



Seed germination and seedling growth responses of toothbrush tree (*Salvadora persica* Linn.) to different interacting abiotic stresses



S. Midhat S. Hadi, Muhammad Zaheer Ahmed, Abdul Hameed, M. Ajmal Khan, Bilquees Gul*

Institute of Sustainable Halophyte Utilization (ISHU), University of Karachi, Karachi, 75270, Pakistan

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ABSTRACT

Toothbrush tree or miswak (*Salvadora persica* Linn.) is an important medicinal species found abundantly on dry saline lands of the warm subtropical region. Seed germination responses of this plant are not fully known. We therefore studied the effects of salinity (0–500 mM NaCl), temperature (10/20, 15/25, 20/30 and 25/35 °C) and photoperiods (12 h photoperiod and 24 h dark) on seed germination and seedling growth. Seeds were scarified before germination to address physical dormancy. Seeds germinated better at low (10/20, 15/25 °C) to moderate (20/30 °C) in comparison to high (25/35 °C) temperature regimes. Seeds in 12 h photoperiod had higher germination percentages than those germinated in complete dark. Germination decreased with increases in salinity and seeds failed to germinate at 400 mM NaCl or higher salinity. However, un-germinated seeds when transferred to distilled water from high (400 mM NaCl) salinity germinated readily. Recovery percentage decreased with increasing temperature and there was no recovery under the highest temperature regime. Seedling growth was optimal at 15/25 °C and decreased with increases in salinity. Dry storage of seeds for 12 months resulted in substantial reductions in both germination and salinity tolerance. Our data indicates that the seeds of *S. persica* are sensitive to variations in different abiotic factors, which seems to act as a possible cue for germination and subsequent seedling growth.

1. Introduction

Salvadora persica Linn. (a.k.a. Toothbrush tree or miswak) is an evergreen small tree from the family Salvadoraceae and is commonly found in saline areas of Nepal, India, Pakistan, Iran, Arabian Peninsula, Egypt, and Africa (Reddy et al., 2008). This woody halophyte has numerous economic usages, such as being used as toothbrush (Miswak) in many Asian and African cultures (Sher et al., 2010). Some information about the salinity tolerance limit under field conditions (65 dS m⁻¹; Rao et al., 2004) and baseline mechanisms of salinity tolerance (Maggio et al., 2000; Upendra and Dagla, 2016; Rangani et al., 2016) at mature vegetative stage exists. However, we found only one report that tested effects of constant temperatures and light on seed germination of this plant (Tripathi and Joshi, 2010).

Location and timing of seedling recruitment and even the possibility of seedling survival depend on germination responses to variations in environmental conditions, such as salinity, photoperiod, and temperature regime (Gul et al., 2013). Generally, higher stress tolerance during seed germination and seedling growth allows weak competitors to occupy stressful sites in a habitat that superior competitors would avoid (Khan and Gul, 2006). Hence, a thorough understanding of seed

germination ecology of plants has been an important research topic in plant sciences (Gul et al., 2013). However, this kind of information about seed germination ecology of halophytes is limited to merely < 100 (Gul et al., 2013) out of 1469 species (<http://www.sussex.ac.uk/affiliates/halophytes/>).

Many morpho-physiological attributes may also influence seed germination responses (Bewley and Black et al., 1994; Baskin and Baskin, 2014). For example, seeds of about 25% plants are physically dormant (PY), owing their dormancy to their hard impermeable testa or endocarp that restricts imbibition. The removal of that constriction is essential for germination completion (Baskin et al., 2000; Baskin and Baskin, 2014; Hudson et al., 2015). This hard seededness is reportedly linked to seed germination regulation, prevention of predation, and facilitation of dispersal (Paulsen et al., 2013; Hudson et al., 2015). However, information about PY in halophytes and methods to overcome it is scanty (Gul et al., 2013).

Halophytes are naturally tolerant to salinity during their mature vegetative stage. However, their seed germination is generally decreased with increases in soil salinity (Gul et al., 2013). Upper limit (≈10% germination) of salinity tolerance of halophyte seeds varies substantially among species and ranges from ≈200 to 1700 mM NaCl

* Corresponding author.

E-mail address: bilqueesgul@uok.edu.pk (B. Gul).

(Gul et al., 2013). Most ungerminated seeds of halophytes generally remain viable under high soil salinity by entering a state of conditional or enforced dormancy and germinate (recover) readily when salinity decreases following adequate rainfall (Pujol et al., 2000). Salinity tolerance and recovery of germination of halophyte seeds are reportedly influenced by variations in temperature and photoperiod. Usually, sub-, supra-optimal temperatures and absence of light decrease final seed germination percentage, salinity tolerance, and recovery of germination (Khan and Gul, 2006). Variations in thermoperiod and photoperiod, in combination with salinity, can result in seed dormancy of various types, which may even lead to seed mortality under extreme conditions (Gul et al., 2013).

