



Effect of salinity and temperature on the germination of *Spergularia marina* seeds and ameliorating effect of ascorbic and salicylic acids

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Abstract: *Spergularia marina* (Caryophyllaceae) is a halophytic species and widely distributed among the sea shores of Turkey. Its seeds may be unwinged or winged. Laboratory experiments were conducted to determine the effects of salinity (0, 50, 100 and 500 mM NaCl) and temperature (10, 15, 25, 30, 35°C) on seed germination. *S. marina* showed 73.3% germination in non-saline controls at 25°C. No germination occurred at 30°C and 35°C. Seed germination of *Spergularia marina* was significantly affected by salinity levels, temperature and their interactions. Maximum final germination percentage occurred under the lowest salinity condition (50 mM) and distilled water at 25°C. Seed germination was completely inhibited by 500 mM NaCl, although seed germination rate was not affected by salinity. Recovery germination was greatest in 500 mM and at 25°C. The rate of germination was significantly affected by temperature ($p < 0.01$). The germination percentage of the seeds pretreated with 40 mM and 60 mM L-ASA in 50 mM and 100 mM NaCl was improved compared with that of untreated L-ASA. Addition of 0.5 mM salicylic acid (SA) improved the germination in 50 mM NaCl.

Key words: *Spergularia marina*, Salinity, Temperature, Germination, Ascorbic acid, Salicylic acid
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Introduction

Salinity stress is a major limiting factor for plants germination growing in coastal habitats as it is one of the most critical periods in life cycle of halophytes (Gilles *et al.*, 2001; Rubio Casal *et al.*, 2003). Salt stress affects germination percentage, germination rate and seedling growth in different ways depending on the plant species (Ungar, 1996; Gul and Weber, 1999). It was reported that maximum germination of the seeds of halophytic plants occurred in distilled water or under reduced salinity (Gul and Weber, 1999; Khan and Gulzar, 2003; Carter and Ungar, 2003), and it has been found that germination percentage is reduced with a high NaCl concentrations (Tobe *et al.*, 2001; Pujol 2000; Rubio-Casal *et al.*, 2003). Germination of halophytes typically occurs after rains which provide moisture and leaching the salt ions (Khan and Ungar, 1997; Vincente, 2004; El Keblawy, 2004; El Keblawy and Al Rawai, 2005; Song *et al.*, 2006).

Temperature is also an important factor that determines germination. The effects of salinity and temperature on germination of many halophytic plants have been studied by several authors and response of the plants to temperature and salinity varies according to salinity response of the halophytic species (Gul and Weber, 1999; Khan and Ungar, 2001; Khan *et al.*, 2001; El Keblawy and Al Rawai, 2005; Al Khateeb, 2006; Gorai *et al.*, 2006; Song *et al.*, 2006; Cicek and Tilki, 2007; Tilki and Dirik, 2007).

Salinity may cause disturbances in plant metabolism (El Tayeb, 2005). Salinity-induced oxidative stress could be a reason for germination inhibition (Amor *et al.*, 2005). The strategy of osmotic adjustment varied from one plant to another as well as tissues. Many trials have been made to help the plants to overcome these disturbances using various treatments in laboratory.

Salicylic acid (SA) plays an important role in abiotic plant stress tolerance increasing their resistance to salinity, water deficit, low temperature as well as detrimental action of heavy metals (Mishra and Chaudhari, 1997; Mishra and Chaudhari, 1999; Senaratra *et al.*, 2000; Bezrukova *et al.*, 2001; Shakirova *et al.*, 2003; Singh and Usha, 2003; Drazic and Mihailovic, 2005; El Tayeb, 2005; Gunes *et al.*, 2007; Chandra *et al.*, 2007).

Exogenously applied salicylic acid may improve germination of halophytes. Similarly application of L-ascorbic acid alleviated the sea salt effects in some halophytes (Khan *et al.*, 2006). Also L-ascorbic acid (L-ASA) ameliorated the toxic effect of heavy metals in animals (Singh and Rana, 2007). Little information is available on the effect of salicylic acid and ascorbic acid on the germination of the seeds under salt stress.

