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Publisher: Taylor & Francis

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Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology: Official Journal of the Societa Botanica Italiana

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/tplb20>

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Accepted author version posted online: 06 Jan 2015. Published online: 28 Jan 2015.

To cite this article: A. Rasheed, A. Hameed, M. A. Khan & B. Gul (2015): Variation in temperature and light but not salinity invokes antioxidant enzyme activities in germinating seeds of *Salsola drummondii*, Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology: Official Journal of the Societa Botanica Italiana, DOI: [10.1080/11263504.2014.1001463](https://doi.org/10.1080/11263504.2014.1001463)

To link to this article: <http://dx.doi.org/10.1080/11263504.2014.1001463>

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ORIGINAL ARTICLE

Variation in temperature and light but not salinity invokes antioxidant enzyme activities in germinating seeds of *Salsola drummondii*

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Abstract

Seeds with efficient antioxidant defence system show higher germination under stress conditions; however, such information is limited for the halophyte seeds. We therefore studied lipid peroxidation and antioxidant responses of a leaf-succulent halophyte *Salsola drummondii* during seed germination under different salinity levels (0, 200 and 800 mM NaCl), temperature (10/20, 20/30 and 25/35°C) and light regimes. Seeds absorbed water and germinated in less than 1 h in non-saline control while increases in salinity decreased the rate of water uptake as well as seed germination. Non-optimal temperatures (10/20 and 25/35°C) and complete dark condition reduced seed germination in comparison to those seeds germinated under optimal temperature (20/30°C) and 12-h photoperiod, respectively. Generally, higher lipid peroxidation and antioxidant enzyme activities were observed in seeds at non-optimal temperature and in those seeds germinated in dark. Decrease in reduced ascorbic acid content was found in highest salinity and temperature treatments, while reduced glutathione content did not change significantly with changes in salinity, temperature and light regimes. These results indicate variation in temperature and light but not salinity enhances antioxidant enzyme activities in germinating seeds of *Salsola drummondii*.

Keywords: Antioxidant, germination, halophyte, reactive oxygen species

Introduction

Germination is the transition of dry seeds from quiescence to active metabolic state which requires energy (Nonogaki et al. 2010). Hence, increase in oxygen consumption for energy production is detected in seeds soon after imbibition (Bewley & Black 1994; Rosental et al. 2014). Mitochondrial membranes in dry seeds are damaged and cause production of reactive oxygen species (ROS) during early imbibitional phase (Crowe & Crowe 1992; Tommasi et al. 2001; Noctor et al. 2007; Nonogaki et al. 2010). Therefore, to prevent oxidative damage, seeds possess a battery of antioxidant enzymes such as superoxide dismutase (SOD) that converts the toxic superoxide radical into hydrogen peroxide (H₂O₂; Kliebenstein et al. 1998), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase which detoxify H₂O₂ (Willekens et al. 1995; Noctor & Foyer 1998). Antioxidant compounds such as ascorbic acid (AsA) and

glutathione (GSH) also play a role in ROS scavenging (Smirnoff 2000; Miller et al. 2010; Foyer & Noctor 2011). However, AsA is generally absent in dry seeds (De Tullio & Arrigoni 2003; Hameed et al. 2014). Generally, little is known about changes in antioxidant components of seeds during germination, particularly of non-crop plants such as halophytes (Kranter & Seal 2013; Hameed et al. 2014).

Environmental stresses such as salinity, drought and temperature cause ROS accumulation and decrease in the capacity of cells to detoxify them, hence result in oxidative damage to different cell components and even cell death (McDonald 1999; Tommasi 2001; Lee et al. 2010; Xu et al. 2013). Therefore, the success of germination is strongly dependent on the quality of antioxidant defence that operates during germination (De Gara et al. 1997; Bailly 2004). However, such studies are mostly focused on crops and other economically important plants (Gidrol et al. 1994; Bailly et al. 1996, 2001;

Oracz et al. 2009). Little is known about halophyte seeds (Khan et al. 2006; Bogdanović et al. 2008). Out of ~2500 halophyte species found in the world, information about seed antioxidant responses of just few halophyte species is known (Dučić et al. 2003; Xing et al. 2013; Hameed et al. 2014). Recently, Hameed et al. (2014) showed that salinity inhibits seeds germination of subtropical coastal halophytes *Suaeda fruticosa* and *Limonium stocksii* by reducing water uptake and ascorbate-dependent antioxidant system. However, to the best of our knowledge, no comprehensive study on effects of different environmental factors such as salinity, temperature and light on antioxidant enzyme activities during seed germination is available.

Salsola drummondii Ulbr. is a leaf-succulent perennial halophyte from the family Amaranthaceae and is a source of soda ash, medicine and forage for local populations (Qureshi et al. 1993; Gilani et al. 2010). This species appears to survive vast fluctuations in soil moisture (~0.5% to 6%), electrical conductivity ($EC_{1:10}$; ~10 to 30 $dS\ m^{-1}$) and extreme high temperatures (>45°C) in natural field conditions and yearly produces large number of seeds. However, details of mechanisms underlying this high stress tolerance of *S. drummondii* are not known. Seeds in laboratory conditions germinated in up to 1000 mM NaCl at optimal light and temperature conditions (Rasheed et al. unpublished data). How halophyte seeds respond biochemically to different environmental variables during their germination is not well understood. In addition, information about antioxidant responses of the seeds of halophytes from warm arid habitats is also missing. Therefore, we investigated the effect of different environmental factors, such as salinity, temperature and light/dark on antioxidant enzyme activities of the seeds of xerohalophyte *S. drummondii* during germination. Findings of this research work would improve our understanding about seed antioxidant responses of Salsoloideae species, which have peculiar seed characteristics and exhibit very fast “cryptoviviparous-like” germination (Liu et al. 2013).

