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Effect of ascorbic acid on seed germination of three halophytic grass species under saline conditions

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Abstract

Grasses on the Pakistani coast are moderately to highly salt tolerant and have potential for utilization as a cash crop. This study was designed to determine whether seed germination of three halophytic grasses (*Phragmites karka, Dichanthium annulatum* and *Eragrostis ciliaris*) could be improved by exogenous application of ascorbic acid (AsA) under saline conditions. Seeds of *P. karka* were germinated in varying concentrations of NaCl and AsA under different temperature regimes, and seeds of *Dichanthium annulatum and Eragrostis ciliaris* were germinated at optimal temperatures only. In *P. karka,* concentrations of AsA (5 and 10 mM) alleviated the salinity effects better at cooler and moderate thermo-periods, whereas higher concentrations (20 mM of AsA) failed to improve germination under all temperature regimes. AsA was ineffective at a warmer thermo-period (25/35°C). The rate of germination also increased at all thermo-periods with the application of AsA except at 25/35°C under saline conditions. Application of AsA improved the germination of *E. ciliaris* seeds under saline conditions but was inhibitory for *D. annulatum* in comparison with the untreated control. The rate of germination followed the similar pattern as that of seed germination. Results indicate that AsA has the ability to partially alleviate the effect of salinity on seed germination of some grass species under optimal temperature regime.

Keywords: grasses, salinity, semi-arid, *Phragmites karka*, *Dichnathium annulatum*, *Eragrostis ciliaris*, Pakistan

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Introduction

Conventional domesticated crops face serious problems in completing their life cycle in regions characterized by severe drought, warm temperatures and high salinity. Inducing salt tolerance in these crops, either through conventional breeding or through modern molecular methods, is difficult to achieve because the trait is under multigenic control (Agarwal *et al.*, 2012). Attention has consequently shifted to those plants that are already adapted to saline conditions and can be utilized as non-conventional cash crops (Koyro *et al.*, 2011). The Institute of Sustainable Halophyte Utilization, the University of Karachi, Pakistan, is screening local halophytes for their fodder, forage, medicinal, oil seed and other economically useful characteristics (Khan *et al.*, 2006b).

Initiation of seed germination is coupled with mitochondrial activity, which leads to the production of reactive oxygen species (ROS; Schopfer *et al.*, 2001; Garnczarska and Wojtyla, 2008; Kranner *et al.*, 2010). This ROS production may protect germinating seeds from pathogens (Schopfer *et al.*, 2001), softening the cell wall (Müller *et al.*, 2009) to promote radicle emergence and oxidize inhibitors (Ogawa and Iwabuchi, 2001).

Ascorbic acid (AsA) is widely distributed in plants and is a potent antioxidant that donates electrons in a wide range of enzymatic and non-enzymatic reactions (Arrigoni and De Tullio, 2000; Horemans *et al.*, 2000; Smirnoff, 2000; Foyer and Noctor, 2011). It scavenges different ROS, such as superoxide, hydroxyl radicals and singlet oxygen, directly or indirectly, for regulating their cellular concentrations within narrow tolerable ranges (Kocsy *et al.*, 2001; Shao *et al.*, 2006, 2008). AsA is also required for the biosynthesis of different plant hormones, such as gibberellins, and ethylene, in addition to its role as an antioxidant, and therefore could be important for germination (De Tullio and Arrigoni, 2003). There is evidence of a correlation between seed germination capacity and the AsA system, because mature orthodox seeds lack AsA (De Tullio and Arrigoni, 2003). Increasing the AsA content

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by exogenous application may therefore be beneficial for seed germination of some species, particularly under salinity stress, which is known to accelerate the ROS production in plants (Noctor and Foyer, 1998; Hernández and Almansam, 2002). Khan *et al.* (2006a) reported such beneficial effects of exogenous AsA on seed germination of halophytes under salt stress; however, the responses were species specific.