Generally, nascent seedlings of halophytes are highly sensitive to salinity and display retarded radicle growth when exposed to high salinity (Malcolm et al., 2003), as seen in *Halocnemum strobilaceum* (Qu et al., 2008). Gutterman (1980) reported that a majority of germinating *Mesembryanthemum nodiflorum* seeds were found in runnels and depressions that had higher water penetration. Similarly, *H. strobilaceum* seeds used a “rain gauge” to escape salinity (Gutterman, 1993). Coupling seedling growth with germination studies would indicate whether seedlings are recruited once germination has occurred. However, availability of such data is rare. This study examined the effects of variations in salinity, temperature, and photoperiod on seed germination responses and seedling growth of *S. persica*. Following hypotheses were addressed: 1) Seeds of *S. persica* are innately dormant. 2) Germination of *S. persica* will be reduced at 400 mM NaCl as compared to that in non-saline control. 3) Seed germination of the test species will be influenced by both temperature and photoperiod. 4) *S. persica* seeds will show recovery of germination after alleviation of salinity. 5) Seedling growth in *S. persica* will be affected by salinity, temperature, and photoperiod. 6) Germination will be reduced, following seed storage.

2. Materials and methods

2.1. Seed collection and habitat

Mature fruits or drupes of *Salvadora persica* were collected from Hawke's Bay Coast, Karachi, Pakistan (Latitude: 24° 51' 33.65" N; Longitude: 66° 52' 9.03" E) in April 2012 and brought to the laboratory. Seeds were separated manually, rinsed briefly with distilled water, air-dried, and dry-stored in a clear plastic bottle at room temperature.

2.2. Effects of scarification on seed germination and imbibition

Freshly collected seeds were divided into two batches. One batch was scarified using concentrated Sulphuric acid for one minute, followed by surface sterilization with 1% commercial bleach and air-drying. The other batch was left untreated. After taking the initial weight, both batches of seeds were provided with 7 mL distilled water. Increase in weight of each batch due to imbibition was noted after a 12 h interval for 2 days at optimal temperature regime 15/25 °C and 12 h light / 12 h dark photoperiod (based on preliminary investigations). Seed germination of the aforementioned seed batches was also monitored using the methods described below.

2.3. Effects of salinity, temperature, and photoperiod on seed germination and imbibition

Seed germination experiments were carried out in programmable incubators (Percival Inc., USA) with the following settings: Temperature (10/20, 15/25, 20/30 and 25/35 °C) and Photoperiod (12 h light / 12 h dark and 24 h dark). Philips cool-white fluorescent tubes ($\approx 25 \mu\text{mol m}^{-2} \text{s}^{-1}$, 400–750 nm) acted as the source of light. Scarified (Acid + Bleach) seeds were used in germination experiments. Clear 6-well plates with 5 mL of test solution per well were used. There were three replicates of 25 seeds per treatment. Different NaCl solutions

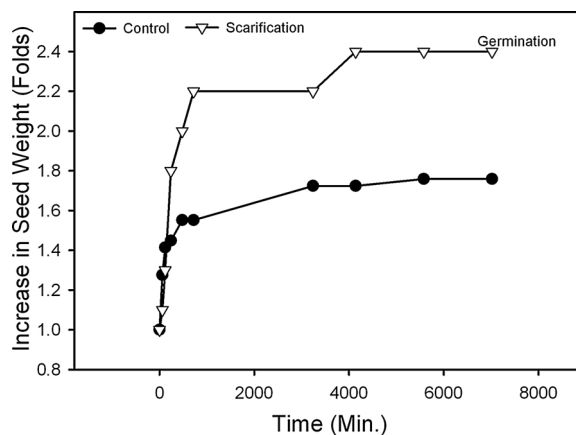


Fig. 1. Increase in weight (folds of initial seed weight) due to water uptake by untreated (control) and treated (scarified) seeds of *Salvadora persica*.

Table 1

Split-Plot Analysis of variance of the effect of various factors on seed germination (A, B) and seedling length (C) in *Salvadora persica*. Numbers represent F values (* = $p < 0.01$).

A				
Factor (Split-Plot ANOVA)	df	Error df	MS	F ^{sig}
Temperature (T)	3	6	10170.559	227.123*
Salinity (S)	5	10	15671.910	302.825*
Photoperiod (P)	1	2	157.607	.722 ^{ns}
T × S	15	30	1393.645	10.233*
T × P	3	6	175.930	1.419 ^{ns}
S × P	5	10	592.162	4.658**
T × S × P	15	30	199.700	2.422**
B				
Factor (Split-Plot ANOVA)	df	Error df	MS	F ^{sig}
Temperature (T)	3	6	1194.977	90.630*
Salinity (S)	5	10	3461.081	521.334*
T × S	15	30	397.577	37.150*
C				
Factor (Split-Plot ANOVA)	df	Error df	MS	F ^{sig}
Temperature	3	18	248.390	20.014*
Salinity	1.008	4.034	6411.833	10.277*
Photoperiod	1	44	9776.044	32.158*

(0, 100, 200, 300, 400, and 500 mM) were used. Radicle emergence was the criterion for germination (Bewley, 1997). The number of germinated seeds from the set that was provided a 12-hour photoperiod was counted on alternate days for 20 days, while final germination was noted once at day 20 in case of dark treatments. Rate of germination (RG) for light-treated seeds was estimated by using a modified Timson's index of germination velocity: $RG = \Sigma G/t$, where G is the seed germination percentage at 2-day intervals and t is the total germination period (Khan and Ungar, 1997). The maximum value possible for our data using this index was 50 (i.e., 1000/20), so the higher the value, the faster the germination.