Materials and methods

Seed collection

Mature seeds of *Salsola drummondii* were collected during February 2011, from a population growing in a salt desert located at Winder, Balochistan (Pakistan). Seeds were separated from perianths, surface sterilized by using 1% commercial bleach, rinsed with distilled water, air dried and stored in clear plastic Petri plates until used.

Seed characteristics

Colour, texture, shape and size (diameter) of the freshly collected seeds were recorded. Fresh weight of 100 seeds was taken. These seeds were then dried in a forced-draft oven at 105°C for 48 h to determine their dry weight. Seed moisture was calculated by subtracting dry weight from fresh weight. Oven-dried seeds were then placed in a furnace at 550°C for 5 h to determine ash contents. Finally, organic weight was calculated by subtracting ash from dry weight.

Seed germination

Seed germination experiments were conducted in programmed incubators (Percival Scientific, Boone, IA, USA). Effects of NaCl treatments (0, 200 and 800 mM NaCl) on seed germination, lipid peroxidation and antioxidant enzyme activities were examined at 20/30°C, where low temperature coincided with 12-h dark and high temperature with 12-h light period ($25\ \mu\text{mol}\ m^{-2}\ s^{-1}$; 400–700 nm, Philips cool-white fluorescent lamps). Seeds were germinated in distilled water under alternative temperature regimes of 10/20 (low), 20/30 (moderate) and 25/35°C (high) both in 12-h light/dark period as well as in dark. There were four replicates of 25 seeds each per treatment. Tight-fitting plastic Petri plates (5 cm Φ) with clear lids were used with 5 ml of test solutions. Germination data were recorded on alternate days for a period of 20 days and expressed as described in Khan and Ungar (1984).

Seed water uptake

Seeds were immersed in three NaCl concentrations (0, 200 and 800 mM), and the relative increase in fresh weight of seeds (W_r) was recorded (Song et al. 2005) after 50 min (time required for embryo protrusion in distilled water).

Lipid peroxidation

Seeds (0.5 g) were ground fine with mortar and pestle using liquid nitrogen and homogenized in 1% ice-cold trichloroacetic acid. Homogenate was then centrifuged at 12,000g for 20 min at 4°C. Supernatant was used for the determination of malondialdehyde (MDA) (Heath & Packer 1968).

Antioxidant enzymes

Extraction of antioxidant enzymes was done according to the method of Polle et al. (1994). Activities of SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), guaiacol peroxidase (GPX, EC 1.11.1.7) and APX (EC

Table I. Seed characteristics of *Salsola drummondii*.

Parameter	
Colour	RAL1011 Brown Beigea
Texture	Rough
Size (mm)	0.2
Fresh weight (g/100 seeds)	0.097
Moisture (%)	4.8
Ash (%)	77.14
Organic content (%)	18.05

aHLC = 74-60-36, RGB = 138-102-066 and CYMK (coated) = 30, 50, 80, 0 values are based on various measurements using D65 light with a standard observer according to International Commission on Illumination (CIE 1964). Source: www.ralcolors.com.

1.11.1.11) were assayed and expressed as described in Hameed et al. (2012).

Antioxidant substances

Reduced AsA was determined in TCA extracts by using the method of Luwe et al. (1993). Reduced GSH contents were estimated in TCA extracts according to Guri (1983).

Statistical analyses

Statistical analyses were conducted by using SPSS version 11.0 for windows (SPSS Inc. 2007). Germination data were arcsine transformed before statistical analysis. Analysis of variance was carried out to determine whether different environmental variables affected germination and antioxidant enzyme activities significantly. *t*-Test and post-hoc Bonferroni test ($P < 0.05$) were conducted to compare individual means of treatments.

Results

Seed characteristics

Seeds were beige-brown, rough textured, round and small (0.2 mm Φ) with a fresh weight of about 97 mg per 100 seeds (Table I). There was about 77% ash, 18% organic content and 4.8% moisture in fresh seeds of *S. drummondii*.

Seed germination

Analysis of variance (ANOVA) indicated a significant (F -value = 113.796; $P < 0.001$) effect of salinity on final germination percentage of *S. drummondii* seeds (Table II). Final seed germination percentage decreased from 91% in distilled water to 55% in 200 mM NaCl and only 8% seeds germinated in high (800 mM NaCl) salinity treatment (Figure 1(a)). Seeds of *S. drummondii* germinated (F -value = 3.74;

Table II. ANOVA showing the effect of different abiotic factors on per cent germination (G), relative water uptake (RWU), MDA content (MDA) and antioxidant enzyme activities of *Salsola drummondii* seeds.

Parameters	Salinity	Temperature	Light/dark
G	113.796***	3.745*	6.607*
RWU	119.644***	–	–
SOD	0.493ns	54.324**	27.996*
CAT	8.227*	34.916**	285.817***
APX	1.830*	189.297***	12.039*
GPX	0.479ns	23.463*	22.364**
MDA	0.155ns	7.348*	10.759*
AsA	160.441**	12.000*	1.394ns
GSH	0.800ns	0.144ns	1.543ns

Note: Values represent *F*-values. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, non-significant.