Phragmites karka (Retz.) is a widely distributed grass in Pakistan found along streams and in wet grasslands and swamp. It is capable of withstanding heavy floods, which makes it an excellent stabilizer of erosion-prone river banks. It is also a source of lignocellulosic biomass for ethanol production (Abidin *et al.*, 2011) and is grazed in some regions of New Guinea (Rosa-Innes, 1977). *Dichanthium annulatum* (Forssk.) Stapf is a perennial halophytic fodder grass eagerly sought by grazing livestock and is commonly found in the salt flats of the Karachi region. *Eragrostis ciliaris* (L.) R. Br. is an annual grass that is also distributed widely in salt flats and usually develops after monsoon rains; it is also readily grazed (Cope, 1982).

We report the role of AsA in alleviating the effect of salinity on seed germination of *P. karka*, *D. annulatum* and *E. ciliaris*. An initial study with *P. karka* helped us to select an optimal temperature regime for the study.

Materials and methods

Seeds of the three grass species were collected during September 2009 from the Karachi University campus, then separated from the inflorescence, cleaned and stored at room temperature. During October, seeds were surface-sterilized with 1% sodium hypochlorite before commencing the experiment. Twenty-five seeds in four replicates were germinated in 5 mL water or saline solution using air-tight plastic Petri dishes kept in growth cabinets. Germination (emergence of radicle) was recorded every alternate day for 20 days. The rate of germination was calculated with the help of a modified Timson's index of germination velocity (=$\sum$G/t) where *G* is the percentage of seed germinated after 20 days and *t* is the total time of germination (Khan and Ungar, 1999).

Seeds of *P. karka* were germinated in six concentrations of NaCl (0, 100, 200, 300, 400 and 500 mM) with or without AsA (5, 10, 20 mM) in 12 h light/12 h dark photoperiod (25 μmol m^{-2} s^{-1}; 400–700 nm cool white fluorescent Philips lamps, Karachi, Pakistan) at four temperature regimes (10/20, 15/25, 20/30 and 25/35°C) simulating night/day conditions. Seeds of *D. annulatum* and *E. ciliaris* were germinated similarly, in incubators as described above, but only at 20/30°C with different concentrations (5, 10, 20, 25 mM) of AsA and salinity 0, 25, 75, 100, 125 and 150 mM NaCl for *E. ciliaris* and 0, 100, 200, 300, 400 and 500 mM NaCl for *D. annulatum*. Most previous studies had indicated that 20/30°C is an optimal temperature regime for the seed germination of

Table 1 Three-way ANOVA for percentage germination and rate of germination in seeds of *Phragmites karka*.

Independent variables	Dependent variables	
	Germination (%)	Germination (Rate)
	F-value	
Temperature (T)	566·43	962·37
NaCl (S)	157·76	220·97
Ascorbic acid (AsA)	214·23	287·75
T × S	4·65	7·96
T × AsA	39·94	68·23
S × AsA	24·82	34·82
T × S × AsA	3·67	4·32

Numbers represent *F*-values significant at $P < 0{\cdot}0001$.

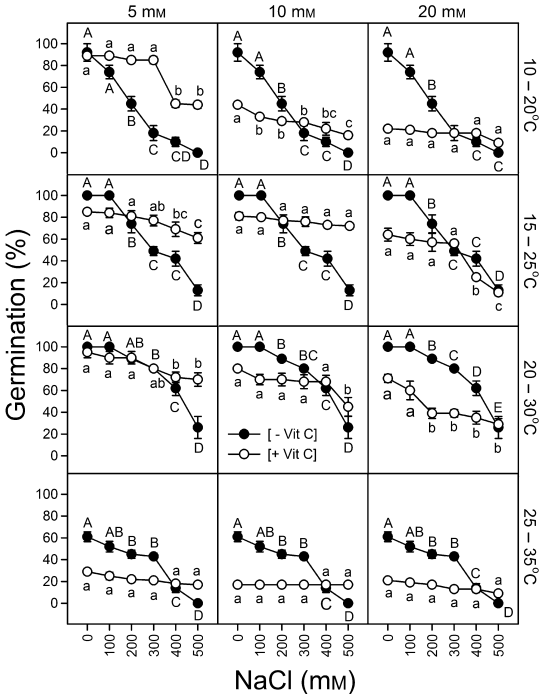

Figure 1 Effect of NaCl with and without various concentrations of ascorbic acid (AsA) on seed germination of *Phragmites karka* at various thermo-periods. The same letters within each AsA concentration indicate non-significant ($P > 0{\cdot}05$) differences (Bonferroni test) at the end of the experiment.

