksii* in different NaCl concentrations at different time intervals. Data is given as mean ± standard error ($n = 4$).

Table 2
A Two-way ANOVA for the effects of salt stress (*S*), time (*T*) and their interaction on different parameters of *S. fruticosa* and *L. stocksii* seeds. (Numbers represent *F-values*.).

Parameters	*Suaeda fruticosa*			*Limonium stocksii*		
	Time (*T*)	Salt (*S*)	*T* × *S*	Time (*T*)	Salt (*S*)	*T* × *S*
H_2O_2	103.41***	179.63***	16.52***	50.33***	105.33***	3.16*
MDA	0.67ns	13.16**	0.33ns	14.29*	22.51***	5.63*
AsA	4731***	3741***	1303***	6942***	7598***	2912***
DHA	26.33***	35.79***	7.38**	72.96***	72.19***	7.84**
GSH	329.69***	834.99***	25.57***	907.88***	1188***	100.01***
GSSG	1.20ns	139.39***	0.11ns	34.69***	17.00***	7.77**
CAT	0.37ns	55.81***	1.16ns	612.86***	674.05***	240.03***
GPX	50.23***	54.87***	12.61***	24.26***	2.93ns	26.64***
APX	201.31***	44.57***	15.32***	28.32***	207.63***	1.54ns
GR	25.51***	20.07***	9.25**	262.80***	2.33 ns	6.33*
SOD	125.78***	2.69ns	4.79*	54.80***	18.72***	1.35ns

ns, non-significant.
* $P < 0.01$.
** $P < 0.001$.
*** $P < 0.0001$.

ANOVA indicated a significant ($P < 0.001$) effect of salinity, time and their interaction on GSH content in both species (Table 2). Reduced glutathione (GSH) was present in dry seeds of both species but the amount in *S. fruticosa* was twice that of *L. stocksii* (Fig. 5A and C). Glutathione increased with time during germination in distilled water as well as in NaCl solutions in both species. However this increase with time was lower under saline conditions (Fig. 5A and C). Oxidized glutathione (GSSG) was significantly ($P < 0.05$) lower than GSH in dry seeds of both species and further declined as seeds imbibed water (Fig. 5B and D). NaCl treatments generally slowed down the temporal decline in GSSG contents.

3.6. Antioxidant enzymes

Superoxide dismutase (SOD) activity increased linearly with time during water imbibition in seeds of both species (Fig. 6A and B). Superoxide dismutase activity was higher in seeds of *S. fruticosa* after 23 h of imbibition in saline solutions compared to 0 mM NaCl and a similar increase was noticed in seeds of *L. stocksii* only at 400 mM NaCl after 18 h of imbibition (Fig. 6A and B).

Highest catalase (CAT) activity in seeds of *L. stocksii* was recorded after 3 h of imbibition and later decreased while little changes in CAT activity were found in seeds of *S. fruticosa* (Fig. 6C and D). Catalase activity increased significantly ($P < 0.05$) in both species with the increase in NaCl concentration (Fig. 6C and D).

Guaiacol peroxidase (GPX) activity increased significantly ($P < 0.05$) 3 h after water imbibition in seeds of *S. fruticosa* but no change was noticed at subsequent time intervals (Fig. 6E). Guaiacol peroxidase activity in *L. stocksii* did not change for 12 h but decreased significantly after 18 h of imbibition (Fig. 6F). Guaiacol peroxidase activity increased in seeds of both species when imbibed in increasing concentration of NaCl with higher values in *S. fruticosa* (Fig. 6E and F).

Ascorbate peroxidase (APX) activity increased progressively with time in water imbibed seeds of both species (Fig. 6G and H). Increases in NaCl concentration decreased APX activity in *L. stocksii* while in *S. fruticosa* this decrease was recorded only in 400 mM (Fig. 6G and H).