Ungerminated seeds from the highest salinity concentration (500 mM NaCl, i.e. the treatment with maximum inhibition of germination) of each thermoperiod were transferred to wells filled with distilled water (5 mL) but kept at the same temperature regimes as before. One week was allotted for recovery of germination. The seeds that failed to recover were then tested for viability. To determine the state of viability according to MacKay (1972), ungerminated seeds were sliced in half and treated with 0.1% (w/v) 2, 3, 5-triphenyltetrazolium

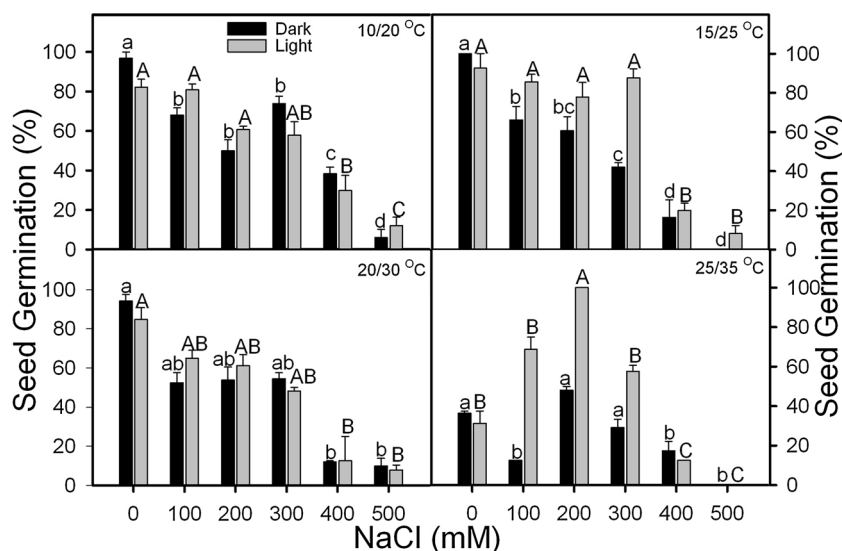


Fig. 2. Effects of salinity, temperature, and photoperiod on seed germination of *Salvadora persica*. Different letters over identical bar types indicate significant differences ($P < 0.05$; Games-Howell Test). Upper case and lower-case letters were used for light and dark treatments, respectively.

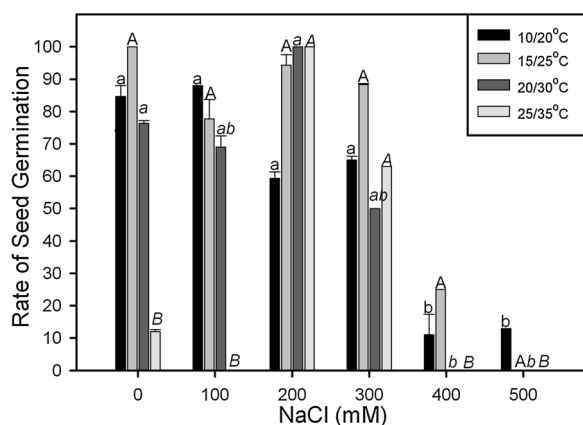


Fig. 3. Effects of salinity and temperature on rate of germination of *Salvadora persica*. Different letters over identical bar types indicate significant differences ($P < 0.05$; Games-Howell Test). Non-italicized lower-case and upper-case letters and italicized lower-case and upper-case letters were used for 10/20 °C, 15/25 °C, 20/30 °C, and 25/35 °C treatments, respectively.

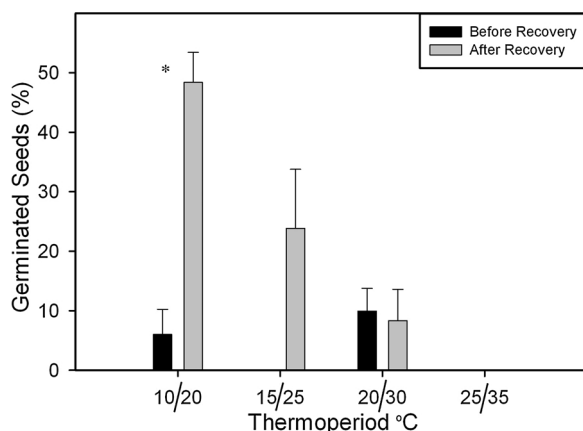


Fig. 4. Percentage of germinated seeds of *Salvadora persica* from 500 mM NaCl at different thermoperiods before and after recovery with distilled water (Asterisk (*) = significant, $P < 0.05$; Paired t -test).

chloride (TTC) solution. After an incubation of 3 h, seeds were checked for pink-red staining they would show, if viable.