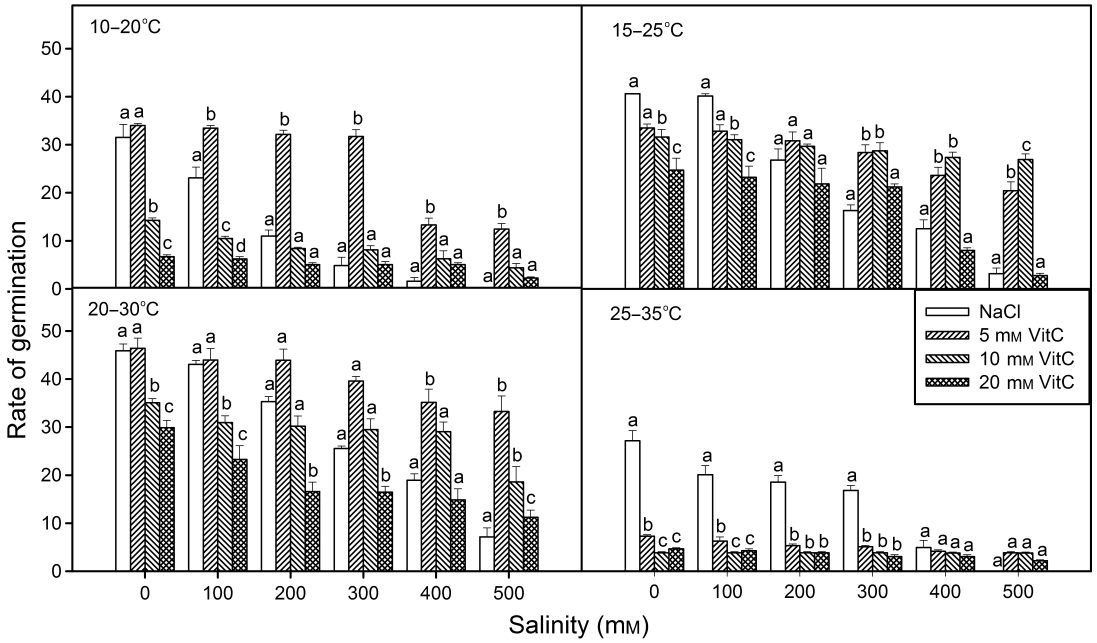

Figure 2 Effect of NaCl with and without various concentrations of ascorbic acid on germination rate of *Phragmites karka* at different temperature regimes. The same letters within each salinity concentration indicate non-significant ($P < 0{\cdot}05$) differences (Bonferroni test) at the end of the experiment.

subtropical species. Analysis of variance (ANOVA) was used to test for significance of differences between treatments (SPSS, 1999) and their interactions. The Bonferroni test (a multiple-range test) was carried out to determine significance ($P < 0{\cdot}05$) between individual treatments.

Results

Phragmites karka

A three-way ANOVA indicated significant ($P < 0{\cdot}0001$) individual effects of salinity, temperature, AsA and their interactions on seed germination of *P. karka* (Table 1). Increase in both salinity and temperature decreased germination (Figure 1). Low levels of AsA (5 mM) significantly alleviated salinity effects, which were prominent especially at 10/20°C. Here, even at the high salinity of 500 mM NaCl, about 50% seeds germinated when treated with AsA in comparison with no germination in the absence of AsA, whereas 10 and 20 mM AsA had generally negative effects. At the higher temperature regime of 15/25°C, both 5 and 10 mM AsA treatments alleviated the salinity effect, but no such effect was observed at 20 mM treatment. The salinity effect on germination of *P. karka* was alleviated in the presence of 5 mM AsA at 20/30°C, at which 80% seeds germinated at 500 mM of NaCl. At the warmer thermo-period of 25/35°C, seed germination was significantly inhibited in both non-saline and saline conditions, and application of AsA further inhibited seed germination. The rate of germination was also significantly improved by the application of lower concentrations (5 and 10 mM) of AsA at all thermo-periods except at 25/35°C (Figure 2).

Table 2 Two-way ANOVA for percentage germination and rate of germination in seeds of *Dichanthium annulatum* and *Eragrostis ciliaris*.