Glutathione reductase (GR) activity increased with time in water-imbibed seeds of both species with higher values in *L. stocksii* (Fig. 6I and J). There was no effect of NaCl on GR activity in *L. stocksii* but it increased in *S. fruticosa* at 400 mM NaCl after 17 and 23 h of imbibition (Fig. 6I and J).

4. Discussion

4.1. Characteristics and antioxidant system of dry seeds

Our results indicate that the fresh seeds of both *S. fruticosa* and *L. stocksii* had low levels of moisture, H_2O_2 and MDA in their dry

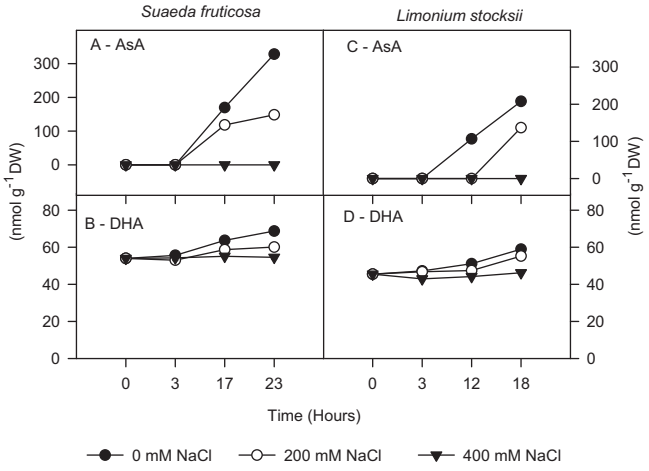

Fig. 4. Endogenous AsA and DHA contents in germinating seeds of *S. fruticosa* and *L. stocksii* in different NaCl concentrations at different time intervals. Data is given as mean ± standard error ($n = 4$).

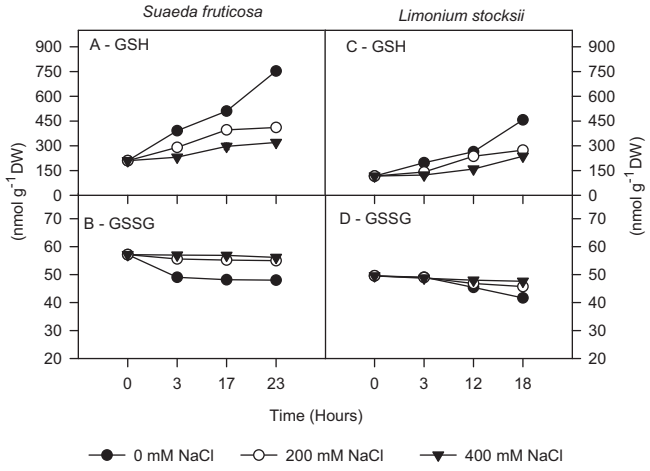

Fig. 5. Endogenous GSH and GSSG contents in germinating seeds of *S. fruticosa* and *L. stocksii* in different NaCl concentrations at different time intervals. Data is given as mean ± standard error ($n = 4$).