Spergularia marina (Caryophyllaceae) is a widely distributed halophytic species through the seashores of Turkey. The seeds of this species may be unwinged or winged. Previous experiments indicated that there is an interaction between the effects of temperature and salinity on germination of *S. marina* (Ungar, 1967). Germination rate was significantly reduced at higher salinity levels (Keiffer and Ungar, 1997) and high salt concentration with increasing temperature displayed an inhibitory effect on germination (Ungar, 1967).

Therefore, the aims of the present study were:

- 1) to investigate the effect of temperature and salinity on the germination of the seeds of *S. marina*,
- 2) to determine other germination characteristics (*i.e.* germination rate, mean daily germination, recovery germination)
- 3) to show improving effect of salicylic acid and L-ascorbic acid on the germination of the seeds under salt stress.

Materials and Methods

Seed collection: The seeds of *S. marina* were collected in July 2004 from pure stand in saline soils in Samsun (41°42' 17" and 41°31' 46" N and 36°05' 40" and 36°03' 01" E). Seeds were dried in laboratory and stored at room temperature before being used (seed weight after drying : 44±2 mg 1000⁻¹ seeds).

Effect of temperature and salinity on seed germination: Germination trials were carried out in sterilized petri dishes on two layers of filter paper moistened with 10 ml solution of 50, 100 or 500 mM NaCl or distilled water (control). Temperature was kept constant at 10, 15, 20, 25, 30 and 35°C. All petri dishes were kept in an incubator under continuous darkness for 15 days. Each of the three replicates of 100 seeds each were used. The solution in each petri dish was renewed every three days. Germinated seeds were counted, then these seeds were removed from petri dishes. Seeds were considered germinating with the emergence of the radicle.

Four parameters of germination were determined:

1. Final germination percentage
2. Germination rate (GR): is a measure of rapidity of germination, with lower values indicating faster germination. It is calculated as follows:

$$GR = (n_1 t_1) + (n_2 t_2) + \dots + (n_x t_x) / x^n$$

Where n_i is the number of germinants at the first day of germination, t_i is the days from start to first germination, and x^n the total numbers of seeds germinated (Rubio-Casal *et al.*, 2003).

3. Mean daily germination (MDG): where

$$MDG = \frac{\text{Final germination percentage}}{\text{number of days to final germination percentage}}$$

(Rubio-Casal *et al.*, 2003).

4. Recovery test: After 15 days, ungerminated seeds from each petri dish with 50, 100 and 500 mM NaCl were transferred to distilled water and the germination was recorded after seven days. The total germination was determined by the following formula:

$$(a + b) / c \times 100$$

where "a" is the total number of seeds germinated in saline solution, "b" is the total number of seeds germinated in distilled water after transferred from NaCl condition from distilled water (Song *et al.*, 2006) and "c" total number of seeds sown. Recovery tests were made at 10, 20 and 25°C.

The seeds of *S. marina* were exposed to NaCl and distilled water applications and kept at 12:12 light/dark photoperiods during the preliminary study which conducted to determine the effects of light on seed germination. After 15 days of the light application it was

determined that there were no significant differences between dark and 12:12 light/dark period applications. So that the study was continued over dark period.

The effect of L-ascorbic acid and salicylic acid on seed germination in NaCl.

L-Ascorbic acid: The seeds of *S. marina* were pretreated with L-ascorbic acid (20, 40 and 60 mM) for 4 hr and air dried before immersing in NaCl solutions (0, 50, 100 and 500 mM).

The experiments were carried out in an incubator at continuous dark and 25±2°C. Germinated seeds were counted and final germination percentage was determined.

Salicylic acid: The seeds were imbibed with salicylic acid (0.5, 1 and 2 mM) for 24 hr and air dried, then were sown in petri dishes with NaCl solution (0, 50, 100 and 500 mM). Final germination percentage was determined.