Scarified (Acid + Bleach) seeds were also soaked in increasing concentrations of NaCl (0, 100, 300 and 500 mM) at optimal temperature regime 15/25 °C and 12 h light / 12 h dark photoperiod. The increase in weight due to imbibition was recorded after every 48 h. for 5 days.

2.4. Effects of salinity, temperature, and photoperiod on viability and growth of seedlings

Scarified (Acid + Bleach) seeds were incubated in above-mentioned salinity, temperature, and photoperiod regimes for 20 days. Thereafter, the length of seedlings from each treatment was measured. The seedlings were also tested for their viability using TTC staining.

2.5. Effects of storage on seed germination

Seeds were stored in clear plastic bottles at room temperature for 4 months. At the end of the time period, they were used for the following experiment.

2.6. Statistical analyses

All statistical analyses were performed by using IBM SPSS Statistics 20 (IBM Corp., Armonk, New York, USA). Data were subjected to split-plot analysis of variance (ANOVA) to evaluate the effects of salinity, temperature, photoperiod, and their interactions on different germination parameters and seedling growth. However, Levene’s F test revealed that the homogeneity of variance assumption was not met ($P < 0.01$). Therefore, post-hoc comparisons to determine which parameter means differed significantly were done using the Games-Howell test. Paired t -tests were carried out for data analysis on pre- and post-effects of recovery and storage on seed germination.

3. Results

3.1. Effects of scarification on seed germination and imbibition

Freshly collected unscarified seeds of *S. persica* were innately dormant, while scarified (Acid + Bleach) seeds germinated maximally ($\approx 100\%$) in distilled water. There was significantly lesser imbibition in

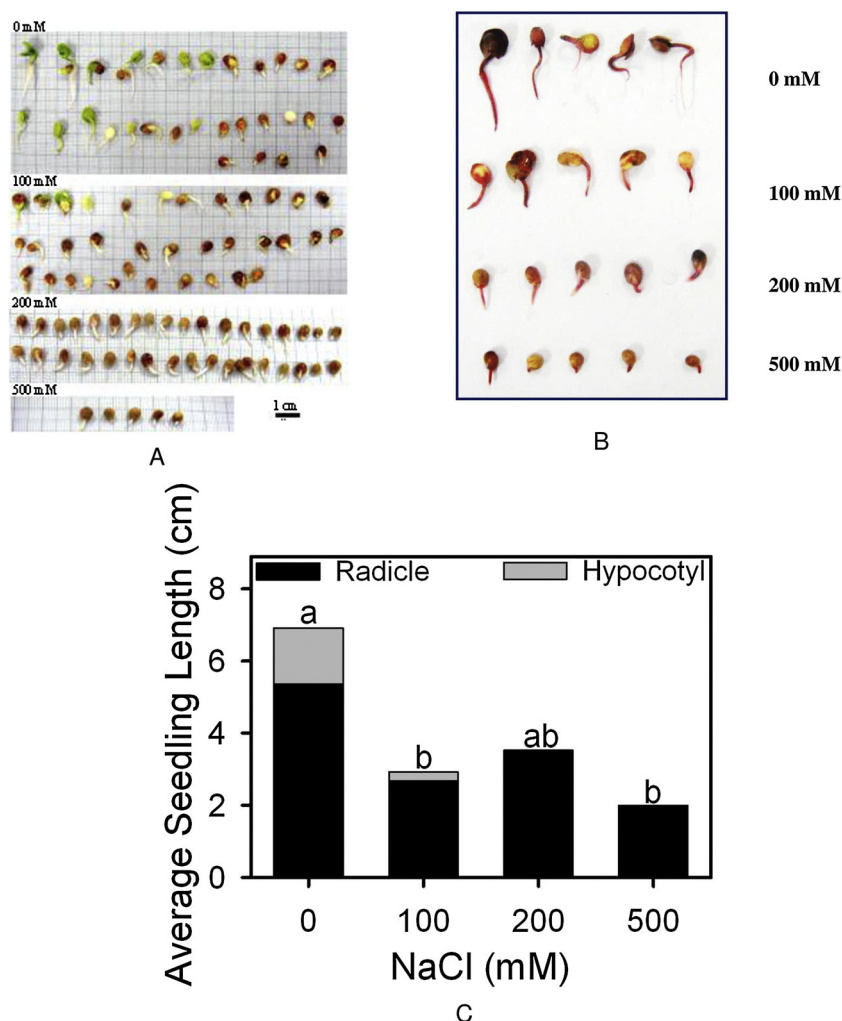


Fig. 5. Germination at 15–25 °C in *Salvadora persica*: (A) all germinated seedlings; (B) viable seedlings; (C) average length per seedling. Different letters represent significant differences between salinity treatments ($P < 0.05$; Games-Howell test).

unscarified seeds, as compared to scarified seeds (Fig. 1).