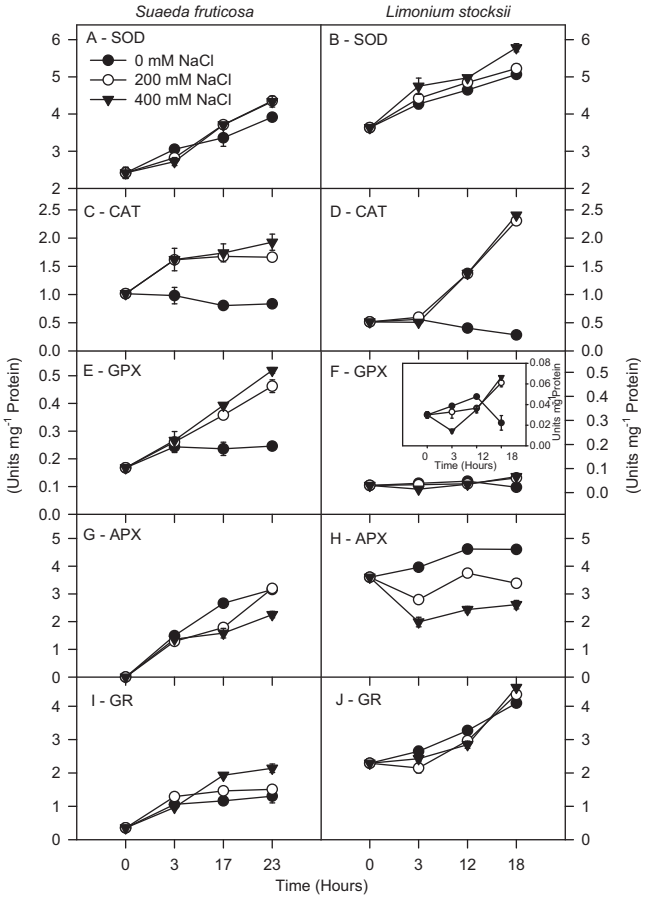

Fig. 6. Antioxidant enzyme activities in germinating seeds of *S. fruticosa* and *L. stocksii* in different NaCl concentrations at different time intervals. Data is given as mean ± standard error ($n = 4$).

state. Amadori and Maillard reactions and lipid autoxidation may be responsible for ROS production and MDA accumulation in dry seeds (McDonald, 2004; Varghese and Naithani, 2007; Bailly et al., 2008). Dry seeds generally lack AsA and APX due to their gradual depletion during maturation drying however, ASA-independent antioxidant system such as SOD and CAT may be present (De Tullio and Arrigoni, 2003; Chen and Arora, 2011). However, such information for halophyte seeds is scanty. Our experiments revealed that the dry seeds of *S. fruticosa* lacked ASA and APX with some APX in *L. stocksii* while both species had higher GSH, SOD, CAT, GPX and GR contents. Weitbrecht et al. (2011) indicated that due to low moisture in dry seeds antioxidant enzyme activities are limited/impossible in most parts of the seeds and GSH probably plays the major antioxidant role.

4.2. Germination and antioxidant system in un-stressed seeds

Seeds of coastal halophytes *S. fruticosa* and *L. stocksii* germinated quickly within 24 h in distilled water. Similarly, seeds of *Salsola affinis* and *Suaeda acuminata* also displayed fast germination and were referred to have "opportunistic strategy" (Wei et al., 2008; Wang et al., 2012). Such a strategy might be useful for quickly exploiting the brief germination window available after sufficient rainfall (Gul et al., 2013; Parsons, 2012).

The reactivation of mitochondrial metabolism during seed germination accompanies ROS production (Pergo and Ishii-Iwamoto, 2011). Likewise, we also observed increase in H_2O_2 levels with time in water-imbibed seeds of both *S. fruticosa* and *L. stocksii*. However, this rise in H_2O_2 levels did not correspond to increase in MDA (oxidative damage marker) perhaps indicating the beneficial roles of H_2O_2 during seed germination as reported for other species (Bailly, 2004; Ishibashi et al., 2012, 2013; Kranner and Seal, 2013).

Ascorbate (AsA) was detected in seeds of both test species soon after attaining complete hydration and continued to increase up to embryo protrusion. Dučić et al. (2003) also reported similar response of the *Chenopodium rubrum* seeds. This increase in AsA could indicate its de novo synthesis (Tommasi et al., 2001) along with recycling from the oxidized form (De Tullio and Arrigoni, 2003). Dehydroascorbate (DHA) contents of *S. fruticosa* and *L. stocksii* seeds also increased during germination, which is in accordance with the finding of De Gara et al. (1997) who observed a similar increase in DHA contents of germinating seeds of wheat. However, this increase was not as much as in case of AsA and at the time of radicle emergence contents of AsA were significantly higher than DHA in both *S. fruticosa* (~4.5 times) and *L. stocksii* (~3 times) seeds. According to Tommasi et al. (2001) increase in AsA during germination is mainly due to the reactivation of its biosynthesis. Ascorbate is also a cofactor for enzymes which synthesize the germination regulating hormones, gibberellins and ethylene (De Tullio and Arrigoni, 2003) and its synthesis during seed germination may promote subsequent cell division for seedling formation (Noctor and Foyer, 1998; De Tullio et al., 1999; De Tullio and Arrigoni, 2003).