Statistical analysis: Statistical analysis was performed using SPSS 10.0 version (Anonymous, 1999). Two-way analysis of variance (ANOVA) was performed to show the differences in seed germination parameters depending on temperature and NaCl concentrations. Tukey's honestly significant difference (HSD) test was used to rank means following analysis of variance.

Results and Discussion

The effects of temperature and salinity on germination: Final germination percentage of *S. marina* seeds was significantly affected by the temperature, salinity and the interactions of both abiotic factors ($p < 0.01$) (Table 1). Germination did not occurred at 30°C and 35°C, in all NaCl concentrations. Seed germination was highest in distilled water at 25°C second highest in 50 mM. Germination decreased at lower temperature (10°C) compared with that of 25°C. Compared with distilled water, germination percentage decreased little in 50 mM NaCl, while it was severely affected by 100 mM NaCl at 25°C. Germination was completely inhibited in 500 mM at 10, 15 and 25°C (Fig. 1).

The rate of germination was the highest at 25°C and the lowest at 10°C (Fig. 2). Changes in temperature significantly effected the rate of germination ($p < 0.01$) while various salinity and interaction of temperature-salinity did not effect the germination rate (Table 1).

There were significant differences between salinity and temperature ($p < 0.01$) in terms of mean daily germination (MDG). The highest MDG was obtained at 25°C in distilled water while it decreased at lower temperature and salinities (Fig. 3).

Recovery germination: Seeds showed recovery from salinity stress at all temperatures. There was relatively lower recovery at lower temperatures (10-15°C) in high salinity treatments (100 - 500 mM). However at 25°C, there was substantial recovery at higher salinities (Fig. 4).

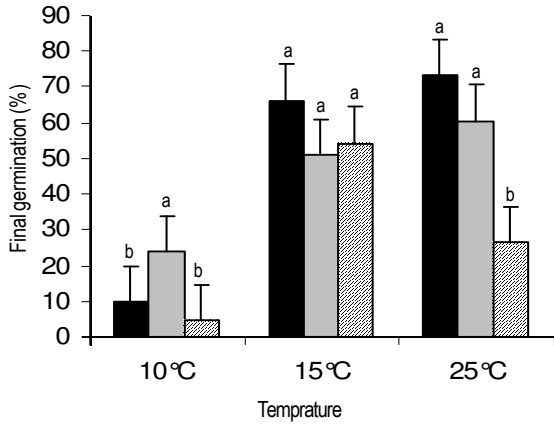


Fig. 1: Final germination percentage (%) with respect to temperature and NaCl concentrations

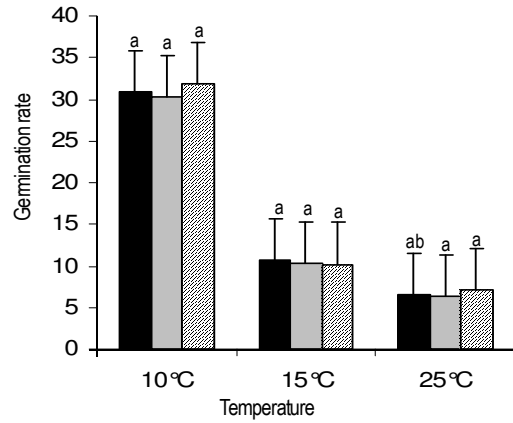


Fig. 2: Germination rate with respect to temperature and NaCl concentrations

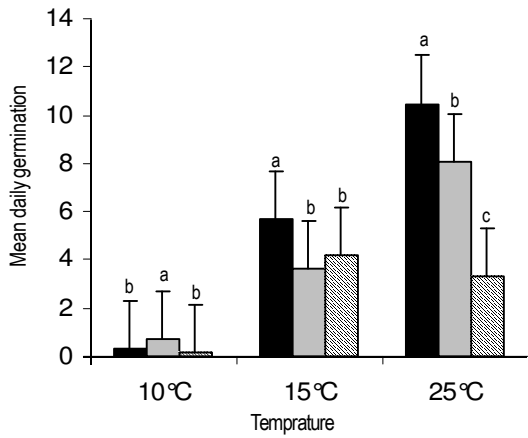


Fig. 3: Mean daily germination (MDG) with respect to temperature and NaCl concentrations