$P < 0.05$) better at 20/30°C than at 10/20°C or 25/35°C (Figure 1(b)). Furthermore, seed germination of *S. drummondii* was (F -value = 6.61; $P < 0.05$) higher (11%) under 12-h photoperiod (12 h dark:12 h light) in comparison to dark (Figure 1(c)).

Water uptake

Seeds absorbed water (135% weight gain compared to dry seeds) and uncoiled in about 50 min (Figure 2(b)). Rate of water absorption and final seed germination decreased with the increase in salinity (Table II; Figure 2(a)) irrespective of light and temperature regimes used.

Lipid peroxidation

ANOVA showed a significant effect of temperature (F -value = 7.35; $P < 0.05$) and light (F -value = 10.76; $P < 0.05$) but not of salinity on lipid peroxidation (Table II; Figure 3). There was significantly higher MDA in seeds incubated at 10/20 and 25/35°C in comparison to 20/30°C, while MDA content in complete dark was higher than that in 12-h photoperiod (Figure 3(b),(c)).

Antioxidant enzymes

After 50 min, CAT activity in salinity-treated seeds (200 and 800 mM NaCl) was lower (F -value = 8.227; $P < 0.05$) as compared to water-imbibed seeds (Figure 5(a)). In contrast, activities of SOD, APX and GPX in salinity-treated seeds were similar to those in water-imbibed seeds (Figures 4(a), 6(a) and 7(a)).

Temperature has a significant effect on SOD (F -value = 54.324; $P < 0.01$), CAT (F -value = 34.916; $P < 0.01$), APX (F -value = 189.297; $P < 0.001$) and GPX (F -value = 23.463; $P < 0.05$) activities.

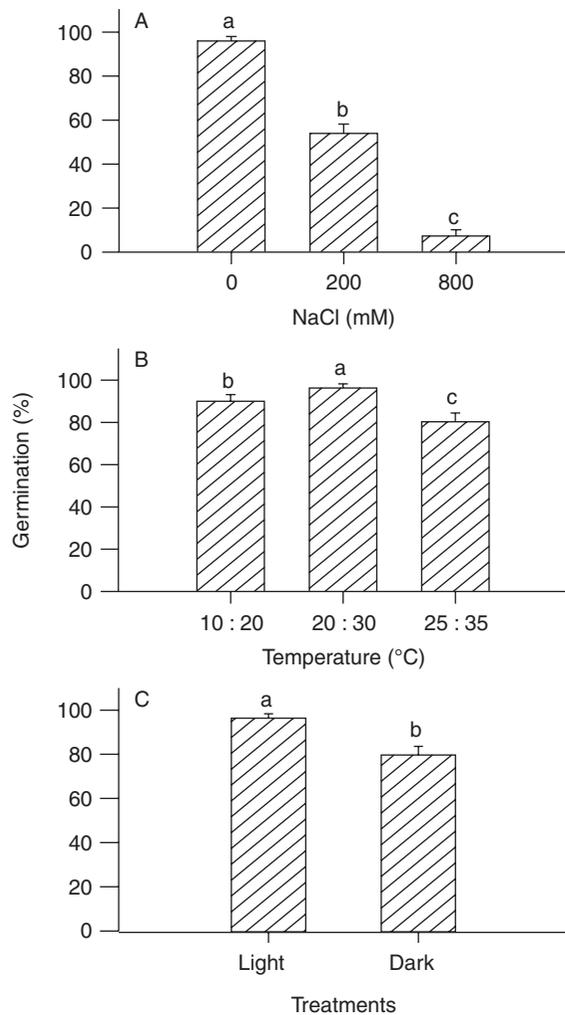


Figure 1. Effect of (A) salinity, (B) temperature and (C) light on the per cent germination in seeds of *Salsola drummondii*. Bars represent mean \pm standard error. Bars with the same alphabet are not significantly different from each other [Bonferroni test (A and B) and *t*-test (C), $P < 0.05$].

SOD and GPX activities were comparable between 10/20 and 20/30°C treatments but increased markedly at 25/35°C (Figures 4(b) and 7(b)). CAT and APX activities were highest at 25/35 and lowest at 20/30°C (Figures 5(b) and 6(b)).

Light regimes exerted a significant effect on antioxidant enzyme activities (Table II). SOD, CAT, APX and GPX activities were substantially higher in dark compared to 12-h photoperiod (Figures 4(c), 5(c), 6(c) and 7(c)).

Antioxidant substances

Reduced AsA contents were affected by salinity (F -value = 160.44; $P < 0.01$) and temperature (F -value = 12.00; $P < 0.01$) but not with light regimes (Table II). Some decrease in AsA contents at the highest salinity and temperature treatments was observed (Figure 8(a),(b)). Contents of GSH did not