Reduced glutathione (GSH) is another non-enzymatic antioxidant which increased progressively in both test species with time after imbibition. GSH was also reported to increase in seeds of pine (Tommasi et al., 2001) and pea (Spragg et al., 1962)) during the initial 24 h of germination period and its concentration peaked at the time of radicle emergence in seed of *C. rubrum* (Dučić et al., 2003). Oxidized form of glutathione (GSSG) declined with time in water-imbibed seeds of both species, which could indicate its recycling to the reduced form (GSH). Likewise, decrease in GSSG along with increase in GSH was observed in germinating seeds of pine (Tommasi et al., 2001).

Superoxide dismutase, APX and GR activities increased while CAT and GPX activities decreased with time in water-imbibed seeds of both *L. stocksii* and *S. fruticosa*. Lower CAT activity may facilitate seed germination by allowing accumulation of H_2O_2 (Taylorson and Hendricks, 1977). Similarly, decrease in GPX activity may also facilitate germination by lowering peroxidase mediated cell wall hardening which may hinder radicle emergence, as osmotically driven radicle emergence requires wall loosening (Dionisio-Sese and Tobita, 1998; Cavalcanti et al., 2004; Bewley et al., 2013). Peroxidase (POD) activity was not detected during seed germination in *Chenopodium murale*, indicating little role of peroxidases during seed germination (Bogdanović et al., 2008). In *Spinacia oleracea*, dry seed maintained an SOD and CAT based antioxidant system which was replaced by APX based system in osmo-primed germinating seeds (Chen and Arora, 2011).

4.3. Germination and antioxidant system in salt-stressed seeds

Seed germination of halophytes as observed in this study, generally decreases with increasing salinity and most un-germinated seeds of test species recovered completely when transferred to distilled water indicating osmotic rather than ionic effects (Gul et al., 2013). Osmotic effects of salinity on seed germination were also reported for *Atriplex prostrata* (Egan et al., 1997), *A. halimus* (Bajji et al., 2002), *Halocnemum strobilaceum*, *Arthrocnemum macrostachyum*, *Sarcocornia fruticosa*, and *Salicornia ramosissima* (Pujol et al., 2000). Lower seed germination with an increase in salinity in our study was perhaps due to decrease in water uptake as in some other halophytes (Song et al., 2005) and reduced de novo synthesis of AsA. Khan et al. (2006) reported that application of exogenous AsA improved the seed germination of *S. fruticosa* under saline conditions thus supporting our basic premise.

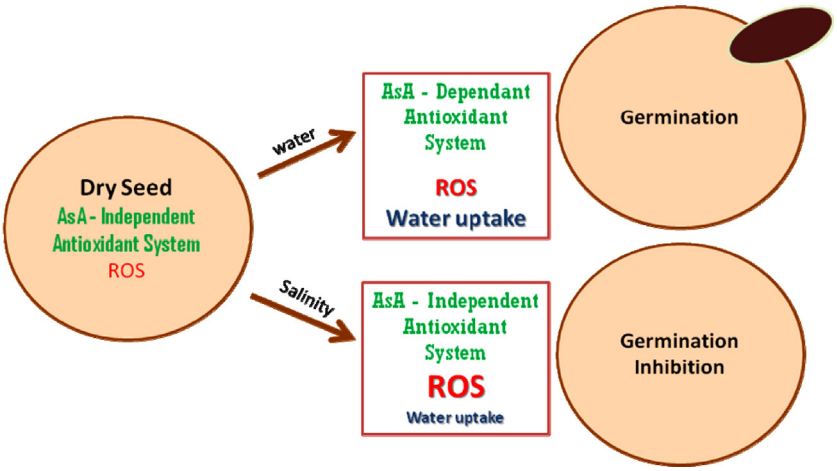

Fig. 7. Graphical sketch of key findings.