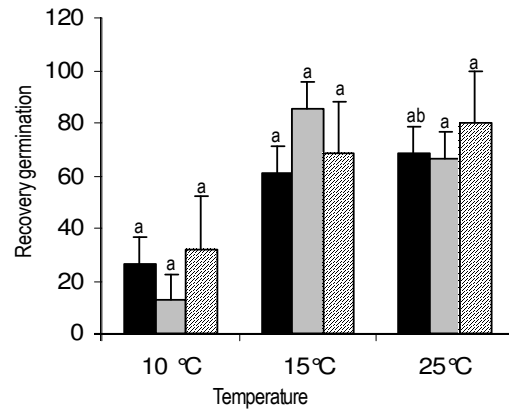


Fig. 4: Recovery germination with respect to temperature and NaCl concentrations

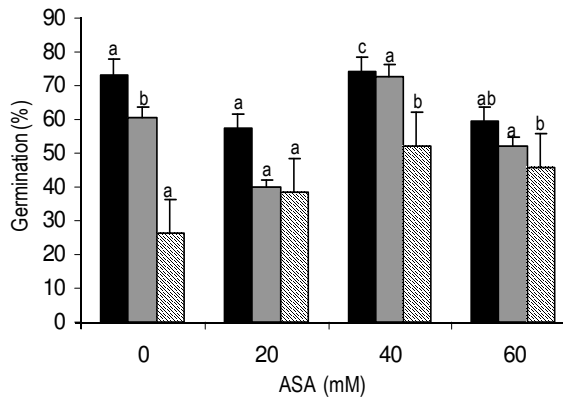


Fig. 5: Germination percentage with respect to L-ascorbic acid and NaCl concentrations

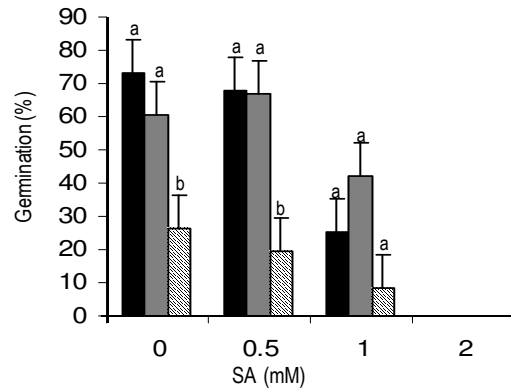


Fig. 6: Germination percentage with respect to salicylic acid and NaCl concentrations

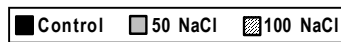


Table - 1: Results of two-way ANOVA of characteristics of seed germination when seeds were moistened with 0, 50, 100 and 500 mM NaCl at constant 5, 10, 15, 25, 30 and 35°C in continuous darkness

Independent variable	Temperature	NaCl	Temp. & NaCl	Total mean \pm SD
Final germination percentage	5436.704**	1144.704**	561.815**	41.18 \pm 24.76
Germination rate	1548.98**	1.437 NS	.780 NS	16.02 \pm 10.95
Mean daily germination	108.010**	19.602**	12.041**	4.05 \pm 3.44
Recovery germination	6912.111	152.111**	383.389*	55.66 \pm 25.62

Note: Number represents mean square (*p < 0.05; **p < 0.01, NS : Not significant), SD: Standard deviation

Table - 2: Statistical analysis of the effect of salicylic acid (SA) on the germination of *S. marina* seeds by two way ANOVA

Independent variable	Salicylic acid			Total mean \pm SD
	SA	NaCl	SA & NaCl	
Final germination percentage (%)	5702.917**	3231.361**	560.472*	32.52 \pm 29.61