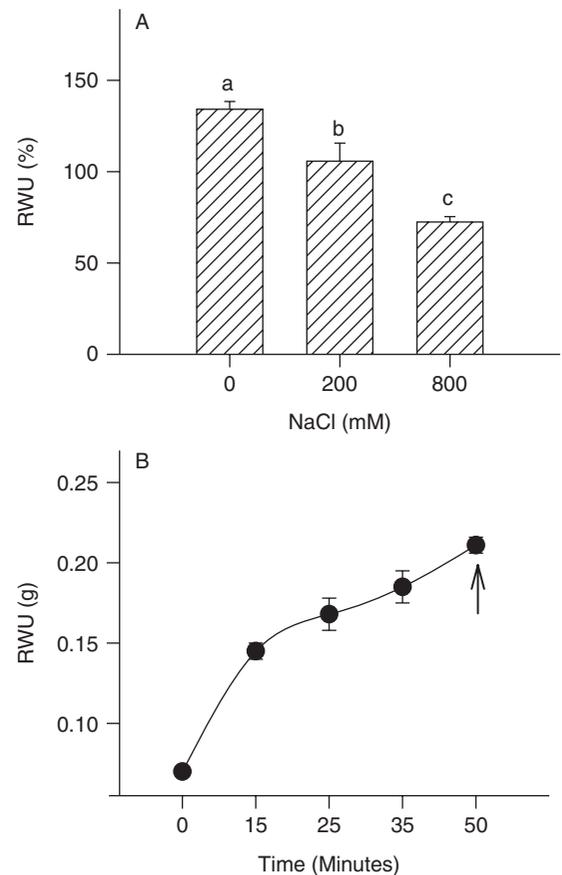


Figure 2. Effect of salinity on the relative water uptake in seeds of *Salsola drummondii* after 50 min of soaking. (B) Arrow indicates embryo protrusion. Bars and circles represent mean \pm standard error. Bars with the same alphabet are not significantly different from each other (Bonferroni test, $P < 0.05$).

vary with changes in salinity, temperature and light regimes (Table II and Figure 9).

Discussion

Seeds of *S. drummondii* were small (0.2 mm Φ) and had lower moisture (4.8%) during the dry state like other perennial halophytes (Song et al. 2005) and germinated rapidly (50 min) on the availability of moisture by uncoiling of embryo. Seeds of *S. tragus* (Wallace et al. 1968) and *Haloxylon stocksii* (Sharma & Sen 1989) similarly germinated within 30 min. Seed imbibition results in elongation of embryo cells and uncoiling of the spiral embryo in salsoloideae species, which ruptures the seed/fruit coat quickly and marks germination completion without cell division (Wallace et al. 1968; Parsons 2012). Liu et al. (2013) proposed that such fast-germinating seeds are “cryptoviviparous-like”, probably to enhance the ability of their seedlings to become rooted quickly in deeper, moist soil before the upper layer of soil dries in arid desert environments.

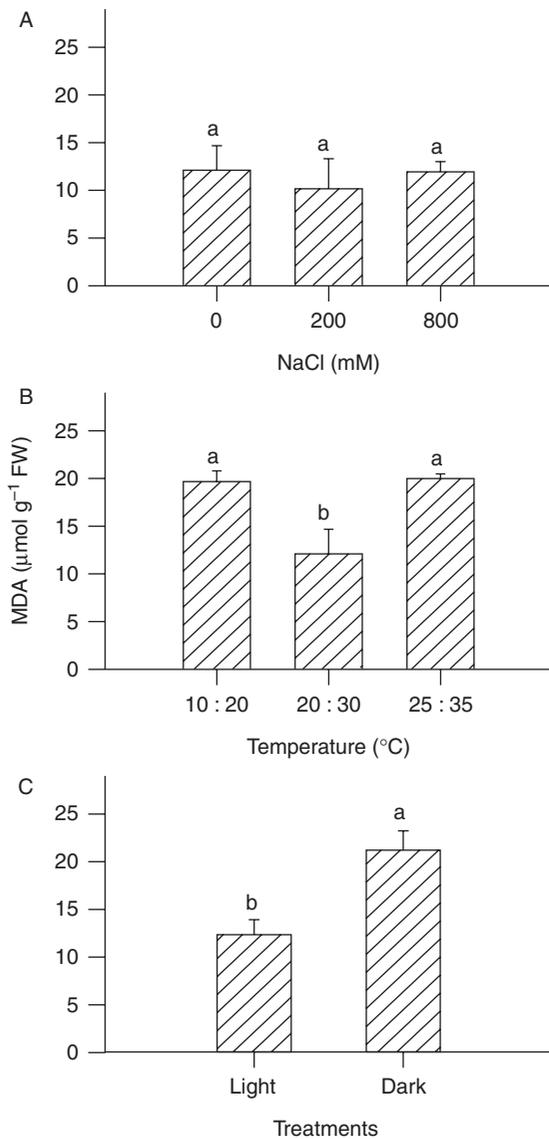


Figure 3. Effects of (A) salinity, (B) temperature and (C) light on the MDA content in the germinating seeds of *S. drummondii*. Bars represent mean \pm standard error. Bars with the same alphabet are not significantly different from each other [Bonferroni test (A and B) and *t*-test (C), $P < 0.05$].

Seeds of *S. drummondii* germinated optimally in distilled water at 20/30 $^{\circ}\text{C}$ and under 12-h photoperiod, like most subtropical halophytes (Gul et al. 2013). Reduction in seed germination of *S. drummondii* with increases in salinity coincided with a decrease in seeds' relative water uptake, as reported for *Suaeda physophora*, *Haloxylon ammodendron* and *H. persicum* (Song et al. 2005). However, reduction in seed germination at sub-optimal temperatures and in dark is not related to reduced water uptake. Recently, oxidative stress is ascribed to as a common consequence of environmental stresses (Greene 2002; Sharma et al. 2012). Therefore, germination inhibition in these conditions could be linked with oxidative stress.