Few reports are available on ROS production and scavenging during seed germination of halophytes under saline conditions. Increasing levels of H_2O_2 and MDA are indicators of oxidative stress in plants. In this study, endogenous H_2O_2 and MDA increased under salt stress in both *S. fruticosa* and *L. stocksii*, as reported for seedlings of *Sorghum bicolor* (Chai et al., 2010). Ascorbate–glutathione cycle is involved in recycling of lipid soluble antioxidant α-tocopherol, which protects membranes from ROS mediated peroxidation (Lushchak and Semchuk, 2012). Salinity decreased AsA and GSH contents of the seeds of both test species. Increase in MDA content (an indicator of lipid peroxidation) might be related to lower recycling of lipid soluble antioxidant α-tocopherol due to decreased AsA in this study under saline conditions. However, most un-germinated seeds of both test species recovered quickly when transferred to distilled water, which might indicate that the increase in H_2O_2 and MDA levels in this study were probably not too damaging. Earlier studies in *S. fruticosa* and *L. stocksii* also showed a high recovery of un-germinated seeds when transferred to distilled water (Gul et al., 2013). In addition, exogenous application of hydrogen peroxide (0.34%) and sodium hypochlorite (2%) improved seed germination of *S. fruticosa* under saline conditions in an earlier study perhaps by increasing the seed permeability to water or oxidizing the inhibitors (Hameed et al., 2009). Dehydroascrobate (DHA) content in salinity-treated seeds of both test species were lesser in comparison to unstressed seeds, while oxidized glutathione (GSSG) content of *S. fruticosa* and *L. stocksii* seeds were higher under saline conditions than in non-saline controls. Fahey et al. (1980) showed that the higher GSSG were involved in prevention of germination in wheat seeds by blocking protein synthesis.

A higher CAT and GPX activities while lower APX, AsA and GSH levels level were recorded in seeds imbibed in saline solutions when compared to those imbibed in distilled water. When seeds during germination are subjected to other extreme environmental conditions a similar CAT-GPX based antioxidant system play its role (Rahnama and Ebrahimzadeh, 2005; Wang et al., 2008; Fedina, Nedeva, & Çiçek, 2009; Chai et al., 2010; Bao et al., 2011).

5. Conclusions

Seeds of test species were characterized by no detectable AsA in dry state. AsA concentration increased in seeds imbibed with distilled water indicating a role of AsA-dependent antioxidant system during germination (Fig. 7). Exposure of seeds to salinity decreased the levels of AsA, which could be indicative of prevalence of AsA-independent antioxidant system when germination is inhibited. Water uptake was also decreased in seeds of both species under saline conditions. Thus, it appears that prevention of seed germination under saline conditions is due to failure of proper hydration of seeds with concomitant decrease in AsA and GSH contents.

Acknowledgements

Provision of fund by Higher Education Commission of Pakistan through a research project entitled “salt-induced oxidative stress, consequences and possible management” is thankfully acknowledged. Authors would also like to thank Ms. Hina Asrar for her help in some biochemical assays.