Sources	Rate of Germination	Rate of Recovery
	F-value	
Dichanthium annulatum		
Ascorbic acid (AsA)	25·951	21·067
NaCl (S)	245·513	345·214
ASA × S	5·561	9·309
Eragrostis ciliaris		
Ascorbic acid (AsA)	16·397	8·063
NaCl (S)	123·992	150·247
ASA × S	4·297	4·520

Numbers represent *F*-values significant at $P < 0{\cdot}001$.

Dichanthium annulatum and *Eragrostis ciliaris*

A two-way ANOVA indicated significant ($P < 0{\cdot}001$) effects for NaCl and AsA on seed germination for both *D. annulatum* and *E. ciliaris* (Table 2). Increase in NaCl concentration progressively decreased germination in both species, whereas the application of AsA stimulated germination of *E. ciliaris* but reduced it in *D. annulatum* (Table 3); this response was dependent on the concentration of AsA applied. Application of AsA alleviated the salinity effect in *E. ciliaris*; in its absence, none of the seeds germinated above 125 mM. Only 4% of seeds germinated at 150 mM NaCl in the presence of 20 mM AsA. At low AsA level (5 mM), only a few seeds germinated above 125 mM NaCl (Table 3). A low germination was observed in *D. annulatum* seeds treated with 400 mM NaCl and 25 mM AsA (Table 3).

The rate of germination progressively decreased with increasing NaCl concentration in both these species (Table 3). Increasing the AsA concentration increased the germination rate, but there was no significant difference between AsA concentrations in *E. ciliaris* seeds, although *D. annulatum* showed significant difference ($P < 0{\cdot}0001$) between AsA and NaCl concentrations on the germination rate (Table 2).

Discussion

Seed germination is generally inhibited under saline conditions, and the effect is accentuated when temperature conditions deviate from optimum (Khan and Gul, 2006). Our results indicated that, in the case of *P. karka*, warmer temperature regimes prevented more seed from germinating. Osmotic and ionic stress

Table 3 Total (%) germination and rate of germination (mean ± standard error) at the end of the experiment for *Dichanthium annulatum* and *Eragrostis ciliaris* in different salinities with various concentrations of ascorbic acid (AsA).

		Dichanthium annulatum			*Eragrostis ciliaris*	
ASA (mM)	**NaCl (mM)**	**Germination (%)**	**Rate of germination**	**NaCl (mM)**	**Germination (%)**	**Rate of germination**
0	0	100·0 ± 0·0	50·0 ± 0·00	0	63·0 ± 1·9	27·2 ± 1·0
	100	85·0 ± 2·5	24·2 ± 1·56	25	54·0 ± 8·8	24·0 ± 3·9
	200	31·0 ± 5·7	10·9 ± 2·31	75	18·0 ± 6·6	8·4 ± 3·0
	300	41·0 ± 1·0	14·6 ± 0·63	100	17·0 ± 1·9	6·2 ± 0·5
	400	24·0 ± 2·8	7·1 ± 1·05	125	6·0 ± 2·0	1·9 ± 1·2
	500	0·0 ± 0·0	0·0 ± 0·00	150	0·0 ± 0·0	0·0 ± 0·0
5	0	58·0 ± 8·0	26·1 ± 3·64	0	76·0 ± 13·9	36·0 ± 6·7
	100	20·0 ± 1·4	26·0 ± 4·16	25	46·0 ± 3·5	17·3 ± 0·6
	200	20·0 ± 6·9	6·9 ± 2·25	75	50·0 ± 3·45	14·8 ± 0·8
	300	20·0 ± 0·6	6·3 ± 2·19	100	30·0 ± 1·2	7·1 ± 0.
	400	0·0 ± 0·0	0·0 ± 0·00	125	10·0 ± 1·2	2·2 ± 0·4
	500	0·0 ± 0·0	0·0 ± 0·00	150	2·0 ± 1·2	0·1 ± 0·1
10	0	78·0 ± 3·5	36·3 ± 2·25	0	100·0 ± 0·0	46·4 ± 0·3
	100	54·0 ± 5·8	21·3 ± 1·67	25	64·0 ± 2·3	23·4 ± 1·0
	200	24·0 ± 4·6	9·7 ± 1·33	75	48·0 ± 2·3	17·1 ± 1·0
	300	16·0 ± 2·3	5·2 ± 0·46	100	56·0 ± 2·3	15·6 ± 0·0
	400	0·0 ± 0·0	0·0 ± 0·00	125	16·0 ± 4·6	4·5 ± 1·2
	500	0·0 ± 0·0	0·000·00	150	10·0 ± 3·5	1·0 ± 0·2
20	0	88·0 ± 6·9	37·5 ± 1·44	0	70·7 ± 13·3	30·0 ± 5·8
	100	46·0 ± 8·1	18·9 ± 2·94	25	76·0 ± 6·9	29·2 ± 3·2
	200	44·0 ± 2·3	19·6 ± 0·92	75	50·0 ± 3·5	15·6 ± 0·6
	300	04·0 ± 2·3	1·6 ± 0·92	100	40·0 ± 4·6	11·6 ± 1·0
	400	0·0 ± 0·0	0·0 ± 0·00	125	28·0 ± 2·3	5·8 ± 1·4
	500	0·0 ± 0·0	0·0 ± 0·00	150	4·0 ± 0·0	1·3 ± 0·2
25	0	54·0 ± 3·5	23·3 ± 1·21	0	52·0 ± 0·7	22·6 ± 2·1
	100	52·0 ± 1·3	22·4 ± 0·46	25	60·0 ± 0·4	25·4 ± 3·4
	200	22·0 ± 0·4	8·2 ± 0·12	75	44·0 ± 0·8	15·6 ± 2·3
	300	14·0 ± 0·5	5·4 ± 2·19	100	56·0 ± 1·0	16·6 ± 2·0
	400	2·0 ± 0·6	0·5 ± 0·29	125	12·0 ± 1·0	2·9 ± 0·8
	500	0·0 ± 0·0	0·0 ± 0·00	150	2·0 ± 1·2	0·6 ± 0·4