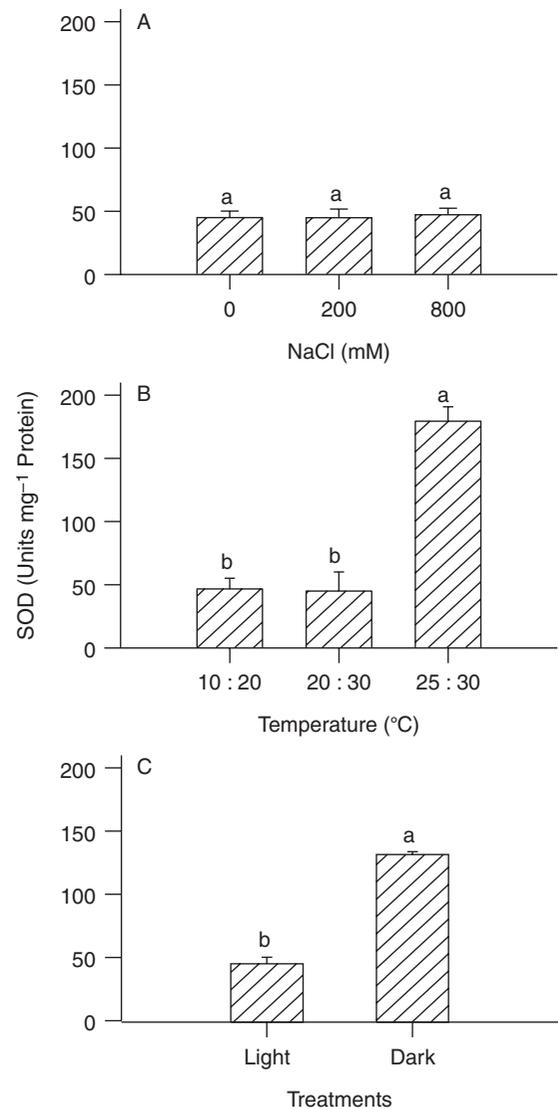


Figure 4. Effects of (A) salinity, (B) temperature and (C) light on the activity of SOD in germinating seeds of *Salsola drummondii*. Bars represent mean \pm standard error. Bars with the same alphabet are not significantly different from each other [Bonferroni test (A and B) and *t*-test (C), $P < 0.05$].

MDA content of germinating seeds of *S. drummondii* did not change with increases in salinity, which might be indicative of unchanged ROS production or efficient antioxidant system during the early phase of germination. However, prolonged exposure to salinity could be detrimental. Likewise, ROS production and MDA content did not change in *Salsola ikonnikovii* seedlings with increases in salinity from 0 to 300 mM NaCl (Xing et al. 2013). In contrast, MDA contents of germinating seeds of *S. drummondii* at sub-optimal temperatures (10/20 and 25/35 $^{\circ}\text{C}$) were higher than those at optimum temperature (20/30 $^{\circ}\text{C}$). Bhattacharjee (2013) also showed that high (40 $^{\circ}\text{C}$) and low (8 $^{\circ}\text{C}$) temperatures during imbibition enhanced MDA content in germinating rice seeds compared

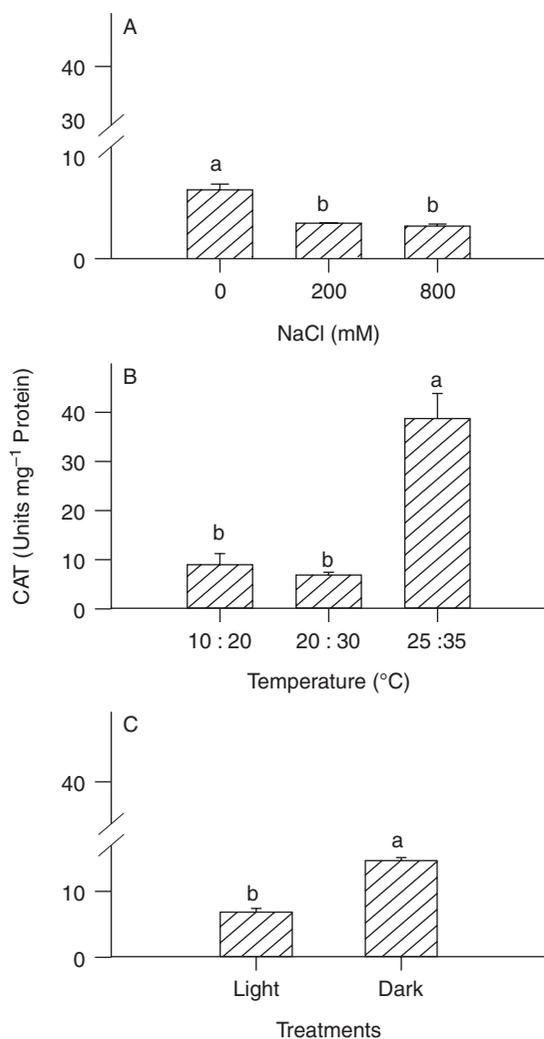


Figure 5. Effects of (A) salinity, (B) temperature and (C) light on the activity of CAT in germinating seeds of *Salsola drummondii*. Bars represent mean \pm standard error. Bars with the same alphabet are not significantly different from each other [Bonferroni test (A and B) and *t*-test (C), $P < 0.05$].

to control (25°C) treatment. MDA accumulated in dark germinated seeds of *S. drummondii*, which coincided with reduced germination in dark than in 12-h photoperiod. Such oxidative damages have been held responsible for poor germination and early seedling establishment in other species under stress conditions (Gong et al. 1997; Bhattacharjee 2013). Both dark and the sub-optimal temperatures caused MDA accumulation in germinating seeds of *S. drummondii*, which warrants the importance to investigate whether any similarity exists in antioxidant responses of species test under these conditions.