References

Anderson, M.E., 1985. Determination of glutathione and glutathione disulphide in biological samples. Methods Enzymol. 113, 548–555.
Bailly, C., 2004. Active oxygen species and antioxidants in seed biology. Seed Sci. Res. 14, 93–107.
Bailly, C., El-Maarouf-Bouteau, H., Corbineau, F., 2008. From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology. C R Biol. 331, 806–814.
Bajji, M., Kinet, J.M., Lutts, S., 2002. Osmotic and ionic effects of NaCl on germination, early seedling growth, and ion content of *Atriplex halimus* (Chenopodiaceae). Can. J. Bot. 80, 297–304.
Bao, J., Sha, S., Zhang, S., 2011. Changes in germinability, lipid peroxidation, and antioxidant enzyme activities in pear stock (*Pyrus betulaefolia* Bge.) seeds during room- and low-temperature storage. Acta Physiol. Plant. 33, 2035–2040.
Bewley, J.D., Black, M., 1994. Seeds Physiology of Development and Germination. Plenum Press, New York.
Bewley, J.D., Bradford, K.J., Hilhorst, H.W.M., Nonogaki, H., 2013. Germination. In: Seeds – Physiology of Development, Germination and Dormancy, 3rd ed. Springer, New York, pp. 133–181.
Bogdanović, J., Radotić, K., Mitrović, A., 2008. Changes in activities of antioxidant enzymes during *Chenopodium murale* seed germination. Biol. Plant. 52, 396–400.
Cavalcanti, F.R., Oliveira, J.T.A., Martins-Miranda, A.S., Viégas, R.A., Silveira, J.A.G., 2004. Superoxide dismutase, catalase and peroxidase activities do not confer protection against oxidative damage in salt-stressed cowpea leaves. New Phytol. 163, 563–571.
Chai, Y.Y., Jiang, C.D., Shi, L., Shi, T.S., Gu, W.B., 2010. Effects of exogenous spermine on sweet sorghum during germination under salinity. Biol. Plant. 54, 145–148.
Chen, K., Arora, R., 2011. Dynamics of the antioxidant system during seed osmopriming, post-priming germination, and seedling establishment in Spinach (*Spinacia oleracea*). Plant Sci. 180, 212–220.
Crowe, J.H., Crowe, L.M., 1992. Membrane integrity in anhydrobiotic organisms: towards a mechanism for stabilizing dry seeds. In: Somero, G.N., Osmond, C.B., Bolis, C.L. (Eds.), Water and Life. Springer-Verlag, Netherlands, pp. 87–103.
De Gara, L., de Pinto, M.C., Arrigoni, O., 1997. Ascorbate synthesis and ascorbate peroxidase activity during the early stage of wheat germination. Physiol. Plant. 100, 894–900.
De Tullio, M.C., Paciolla, C., Vecchia, F.D., Rascio, N., D'Emerico, S., De Gara, L., Liso, R., Arrigoni, O., 1999. Changes in onion root development induced by the inhibition of peptidyl-proyl hydroxylase and influence of the ascorbate system on cell division and elongation. Planta 209, 424–434.
De Tullio, M.C., Arrigoni, O., 2003. The ascorbic acid system in seeds: to protect and to serve. Seed Sci. Res. 13, 249–260.