3.2. Effects of salinity, temperature and photoperiod on seed germination and imbibition

Split-plot ANOVA revealed that salinity and temperature and the interaction between salinity, photoperiod, and temperature ($P < 0.05$) had significant effects on germination of *S. persica* (Table 1A and B). Mean final germination percentage (MFG) was lowest in the presence of high salinity (400 and 500 mM NaCl) at all temperature regimes (Fig. 2). Increases in salinity for seeds at 10/20 °C led to differential changes in dark and a progressive decrease of MFG in light. At 15/25 °C, MFG decreased progressively in dark while remaining unaffected in the presence of low to moderate salinity (100–300 mM NaCl). MFG for seeds at 20/30 °C remained unaffected by low-moderate salinity concentrations. MFG showed maximum values at moderate salinity for seeds exposed to high temperature, especially in the light. The lowest values for both MFG and RG were observed in 400 and 500 mM NaCl (Figs. 2 and 3). RG was not affected by low-moderate salinity at all temperatures, except at 25/35 °C. Ungerminated seeds showed significant recovery ($F = 7.65$, Mean = 42.36, SD = 13.57; $P < 0.05$) when transferred from salinity to distilled water only at 10/20 °C (Fig. 4). All the seeds that failed to recover were tested and found to be viable.

Seeds immersed in distilled water showed higher relative water uptake as compared to those soaked in various NaCl solutions (data not

shown). Higher imbibition in seeds under non-saline condition led to germination initiation (radicle extension) after 96 h (4 days), while those under saline conditions were still un-germinated after 96 h.

3.3. Effects of salinity, temperature, and photoperiod on viability and growth of seedlings

Split-plot ANOVA indicated that salinity ($P < 0.0001$), temperature ($P < 0.0001$) and light ($P < 0.0001$) affected seedling growth of *S. persica* significantly (Table 1C). All the seedlings were viable (Figs. 5 and 6B). Optimal seedling growth was observed at 15/25 °C, followed by other temperatures in the order: 20/30 > 10/20 > 25/35 °C (Fig. 6). Seedling growth was maximal under non-saline condition and radicle length was generally longer than the length of hypocotyl (Fig. 5C). Salinity treatments inhibited seedling growth (particularly hypocotyl length) with most negative effects in 500 mM NaCl, wherein seedling length was the shortest (Fig. 5A). Seedlings exhibited etiolated growth under complete dark conditions, with seedling length being about 5 times that of the length of seedlings provided with alternating light and dark conditions (Fig. 7).

3.4. Effects of storage on seed germination

Storage of seeds led to significant decrease in RG ($F = 19.73$, Mean = 40.45, SD = 2.90) and salinity tolerance to 400 mM NaCl ($F = 12.00$, Mean = 12.00, SD = 1.41) ($P < 0.05$) for the seeds of *S.*