Enhanced activities of different antioxidant enzymes are reportedly associated with successful completion of germination and stress tolerance of seeds (Puntarulo et al. 1991; Guan & Scandalios 1995; Bailly 2004; Kranner & Seal 2013; Hameed et al. 2014). SOD acts as

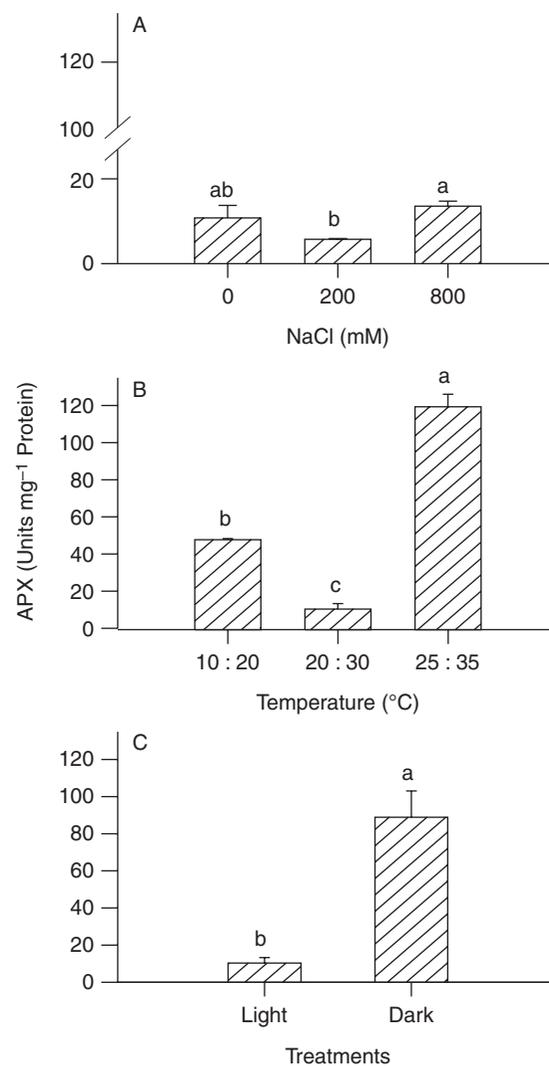


Figure 6. Effects of (A) salinity, (B) temperature and (C) light on the activity of APX in germinating seeds of *Salsola drummondii*. Bars represent mean \pm standard error. Bars with the same alphabet are not significantly different from each other [Bonferroni test (A and B) and *t*-test (C), $P < 0.05$].

the first line of defence against ROS in plants (Alscher et al. 2002). A higher SOD activity was observed in germinating seeds of many plants under salinity (Zheng et al. 2009; Wang et al. 2012), temperature (Mei & Song 2010) and other stresses (Alscher et al. 2002; Guo et al. 2012). However, such information on halophyte seeds is scanty (Sekmen et al. 2012; Kranner & Seal 2013). SOD activity in germinating seeds of *S. drummondii* did not change with increasing salinity but increased significantly at high temperature (25/35°C) and in complete darkness compared to optimal temperature (20/30°C) and 12-h photoperiod, respectively. An increase in MDA content in germinating seeds of *S. drummondii* under sub-optimal temperatures and dark but not in response to salinity also indicated the need for enhanced antioxidant enzyme activities as observed in the case of SOD.

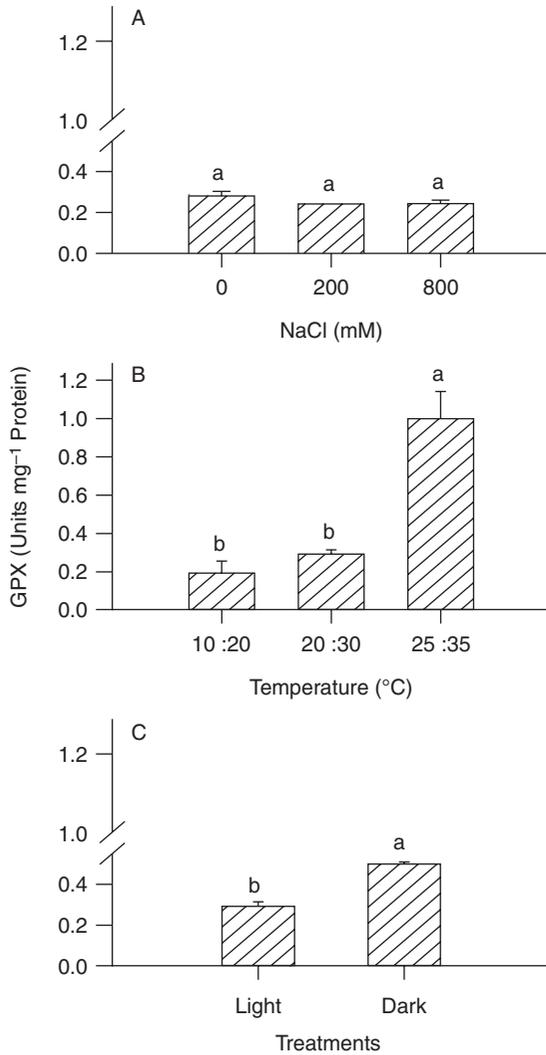


Figure 7. Effects of (A) salinity, (B) temperature and (C) light on the activity of GPX in germinating seeds of *Salsola drummondii*. Bars represent mean \pm standard error. Bars with the same alphabet are not significantly different from each other [Bonferroni test (A and B) and *t*-test (C), $P < 0.05$].