Note: Number represents mean square (*p < 0.05; ** 0.01) SD: Standard deviation

Table - 3: Statistical analysis of the effect of ascorbic acid (A) on the germination of *S. marina* seeds by two way ANOVA

Independent variable	Ascorbic acid			Total mean \pm SD
	L-ASA	NaCl	L-ASA & NaCl	
Final germination percentage (%)	413.731**	1210.583**	497.620*	56.08 \pm 15.36

Note: Number represents mean square (**p < 0.05), SD: Standard deviation

Recovery of germination was significantly affected by temperature (p < 0.01) and the interactions of temperature with salinity (p < 0.05) (Table 1). However there were no relation between recovery and various salt concentrations.

Effect of L- ascorbic acid (L-ASA) and salicylic acid (SA) on the seed germination: L-ascorbic acid pretreatments alleviated the inhibitory effect of salt (50 mM and 100 mM) on seed germination of *S. marina* (Fig. 5). The germination percentage pretreated with 40 mM and 60 mM L-ASA in 50 mM and 100 mM NaCl was similar with that of untreated seeds. The effect of L-ASA, NaCl and the interaction of L-ASA and NaCl were statistically significant (p < 0.05) (Table 3). Improved effect of L-ASA was not observed in 500 mM NaCl. The germination percentage of the seeds pretreated with L-ASA (20, 40 and 60 mM) decreased in distilled water (Fig. 5). Final germination percentage was significantly improved by L-ascorbic acid pretreatments at 50 and 100 mM salt concentrations. Effects of L- ascorbic acid pretreatments on seed germination of halophytic species were found to be species- specific (Khan *et al.*, 2006). Final germination stage of *S. marina* seeds embedded in 40 and 50 mM ascorbic acid was found very close to the control group when they were incubated in 50 mM NaCl media. A very slight improved effect was observed when they were incubated with 100 mM NaCl.

Improving effect of 0.5 mM salicylic acid on the germination was observed in 50 mM NaCl (Fig. 6). But germination percentage of the seeds pretreated with 1 mM SA decreased in distilled water and all salinity conditions tested. SA, NaCl and the interaction of both

the factors were significant (Table 2). The germination of the seeds pretreated with 2 mM SA was completely inhibited under all conditions (Fig. 6).

Spergularia marina is an annual plant and it has a cosmopolitan distribution and occurred in coastal habitats all over the world. *S. marina* has occupied in saline areas with soil total salinity concentrations about 4.0% (Ungar, 1992). But seeds have been reported to germinate in NaCl concentrations of 1.0% but not at 2.0% under laboratory conditions (Keiffer and Ungar, 1997). Ungar (1967) was determined that the maximum germination temperature was 13-21°C in non-saline and low salt concentration (0.5%).

During the preliminary study it was determined that light has no dominant effect on the germination of *S. marina* seeds (data not shown). However, Carter and Ungar (2004) explained that light positively effects on the germination of *S. marina* seeds. This difference may be due to geographical differences.

The results of this study indicate that the seeds of *S. marina* were susceptible to salinity at germination stage. Halophyte seed germination has been reported to occur optimally under reduced salinity stress (Ungar, 1967; Khan and Ungar, 1997; Gul and Weber, 1999; Aiazzi *et al.*, 2002; Khan and Ungar, 2001; Khan *et al.*, 2001; Khan and Gulzar, 2003; Rubio-Casal *et al.*, 2003; El Keblawy and Al Rawai, 2005). However the effect of salinity and temperature was different on the germination of some halophyte seeds. For example, reports indicate that some halophyte seeds germinated at higher

temperatures, and maximum germination occurred under both non-saline and saline conditions (Khan *et al.*, 2000; Khan *et al.*, 2001; Song *et al.*, 2006). High salinity cause germination inhibition at higher temperatures, it suggests that the mechanism of Na⁺ toxicity may not be present in some species (Aiuzzi *et al.*, 2002). In our study, the increase of temperature and salt concentration effects germination rate (GR). Exposure to lower or higher temperatures and higher salt concentrations not only provoke inhibition of germination percentage but also a decrease in germination rate.