Dionisio-Sese, M.L., Tobita, S., 1998. Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135, 1–9.
Dučić, T., Lirić-rajlić, I., Mitrović, A., Radotić, K., 2003. Activities of antioxidant systems during germination of *Chenopodium rubrum* seeds. Biol. Plant. 47, 527–533.
Egan, T.P., Ungar, I.A., Meekins, J.F., 1997. The effects of NaCl, KCl, Na_2SO_4 and K_2NO_4 on the germination of *Ayriplex prostrata* (Chenopodiaceae). J. Plant Nutr. 20, 1723–1730.
El-Maarouf-Bouteau, H., Bailly, C., 2008. Oxidative signaling in seed dormancy and germination. Plant Signal. Behav. 3, 1–8.
Fahey, R.C., Deena, L., Di Stefano, G., Meier, P., Bryan, R.N., 1980. Role of hydration state and thiosulphide status in the control of thermal stability and protein synthesis in wheat embryo. Plant Physiol. 65, 1062–1066.
Fedina, I.S., Nedeva, D., Çiçek, N., 2009. Pre-treatment with H_2O_2 induces salt tolerance in barley seedlings. Biol. Plant. 53, 321–324.
Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48, 909–930.
Grant, J.J., Loake, G.J., 2000. Role of reactive oxygen intermediates and cognate redox signaling in disease resistance. Plant Physiol. 124, 21–30.
Gul, B., Ansari, R., Flowers, T.J., Khan, M.A., 2013. Germination strategies of halophyte seeds under salinity. Environ. Exp. Bot. 91, 22–29.
Hameed, A., Ahmed, M.Z., Gulzar, S., Khan, M.A., 2009. Effect of disinfectants in improving seed germination of *Suaeda fruticosa* under saline conditions. Pak. J. Bot. 41, 2639–2644.
Hameed, A., Hussain, T., Gulzar, S., Aziz, I., Gul, B., Khan, M.A., 2012. Salt tolerance of a cash crop halophyte *Suaeda fruticosa*: biochemical responses to salt and exogenous chemical treatments. Acta Physiol. Plant. 34, 2331–2340.
Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125, 189–198.
Huang, A.H.C., Trelease, R.N., Moore, T.S., 1983. Plant Peroxisomes. Academic Press, New York.
Ishibashi, Y., Koda, Y., Zheng, S.-H., Yuasa, T., Iwaya-Inoue, M., 2013. Regulation of soybean seed germination through ethylene production in response to reactive oxygen species. Ann. Bot. (London) 111, 95–102.
Ishibashi, Y., Tawaratsumida, T., Kondo, K., Kasa, S., Sakamoto, M., Aoki, N., Zheng, S-H., Yuasa, T., Iwaya-Inoue, M., 2012. Reactive oxygen species are involved in gibberellin/abscisic acid signaling in barley aleurone cells. Plant Physiol. 158, 1705–1714.
Khan, M.A., Ahmed, M.Z., Hameed, A., 2006. Effect of sea salt and L-ascorbic acid on the seed germination of halophytes. J. Arid Environ. 67, 535–540.
Khan, M.A., Qaiser, M., 2006. Halophytes of Pakistan: distribution, ecology and economic importance. In: Khan, M.A., Barth, H., Kust, G.C., Boer, B. (Eds.), Sabkha Ecosystems: Volume II: The South and Central Asian Countries. Springer, The Netherlands, pp. 129–153.
Khan, M.A., Ungar, I.A., 1984. Effects of salinity and temperature on the germination and growth of *Atriplex triangularis* Willd. Am. J. Bot. 71, 481–489.
Kranner, I., Seal, C.E., 2013. Salt stress, signaling and redox control in seeds. Funct. Plant Biol. http://dx.doi.org/10.1071/FP13017
Lamb, C., Dixon, R.A., 1997. The oxidative burst in plant disease resistance. Annu. Rev. Plant Physiol. 48, 251–275.
Law, M.Y., Charles, S.A., Halliwell, B., 1983. Glutathione and ascorbic acid in spinach (*Spinacea oleracea*) chloroplasts: the effect of hydrogen peroxide and paraquat. Biochem. J. 210, 899–903.
Loreto, F., Velikova, V., 2001. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiol. 127, 1781–1787.
Lushchak, V.I., Semchuk, N.M., 2012. Tocopherol biosynthesis: chemistry, regulation and effects of environmental factors. Acta Physiol. Plant. 34, 1607–1628.
McDonald, M.B., 2004. Orthodox seed deterioration and its repair. In: Benech-Arnold, R., Sanchez, R.A. (Eds.), Handbook of Seed Physiology – Applications to Agriculture. The Haworth Press, Inc., New York, pp. 273–304.