CAT is a haem-containing antioxidant enzyme that dismutates hydrogen peroxide into oxygen and water (Mhamdi et al. 2010). CAT activity in *S. drummondii* seeds declined under salinity, which could indicate unaltered ROS levels as indicated by unchanged MDA contents in this study. Apel and Hirt (2004) also indicated that the expression and activities of most antioxidant enzymes are stimulated by ROS accumulation. Similarly, the expression of *Cat1* and *Cat3* genes was also ROS dependent in maize (Polidoros & Scandalios 1999). In this study, suboptimal temperatures (10/20 and 25/35°C) and complete darkness resulted in MDA accumulation, which coincided with a rise in CAT activity, indicating higher production of ROS in these conditions. Enhanced SOD activity under higher temperature (25/35°C) and in complete darkness is also in agreement with CAT data in this study.

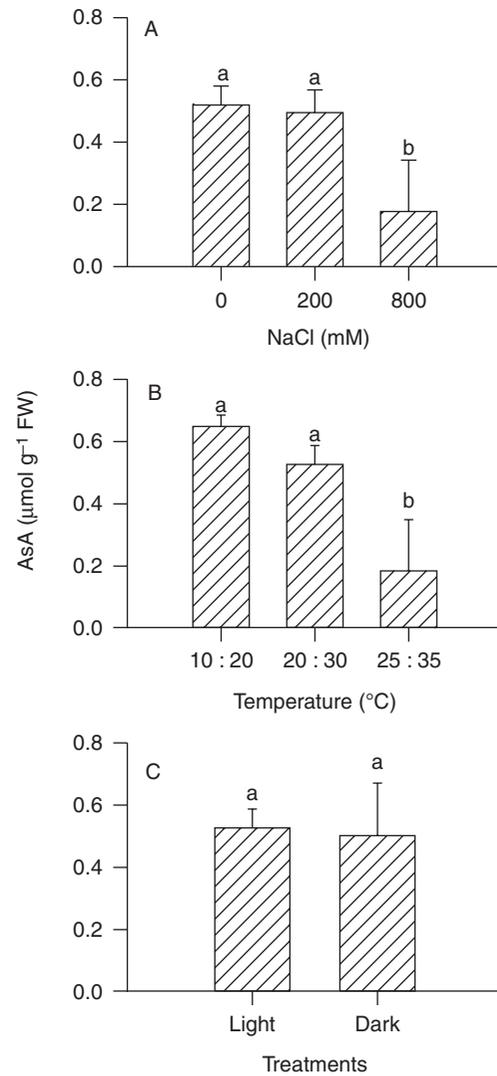


Figure 8. Effects of (A) salinity, (B) temperature and (C) light on the AsA levels in germinating seeds of *Salsola drummondii*. Bars represent mean \pm standard error. Bars with the same alphabet are not significantly different from each other [Bonferroni test (A and B) and *t*-test (C), $P < 0.05$].

GPXs are class III peroxidases, which detoxify hydrogen peroxide by utilizing any phenolic compound such as guaiacol (Siegel 1993; Jouili et al. 2011). Little is known about the GPX activity during seed germination, especially under stress conditions. However, some data on early seedlings are available. For instance, an increase in GPX activity was reported in seedlings of *Brassica napus* cultivars under drought stress (Abedi & Pakniyat 2010), in *Betula pendula* seedlings upon UV exposure (Tegelberg et al. 2008) and in *Morus alba* plants in response to high-temperature treatment (Chaitanya et al. 2001). Recently, an increase in GPX activity was reported in germinating seeds of *Suaeda fruticosa* and *Limonium stocksii* under saline conditions (Hameed et al. 2014). However, in this study, GPX activity did not change in germinating seeds of *S. drummondii*.