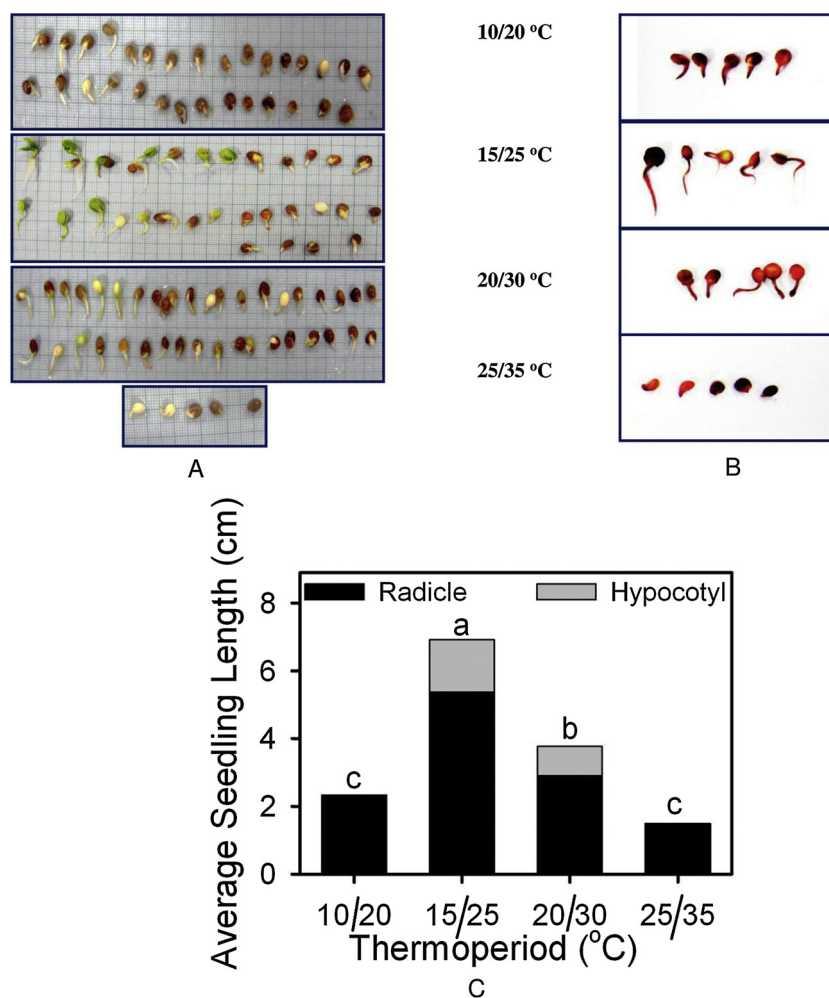


Fig. 6. Germination of *Salvadora persica* seeds at various thermoperiods (A) all germinated seedlings; (B) viable seedlings; (C) average length per seedling. Different letters represent significant ($P < 0.05$) differences between treatments (Games-Howell test).

persica (Fig. 8).

4. Discussion

Fresh seeds of *S. persica* failed to germinate in distilled water and showed little imbibition; however, after scarification, there was high imbibition (2.4 folds to dry weight) with 100% seed germination, indicating the prevalence of innate physical dormancy (Baskin et al., 2000; Baskin and Baskin, 2014). Similarly, seeds of some other halophytes such as *Cressa cretica* (Khan, 1991) and *Atriplex centralasiatica* (Li et al., 2011) also germinated better following scarification. Hudson et al. (2015) reviewed that about 25% of the plant species have physically dormant seeds owing to a hard water-impermeable testa. Physical dormancy enables seeds to survive temporal heterogeneity, predation, and harsh environments (Baskin et al., 2000; Baskin and Baskin, 2014; Hudson et al., 2015). According to Crypsis hypothesis of Paulsen et al. (2013), plants of dry habitats have adapted to produce large quantities of seeds with hard-coats for protection from predation.

Scarified seeds of *S. persica* germinated maximally ($\approx 100\%$) in distilled water and an increase in salinity decreased their germination. However, some ($\approx 10\%$) seeds of *S. persica* could germinate in 500 mM NaCl solution, which is equivalent to seawater salinity (Khan and Gul, 2006). On the other hand, seeds of *S. oleoides* germinated ($\approx 20\%$) in only up to 16.5 dS m^{-1} or equivalent to 160 mM NaCl (Ramoliya and Pandey, 2002). Nevertheless, seeds of many co-occurring species, such as *Suaeda fruticosa*, *Limonium stocksii*, and *Aeluropus lagopoides* also germinate in/above 500 mM NaCl (Gul et al., 2013). Based on these

data, it can be hypothesized that although most seeds would germinate following sufficient rainfall, some (10%) germination under high or seawater salinity might provide these subtropical halophytes with a chance to produce some seedlings during years with low rainfall.

Inhibition of seed germination under saline conditions could result from either osmotic or/and ionic effects of salinity (Ungar, 1978; Bajji et al., 2002; Song et al., 2005; Hameed et al., 2013). However, in the case of *S. persica*, seeds exposed to 500 mM NaCl were found to be viable, suggesting that germination was prevented by the osmotic effect of salinity. Similar behavior has been reported for other species found in hot climates, such as the Mediterranean *Silene mollissima* and *S. hifacensis* (Murrú et al., 2017) and *Zygophyllum propinquum* (Manzoor et al., 2017). When coupled with recovery, this response could result in resumption of germination on removal of stress, such as after rainfall. Additionally, lower imbibition of *S. persica* seeds under saline condition also pointed towards the osmotic constraint of salinity. Inhibition of seed germination in the presence of salinity was also reported in *Suaeda physophora*, *Haloxylon ammodendron*, *Haloxylon persicum* (Song et al., 2005), *Suaeda fruticosa*, and *Limonium stocksii* (Hameed et al., 2014). These data indicate that germination inhibition in most halophytes is due to osmotic constraint rather than ionic toxicity. However, detailed physiological and biochemical studies are required to decipher the mechanisms preventing ionic toxicity in seeds of these halophytes.