In this research, germination rate was significantly reduced at 10 and 15°C, but the effect of increase in salt concentration was not statistically significant. In the seeds of *Arthrocnemum macrostachyum*, GR was related to salinity and slowed in high salt concentration (Rubio-Casal *et al.*, 2003). Similar results were also obtained from *Prosopis juliflora* seeds (El Keblawy and Al Rawai, 2005)

Salinity inhibits germination of halophyte seeds in one of two ways: (1) preventing germination without loss of viability at higher salinities; and (2) delaying germination of seeds at salinities that cause some stress to seeds but not prevent germination (Gulzar and Khan, 2001).

The response of seeds of *S. marina* transferred to distilled water after 15 d at various salinities varied depending on the temperature. Seeds exposed to higher salinity (100-500 mM NaCl) recovered quickly at moderate temperature (25°C). Under hypersaline conditions, seed survival rather than germinability may be an appropriate criterion for success, since recovery germination occur in seeds of halophytes when hypersaline conditions are alleviated (Pujol *et al.*, 2000; Gulzar and Khan 2001; Huang *et al.*, 2003). However the recovery germination of seeds that were previously exposed to hypersaline conditions was affected by temperature regime (Keiffer and Ungar, 1997; Gulzar and Khan, 2001; Song *et al.*, 2006). Similar results were also reported for *Salicornia ramosissima* and *Arthrocnemum macrostachyum* (Rubio Casal *et al.*, 2003) and *H. ammodendron* (Huang *et al.*, 2003). Seed viability was not affected by exposure to high salt concentrations and the seeds of the species exposed to higher salinity showed a greater germination percentage in recovery germination.

At germination level reactive oxygen species (ROS) are generated mainly during depletion of food reserves and oxidative phosphorylation, while their quantitative level is controlled by seed's protective antioxidant system. However, it has been reported that seeds contain various antioxidants in small amounts and compounds like ascorbic acid are not present. An artificial increase in cellular level of an antioxidant such as ascorbic acid should be beneficial in improving stress tolerance at germination level (Khan *et al.*, 2006).

Detailed studies on components of antioxidant defense systems of seeds of halophytes might provide satisfying evidence.

Our results are consistent with those of Shakirova *et al.*, (2003) and El Tayeb (2005), who showed an encouragement in seed germination with SA application. Improved effect of salicylic acid on germination of *S. marina* seeds were observed. It has been found that final germination rate was higher when 0.5 M salicylic acid embedded seeds were incubated in 50 mM NaCl containing media and control media (without NaCl) as compared to seeds without embedded in salicylic acid. The effect of NaCl and salicylic acid interaction on final germination rate was statistically significant. Since salicylic acid is an endogenous growth regulator, it was important to study the influence of exogenous SA on seed germination (El Tayeb, 2005).

Salicylic acid (SA) is an endogenous growth regulator of phenolic nature. Exogenous application of SA may influence a range of diverse processes in plants, including seed germination, stomatal closure, ion uptake and transport, photosynthesis and growth (El Tayeb, 2005). We suggested that SA and ASA application should be used in germination studies with halophytic plants for improving germination.