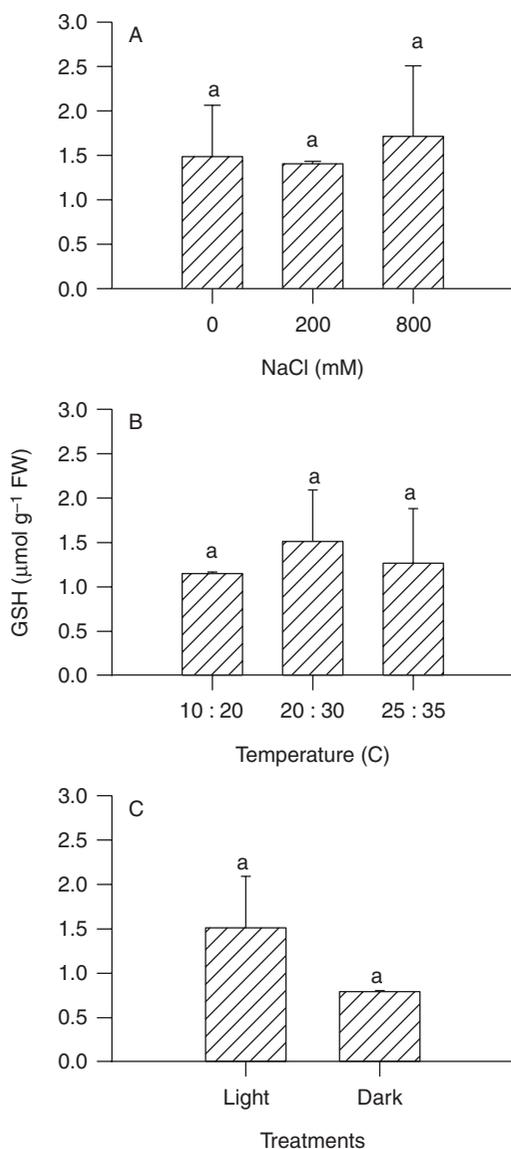


Figure 9. Effects of (A) salinity, (B) temperature and (C) light on the GSH levels in germinating seeds of *Salsola drummondii*. Bars represent mean \pm standard error. Bars with the same alphabet are not significantly different from each other [Bonferroni test (A and B) and *t*-test (C), $P < 0.05$].

under saline conditions, which coincides well with unchanged SOD and MDA levels, indicating less ROS production and thus no need for higher activities of ROS-scavenging enzymes such as GPX. However, GPX activity increased in *S. drummondii* seeds at higher temperature (25/35°C) and in complete darkness, probably to quench higher ROS levels as indicated by higher SOD activity at higher temperature (25/35°C) and in complete darkness. Likewise, GPX activity increased in *Erythrina velutina* seeds at high-temperature (30 and 35°C) treatments (Ribeiro et al. 2014).

APX is another antioxidant enzyme of plants that scavenges hydrogen peroxide by using ascorbate as reductant (Asada 1992). Its involvement in stress-

mediated oxidative stress tolerance in both halophytes and non-halophytes is well documented (Bartosz 1997; Jithesh et al. 2006; Munns & Tester 2008). However, information on the role of APX in stress tolerance of halophyte seeds is scanty. In this study, APX activity like most other antioxidant enzymes remained unaffected by salinity but increased in response to temperature and dark stresses. Increased APX activity was observed in *Erythrina velutina* seeds at high-temperature (30 and 35°C) treatments (Ribeiro et al. 2014). Over-expression of APX along with SOD increased stress tolerance of tobacco seeds (Lee et al. 2010). Similarly, APX activities increased under drought stress in alfalfa cultivars during germination (Wang et al. 2009).

AsA is a key antioxidant compound of plants which quenches ROS directly as well as through Asada–Halliwell–Foyer pathway (Noctor & Foyer 1998; Gest et al. 2013) and is also known to recycle lipid-soluble antioxidant α -tocopherol (Lushchak & Semchuk 2012). Ascorbate is also a cofactor for enzymes which synthesize two important seed germination regulating hormones, gibberellins and ethylene (De Tullio & Arrigoni 2003). Although absent in dry seeds, AsA is rapidly synthesized in germinating seeds (De Tullio & Arrigoni 2003; Dučić et al. 2003; Hameed et al. 2014). Synthesis of AsA during seed germination could facilitate subsequent cell division for seedling formation (Noctor & Foyer 1998; De Tullio et al. 1999; De Tullio & Arrigoni 2003). AsA was also detected in germinating seeds of *S. drummondii* and some decrease in AsA content was observed in response to high salinity and temperature treatments. Likewise, decline in AsA content was reported for the seeds of two other halophytes *Suaeda fruticosa* and *Limonium stocksii* under saline conditions (Hameed et al. 2014). These data including ours thus hint at the importance of AsA in seed germination.

γ -Glutamyl-cysteinyl-glycine (GSH) is another important antioxidant substance in plants, which can directly and indirectly quench various ROS, hence plays an important role in their homeostasis (Noctor et al. 2012; Gill et al. 2013; Kranner & Seal 2013). In the absence of AsA, GSH appears one of the main antioxidants in most orthodox seeds and seems important for success of germination (Tommasi et al. 2001; De Gara et al. 2003; Kranner & Seal 2013; Hameed et al. 2014). However, little information is available on GSH changes during seed germination of halophytes. Recently, Hameed et al. (2014) reported that GSH content decreased in *Suaeda fruticosa* and *Limonium stocksii* seeds during germination under saline conditions. In contrary, GSH content in *S. drummondii* did not vary with changes in salinity nor with temperature and light regimes,

indicating that GSH levels in the seeds of this highly tolerant halophyte are not affected by abiotic stresses. Similarly, GSH contents in seedlings of another highly tolerant halophyte *Salsola crassa* also remained unchanged upon exposure to 30 days salinity compared to control (Yildiztugay et al. 2014).

Salsola drummondii seeds absorbed water and germinated quickly in control, and both water uptake and germination decreased with increases in salinity while MDA and antioxidant activity remained unaffected. Seed germination was reduced at sub-optimal temperatures and in dark with concomitant increase in MDA and antioxidant enzymes. These data hence show that salinity causes water stress while light and temperature stresses upset ROS balance in seeds of our test species. Although antioxidant enzyme activities are enhanced under sub-optimal temperature and in dark, but MDA accumulation indicates that induction in antioxidant system was not sufficient enough to prevent oxidative damages. Therefore, it seems that seeds of halophyte *S. drummondii* are better adapted for salinity than temperature and dark. Further investigations are needed to fully understand these mechanisms.

Acknowledgements

Higher Education Commission of Pakistan is thankfully acknowledged for provision of funds through project entitled “Antioxidant responses of halophyte seeds and seedlings to salinity induced oxidative stress”.

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