Seeds of *S. persica* germinated better under low (10/20, 15/25 °C) to moderate (20/30 °C) temperatures than at high (25/35 °C) temperature, indicating a broad temperature window for seed germination. Seeds of *Salsola drummondii* (Rasheed et al., 2015) and *S. affinis* (Wei et al.,

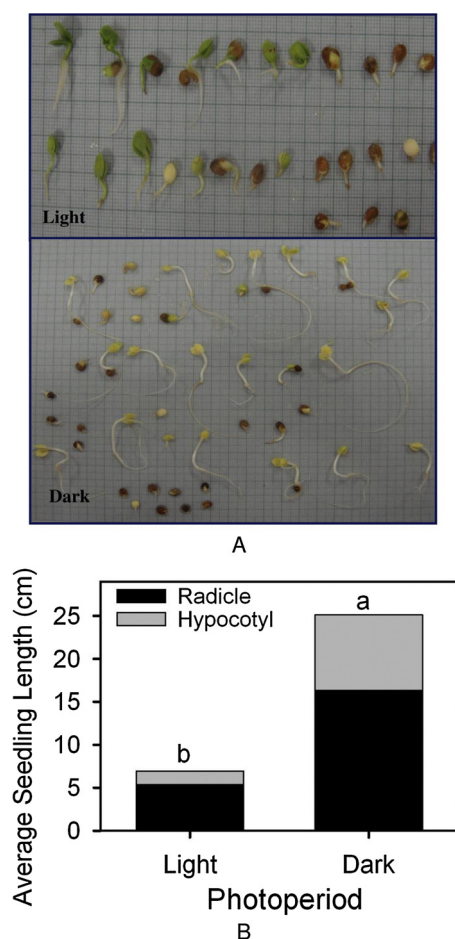


Fig. 7. Germination of *Salvadora persica* seeds in two photoperiods: (A) all germinated seedlings (B) average length per seedling. Different letters represent significant ($P < 0.05$) differences between treatments (Games-Howell test).

2008) also showed the same germination strategy, termed as “opportunistic” by Wei et al. (2008). It would seem its purpose is for a plant to produce seedlings whenever conditions are favorable for seedling growth. Contrary to this strategy, most co-occurring species, such as *S. imbricata* (10/20 °C; Khan and Weber, 2007), *Limonium stocksii* (20/30 °C; Zia and Khan, 2004) and *Desmostachya bipinnata* (25/35 °C; Gulzar et al., 2007) had narrow temperature preferences. Hence, a broad “temperature window” for germination as compared to the narrow ones among co-occurring species might be another adaptation that helps *S. persica* seeds to colonize open-sites in its habitat.

Although high temperature (25/35 °C) was substantially inhibitory for the germination of *S. persica* seeds under non-saline condition, it resulted in enhanced seed germination in solutions of low to moderate salinity (100–300 mM NaCl), as compared to non-saline control. This enhanced germination might be attributed to the phenomenon of cross-tolerance under high temperatures by salinity. Cross-tolerance is a phenomenon in which exposure of plants to a particular stress enhances their tolerance to another stress (Genoud and Metraux, 1999; Capiati et al., 2006). Salinity is often reported to improve tolerance of halophytes to other stresses. For example, NaCl improved responses of *Sesuvium portulacastrum* under mannitol-induced water stress (Slama et al., 2007). Similarly, increased germination of wheat grains occurred at elevated temperatures in the presence of salinity (Song et al., 2005). Pre-treating wheat seeds in the presence of high NaCl concentrations and high temperatures also increased their ability to tolerate higher temperatures and salt stress levels, respectively (Lei et al., 2005). Lei et al. (2005) postulated that an efficient antioxidant system might be responsible for the development of cross-tolerance. We hypothesize that

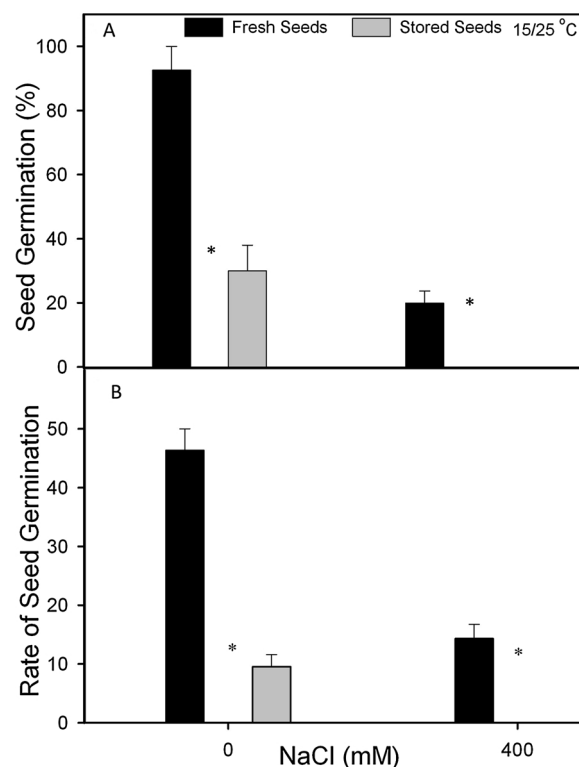


Fig. 8. Germination (A) and Rate of Germination (B) in fresh and stored seeds of *Salvadora persica* at 15/25 °C in light treatment (Asterisk (*) = significant, $P < 0.05$; Paired *t*-test).

similar cross-tolerance might be involved in enhancing seed germination of *S. persica* at high temperature under low to moderate salinity.