References

- Aiuzzi, M.T., P.D. Carbone, J.A. Rienzo and J.A. Arguello: Effects of salinity and temperature on the germination and early seedling growth of *Atriplex cordobensis* Gandoger et Stuckert (Chenopodiaceae). *Seed Sci. Technol.*, **30**, 329-338 (2002).
- Al Khateeb, S.A.: Effect of salinity and temperature on germination, growth and ion relations of *Panicum turgidum* Forssk. *Biores. Tech.*, **97**, 292-298 (2006).
- Amor, N.B., K.B. Hamed, A. Debez, C. Grignon and C. Abdelly: Physiological and antioxidant responses of perennial halophyte *Crithmum maritimum* to salinity. *Plant Sci.*, **4**, 889-899 (2005).
- Anonymous: SPSS 10.0 for Windows. SPSS Inc., New York (1999).
- Bezrukova, M.V., A.R. Sakhabutdinova, R.A. Fathutdinova, I.A. Kyldiarova and F.M. Shakirova: The role of hormonal changes in protective action of salicylic acid on growth of wheat seedlings under water deficit. *Agrochemya* (Russ.), **2**, 51-54 (2001).
- Chandra, A., A. Anand and A. Dubey: Effect of salicylic acid on morphological and biochemical attributes in cowpea *J. Environ. Biol.*, **28**, 193-196 (2007).
- Carter, C.T. and I.A. Ungar: Germination response of dimorphic seeds of two halophyte species to environmentally controlled and natural conditions. *Can. J. Bot.*, **81**, 918-926 (2003).
- Carter, C.T. and I.A. Ungar: Relationships between seed germinability of *Spergularia marina* (Caryophyllaceae) and the formation of zonal communities in an Inland salt marsh. *Ann. Bot.*, **93**, 119-125 (2004).
- Cicek, Emrah and Fahrettin Tilki: Seed germination of three *Ulmus* species from Turkey as influenced by temperature and light. *J. Environ. Biol.*, **28**, 423-425 (2007).
- Drazic, G. and N. Mihailovic: Modification of cadmium toxicity in soybean seedlings by salicylic acid. *Plant Sci.*, **2**, 511-517 (2005).
- El Keblawy, A.: Salinity effects on seed germination of the common desert range grass, *Panicum turgidum*. *Seed Sci. Technol.*, **32**, 943-948 (2004).
- El Keblawy, A. and A. Al-Rawai: Effect of salinity, temperature and light on germination of invasive *Prosopis juliflora* (Sw.) D.C. *J. Arid Environ.*, **61**, 555-565 (2005).
- El-Tayeb, M.A.: Response of barley grains to interactive effect of salinity and salicylic acid. *Plant Growth Regul.*, **45**, 215-224 (2005).
- Gilles, H., L. Morel and C.E. Reynolds: The effect of salinity on different developmental stages of an endemic annual plant, *Aster laurentianus* (Asteraceae). *Amer. J. Bot.*, **88**, 62-67 (2001).