increased under suboptimal temperature conditions and inhibited seed germination. These stresses may cause imbalance in growth regulators and possible increase in ROS production (Sairam and Tyagi, 2004; Wang *et al.*, 2004; Neto *et al.*, 2006). Accumulation of Na^+ may enhance electrolyte leakage, decrease hydrolytic enzyme activity and increase peroxidase activity (Dionisio-Sese and Tobita, 1998; Imlay, 2003; Demiral and Türkan, 2004; Neto *et al.*, 2006).

In our study, inclusion of AsA (at 5 and 10 mM) in the growth medium alleviated the salinity effects on the germination of *P. karka* at lower temperature regimes. In the case of *D. annulatum* and *E. ciliaris*, the effect of AsA was found to be species specific (i.e. pretreatment with AsA alleviated the salinity effect of NaCl on seed germination of *E. ciliaris*, whereas it suppressed the effect in *D. annulatum* at all salinity levels). Khan and Gul (2006) indicated that the effect of AsA on salinity tolerance varied depending on plant species (i.e. its application alleviated salinity effects on *Atriplex stocksii* and *Suaeda fruticosa*, whereas no effect was found on *Aeluropus lagopoides, Arthrocnemum macrostachyum*, *Desmostachya bipinnata* and *Haloxylon stocksii*).

In this study, the application of AsA significantly alleviated salinity stress on the germination of *P. karka* seeds, but it failed to compensate for warmer temperature stress. It can be argued that the response of a system to a particular stress may be different from a combination of stresses as observed here in the presence of both salinity and high temperature.

Seeds pre-treated with 20 mM AsA showed high germination in *E. ciliaris* at each salinity level compared to the nil-AsA control. However, seed germination of *D. annulatum* was inhibited by AsA application, thereby suggesting that a single antioxidant is not responsible for successful protection against ROS in a stressed plant (Foyer *et al.*, 1994) and a complete set of antioxidant defence systems is required to increase stress tolerance. From the present research, it is concluded that the effect of AsA on seed germination is species specific and it may suppress or alleviate the salinity stress. There is no universality in response; therefore, this warrants cautious and careful consideration not only of the nature of a particular antioxidant but also of its concentration for the species under test. Furthermore, environmental factors such as salinity and temperature may become significant determinants of the response in individual cases.

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