Salvadora persica seeds were weakly photoblastic and showed no uniform response to photoperiod during germination. This behavior of the seeds of test species towards light during germination could be linked to their relatively larger seed size. Often, large seeded species have a weaker light requirement for seed germination than the small seeded species do (Koutsovoulou et al., 2014; Xia et al., 2015). Different studies have indicated that the seedlings of large-seeded species can emerge from deeper soil or litter after rapid initial shoot growth, owing to a large amount of seed reserves (Seiwa et al., 2002; Xia et al., 2015). This strategy provides them with an adaptive advantage over smaller-seeded species when colonizing the community gaps. Rapid changes in temperature can occur in the soil layers closer to the surface (Podda et al., 2017). The ability to withstand alternating temperature might indicate successful germination of *S. persica* seeds even when not buried deeply.

Our experiment indicated that when stored, seeds of *S. persica* started to lose their vigor. Storage of seeds reduced the germination of seeds by more than half of that in fresh state. Seed storage of two other halophytes *Salsola imbricata* and *Haloxylon salicornicum* for longer than nine months at room and high temperatures caused significant reductions or complete inhibition in germination (El-Keblawy, 2013). Similarly, storage of seeds for more than 3 months caused substantial reductions in the germination of *Tamarix africana*, *T. boveana* and *T. gallica* (Terrones et al., 2016). Seed deterioration during storage is an inevitable and irreversible process (Bewley and Black et al., 1994). When seeds deteriorate during storage, they lose vigor, become more sensitive to stresses during germination, and ultimately become unable to germinate (Rajjou and Debeaujon, 2008). Evidence suggests that the seed deterioration during storage could be linked to oxidative damages caused by excessively produced reactive oxygen species (ROS) (Tommasi et al., 2006; Rajjou and Debeaujon, 2008). Reduction in germination upon storage in *S. persica* could be linked to its less

dependence on a seed bank for regeneration. Hegazy et al. (2009) reported that despite being an abundant plant in the northwestern Red Sea region, *S. persica* lacks a persistent seed bank, probably owing to seed predation. This matches the behavior of most perennial species growing in warm areas, which have minimal dependence on a seed bank. Instead, such plants adopt a strategy of producing few seeds every year, most of which do not persist in the seed bank (Khan and Gul, 2006; Hegazy et al., 2009).

Salinity also inhibited seedling growth (particularly hypocotyl length) of *S. persica*, with most negative effects seen in 500 mM NaCl. In an earlier study, Vaghela et al. (2010) also reported similar results for *S. persica* seedlings exposed to various NaCl concentrations. Similarly, seedling growth of other perennial halophytes, such as *Atriplex lentiformis* (Malcolm et al., 2003), *Kalidium capsicum* (Tobe et al., 2000) and *Halocnemum strobilaceum* (Qu et al., 2008) was also retarded under saline conditions. However, all the seedlings of *S. persica*, even those produced under high (500 mM NaCl) salinity, were viable, as shown by tetrazolium chloride staining. This indicated that the cessation of growth was a result of osmotic effects rather than ionic toxicity of NaCl. Keshavarzi and Moussavinik (2011) also reported that less water absorption, which is a consequence of the presence of NaCl, leads to reduced cell division and differentiation of plumule and radicle length in seedlings.

Seedlings of *S. persica* showed optimal growth at 15/25 °C as compared to growth at other temperatures. Similar results were also obtained for the seedlings of *Atriplex griffithii* var. *stocksii* (Khan and Rizvi, 1994) and *Panicum turgidum* (Al-Khateeb, 2006). Better growth under 15/25 °C could be ascribed to higher enzyme activities, which would be affected under sub-/supra-optimal temperatures (Taiz et al., 2006). High (25/35 °C) temperature severely retarded seedling growth of *S. persica* in this study, indicating that seedling establishment might be the most temperature-sensitive stage of this plant. This becomes even more pertinent information when viewed in the light of global warming, resulting from recent climatic changes. Seedlings of *S. persica* produced under complete darkness were etiolated but viable. Seedlings grown under dark had radicles that were about five times longer than those produced under 12 h-photoperiod. Since all the seedlings were viable, subsequent leaf production seems highly possible (Burgess, 1985).

5. Conclusions

Seeds of *Salvadora persica* had coat-imposed physical dormancy, which might help in dispersal and enduring predation by birds (personal observation). Acid scarification can break this innate dormancy. At this stage, sensitivity of seeds to extremes of salinity, temperature, and darkness can confine germination to occur only under optimum environmental conditions, such as after sufficient rainfall, that increase the chance of seedling survival. Storage of seeds reduced their germination by more than half of that in fresh state that matches the behavior of most perennial species growing in warm areas, which have minimal dependence on a seed bank.

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