- Gorai, M., A.M. Vadel and M. Neffati: Seed germination characteristics of *Phragmites communis*: Effects of temperature and salinity. *Belg. J. Bot.*, **139**, 78-86 (2006).
- Gul, B. and D.J. Weber: Effect of salinity, light, and temperature on germination in *Allenrolfea occidentalis*. *Can. J. Bot.*, **77**, 240-246 (1999).
- Gulzar, S. and M.A. Khan: Seed germination of a halophytic grass *Aeluropus lagopoides*. *Ann. Bot.*, **87**, 319-324 (2001).
- Gunes, A., A. Inal, M. Alpaslan, F. Eraslan, E. Guneri Bagc and N. Cicek: Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. *J. Plant Physiol.*, **164**, 728-736 (2007).
- Huang, Z., X. Zhang, G. Zheng and Y. Guterman: Influence of light, temperature, salinity and storage on seed germination of *Haloxylon ammodendron*. *J. Arid Environ.*, **55**, 453-464 (2003).
- Keiffer, C.H and I.A. Ungar: The effect of extended exposure to hypersaline conditions on the germination of five inland halophyte species. *Am. J. Bot.*, **84**, 104 (1997).
- Khan, M.A and I.A. Ungar: Effect of thermoperiod on recovery of seed germination of halophytes from saline conditions. *Amer. J. Bot.*, **84**, 279-283 (1997).
- Khan, M.A., B. Gul and D.J. Weber: Germination responses of *Salicornia rubra* to temperature and salinity. *J. Arid Environ.*, **45**, 207-214 (2000).
- Khan, M.A and I.A. Ungar: Seed germination of *Triclochin maritima* as influenced by salinity and dormancy relieving compounds. *Biol. Plant*, **44**, 301-303 (2001).
- Khan, M.A., B. Gul and D.J. Weber: Seed germination in relation to salinity and temperature in *Sarcobatus vermiculatus*. *Biol. Plant*, **45**, 133-135 (2001).
- Khan, M.A. and S. Gulzar: Germination responses of *Sporobolus ioclados*: a saline desert grass. *J. Arid Environ.*, **53**, 387-394 (2003).
- Khan, M.A., M.Z. Ahmed and A. Hameed: Effect of sea salt and L-ascorbic acid on the seed germination of halophytes. *J. Arid Environ.*, **67**, 535-540 (2006).
- Mishra, A. and M.A. Choudhuri: Ameliorating effects of salicylic acid on lead and mercury-induced inhibition of germination and early seedling growth of two rice cultivars. *Seed Sci. Technol.*, **25**, 263-270 (1997).
- Mishra, A. and M.A. Choudhuri: Effects of salicylic acid on heavy metal-induced membran degradation mediated by lipoxigenase in rice. *Biol. Plant*, **42**, 409-415 (1999).
- Pujol, A.J., J.F. Calvo and L. Ramiraz-Diaz: Recovery germination from different osmotic conditions by four halophytes from southeastern Spain. *Ann. Bot.*, **85**, 279-286 (2000).
- Rubio-Casal, A.E., J.M. Castillo, C.J. Luque and M.E. Figueroa: Influence of salinity on germination and seeds viability of two primary colonizers of Mediterranean salt pans. *J. Arid Environ.*, **53**, 145-154 (2003).
- Senaratra, T., D. Touchell, E. Bunn and K. Dixon: Acetyl salicylic acid (aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. *Plant Growth Regul.*, **30**, 157-161 (2000).
- Shakirova, F.M., A.R. Sakhabutdinova, M.V. Bezrukova, R.A. Fatkhutdinova and D.R. Fatkhutdinova: Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Sci.*, **164**, 317-322 (2003).
- Singh, B. and K. Usha: Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regul.*, **39**, 137-141 (2003).
- Singh, S. and S.V.S. Rana: Amelioration of arsenic toxicity by L-Ascorbic acid in laboratory rat. *J. Environ. Biol.*, **28**, 377-384 (2007).
- Song, J., G. Feng and F. Zhang: Salinity and temperature effects on germination for three salt-resistant eahalophytes, *Halostachys caspica*, *Kalidium foliatum* and *Halocnemum strobilaceum*. *Plant Soil*, **279**, 201-207 (2006).
- Tilki, Fahrettin and Huseyin Dirik: Seed germination of three provenances of *Pinus brutia* (Ten.) as influenced by stratification, temperature and water stress. *J. Environ. Biol.*, **28**, 133-136 (2007).
- Tobe, K., L. Zhang, G.Y. Qui, H. Shimizu and K. Omasa: Characteristics of seed germination in five non-halophytic Chinese desert shrub species. *J. Arid Environ.*, **47**, 191-201 (2001).
- Ungar, I.A.: Influence of salinity and temperature on seed germination. *Ohio J. Sci.*, **67**, 120-23 (1967).
- Ungar, I.A.: The effect of intraspecific competition on growth, reproduction, and survival of the halophyte *Spergularia marina*. *Int. J. Plant Sci.*, **153**, 421-424 (1992).
- Ungar, I.A.: Effects of salinity on seed germination, growth and ion accumulation of *Atriplex patula* (Chenopodiaceae). *Am. J. Bot.*, **83**, 604-607 (1996).
- Vincente, O., M. Boscaiu, M.A. Naranjo, E. Estrelles, J.M. Belles and P. Soriano: Responses to salt stress in halophyte *Plantago crassifolia* (Plantaginaceae). *J. Arid Environ.*, **58**, 463-481 (2004).