Müller, K., Linkies, A., Vreeburg, R.A.M., Fry, S.C., Krieger-Liszkay, A., Leubner-Metzger, G., 2009. In vivo cell wall loosening by hydroxyl radicals during cress seed germination and elongation growth. Plant Physiol. 150, 1855–1865.
Noctor, G., De Paepe, R., Foyer, C.H., 2007. Mitochondrial redox biology and homeostasis in plants. Trends Plant Sci. 12, 125–134.
Noctor, G., Foyer, C.H., 1998. Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. Plant Phys. 49, 249–279.
Nonogaki, H., Bassel, G.W., Bewley, J.D., 2010. Germination – still a mystery. Plant Sci. 179, 574–581.
Parsons, R.F., 2012. Incidence and ecology of very fast germination. Seed Sci. Res. 22, 161–167.
Pergo, E.M., Ishii-Iwamoto, E.L., 2011. Changes in energy metabolism and antioxidant defense systems during seed germination of the weed species *Ipomoea triloba* L. and the responses to allelo-chemicals. J. Chem. Ecol. 37, 500–513.
Pujol, J.A., Calvo, J.F., Ramírez-Díaz, L., 2000. Recovery of germination from different osmotic conditions by four halophytes from south-eastern Spain. Ann. Bot. (London) 85, 279–286.
Rahnama, H., Ebrahimzadeh, H., 2005. The effect of NaCl on antioxidant enzyme activities in potato seedlings. Biol. Plant. 49, 93–97.
Sekmen, A.H., Türkan, I., Tanyolac, Z.O., Ozfidan, C., Dinc, A., 2012. Different antioxidant defense responses to salt stress during germination and vegetative stages of endemic halophyte *Gypsophila oblanceolata* BARK. Environ. Exp. Bot. 77, 63–76.
Song, J., Feng, G., Tian, C., Zhang, F., 2005. Strategies for adaptation of *Suaeda physophora*, *Haloxylon ammodendron* and *Haloxylon persicum* to a saline environment during seed germination stage. Ann. Bot. (London) 96, 399–405.
Spragg, S.P., Lievesley, P.M., Wilson, K.M., 1962. The relationship between glutathione and protein sulphydryl groups in germinating pea seeds. Biochem. J. 83, 314–318.
2001. SPSS version 11.0 for Windows. SPSS Inc.
Taylorson, R.B., Hendricks, S.B., 1977. Dormancy in seeds. Annu. Rev. Plant Physiol. 28, 331–354.
Tommasi, F., Paciolla, C., de Pinto, M.C., De Gara, L., 2001. A comparative study of glutathione and ascorbate metabolism during germination of *Pinus pinea* L. seeds. J. Exp. Bot. 52, 1647–1654.
Vallejo, A.J., Yanovsky, M.J., Botto, J.F., 2010. Germination variation in *Arabidopsis thaliana* accessions under moderate osmotic and salt stresses. Ann. Bot. (London) 106, 833–842.
Varghese, B., Naithani, S.C., 2007. Oxidative metabolism-related changes in cryogenically stored Neem (*Azadirachta indica* A. Juss) seed. J. Plant Physiol. 165, 755–765.
Wang, L., Huang, X., Zhou, Q., 2008. Response of peroxidase and catalase to acid rain stress during seed germination of rice, wheat, and rape. Front. Environ. Sci. Eng. China 2, 364–369.
Wang, M., Heimovaara-Dijkstra, S., Van Duijn, B., 1995. Modulation of germination of embryos isolated from dormant and non dormant grains by manipulation of endogenous abscisic acid. Planta 195, 586–592.
Wang, M., van der Meulen, R-M., Visser van Schaik, H-P., van Duijn, B., de Boer, A-H., 1998. Effects of dormancy-breaking chemicals on ABA levels in barley grain embryos. Seed Sci. Res. 8, 129–137.
Wang, H-L., Wang, L., Tian, C-Y., Huang, Z-Y., 2012. Germination dimorphism in *Suaeda acuminata*: a new combination of dormancy types for heteromorphic seeds. S. Afr. J. Bot. 78, 270–275.
Wei, Y., Dong, M., Huang, Z., Tan, D., 2008. Factors influencing seed germination of *Salsola affinis* (Chenopodiaceae), a dominant annual halophyte inhabiting the deserts of Xinjiang, China. Flora 203, 134–140.
Weitbrecht, K., Müller, K., Leubner-Metzger, G., 2011. First off the mark: early seed germination. J. Exp. Bot. 62, 3289–3309.
Zia, S., Khan, M.A., 2007. Alleviation of salinity effects on the seed germination of *Limonium stocksii* by sodium hypochlorite. Pak. J. Bot. 39, 503–511.