SENSITIVITY AND GERMINATION CHARACTERISTICS OF SUMMER AND WINTER SEEDS OF A COASTAL DUNE GRASS *HALOPYRUM MUCRONATUM* (L.) STAPF.

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ABSTRACT

Halopyrum mucronatum (L.) Stapf. (Poaceae) is a perennial halophyte grass dominating the coastal dune vegetation along Arabian sea coasts. This stoloniferous grass produces black and brown seeds (caryopses) during summer and winter, respectively. This study examined germination characteristics and sensitivity of two seed morphs to salinity (0,150 and 300 mM NaCl), thermoperiods (10/20, 15/25, 20/30 and 25/35°C) and photoperiod (12-h photoperiod and 24-h dark) environments. Both seed morphs lacked innate dormancy and germinated maximally in distilled water. Cooler thermoperiod (10/20 °C) was most inhibitory for the germination of both seed morphs and their optimal germination occurred at warmer thermoperiod (25/35 °C). Under moderate to warmer thermoperiods, germination of both seed types decreased only in high (300 mM NaCl) salinity. Whereas at cooler thermoperiod, salinity increment led to a gradual decline in germination of both summer and winter seeds. In general, summer seeds were more tolerant to salinity than the winter seeds. Summer seeds also germinated to relatively higher percentage in dark under high salinity compared to winter seeds. In addition, summer seeds showed substantially lesser mean germination time under cooler thermoperiod than the brown seeds. This study unveils many similarities and differences in the germination characteristics and sensitivity patterns of two seed morphs.

Keywords: Coastal Dune, Dimorphic Seeds, Germination, Halophyte, Salinity, Thermoperiod.

INTRODUCTION

Many plants of stressful and heterogenous habitats produce morpho-physiologically different or heteromorphic seeds, which vary in size, shape, color, texture/structure and germination/dormancy responses (Imbert, 2002; Liu *et al*., 2018; Rasheed *et al*., 2019). As a result, chances of their survival are maximized under fluctuating/unpredictable stressful conditions (Venable, 1985; Telenius, 1992; Imbert, 2002: Nisar *et al*., 2019). At present, seed heteromorphism is known in about 292 species from 26 families with relatively higher abundance in dicots compared to monocots (Imbert, 2002; Wang *et al*., 2010; Rasheed *et al*., 2019).

A number of halophytes also produce heteromorphic seeds, which often vary in dormancy and germination responses (Wang *et al*., 2008; Nisar *et al*., 2019). For instance, brown seeds of *Suaeda aralocaspica* were non-dormant, while black seeds had non-deep physiological dormancy (Wang *et al*., 2017). Similarly, heteromorphic seeds of *Atriplex centralasiatica* (Li *et al*., 2008) and *Cakile edentula* (Zhang, 1993) varied in final germination percentage. A significant variation in sensitivity of heteromorphic seeds to environmental stress may also be observed. For instance, brown compared to black seeds of *S. aralocaspica* had substantially higher salinity tolerance (Wang *et al*., 2008). Likewise, heteromorphic seeds may also display differential germination responses to variations in temperature and photoperiod (Rasheed *et al*., 2019; Nisar *et al*., 2019). Owing to these differences in germination and sensitivity to various environmental factors production of heteromorphic seeds might be advantageous under varying habitat conditions (Böer 1996; Khan and Gul, 1998).

Often seed heteromorphism of color is reported among halophytes (Wang *et al*., 2008; Gul *et al*., 2013; Rasheed *et al*., 2019). For example, *Arthrocnemum macrostachyum* (Nisar *et al*., 2019), *Chenopodium album* (Yao *et al*., 2010), *S. aralocaspica* (Wang *et al*., 2008), *S. salsa* (Song and Wang, 2014), and *Atriplex canescens* (Bhatt and Santo, 2016) produce black and brown colored seeds. Seed heteromorphism of color results from differential chemical composition of the testa/seed coast (Rasheed *et al*., 2019). In many cases, black seeds contain higher

contents of phenolic compounds and hence show differential germination patterns in comparison to their brown counter-parts (Khan and Ungar, 1986; Wang *et al.*, 2008; Siddiqui and Khan, 2011; Gul *et al.*, 2013; Rasheed *et al.*, 2019). Bulk of this information is based on the data from the dicots and generally there is a dearth of such knowledge about monocots.

Halopyrum mucronatum (L.) Stapf. (Poaceae) is a perennial halophyte grass, commonly found on the sand dunes along Arabian Sea coast (Khan and Qaiser, 2006). This stoloniferous grass could be an excellent candidate for bioethanol production as its lignocellulosic biomass is composed of 37% cellulose, 28% hemicellulose and 5% lignin. Since *H. mucronatum* is one of the few plants growing on coastal dune, it can also be used as sand dune binder to check land erosion and for carbon sequestration purposes. It can endure as high as 360 mM NaCl equivalent salinity during mature vegetative stage (Khan *et al.*, 1999) and produces two types of seeds in two seasons (Noor and Khan, 1995). Seeds with black color are produced during the summer, while brown colored seeds are formed during the winter season (Noor and Khan, 1995). Whether or not following the definition of seed heteromorphism in *sensu stricto* (Imbert, 2002; Liu *et al.*, 2018; Rasheed *et al.*, 2019) as they are produced in two seasons, the two seed morphs differ in weight, temperature requirements and salinity tolerance (Khan and Ungar, 2001; Khan and Gulzar, 2003; Siddiqui and Khan, 2011). According to Siddiqui and Khan (2011) the differential phenolic composition could be responsible for differences in morphology and germination responses of the two morphs of *H. mucronatum* seeds. However, a number of germination characteristics such as mean germination time (MGT) and sensitivity-index (SI) have not been determined. This study was hence carried out to fill aforementioned gaps in knowledge. This study is based on following questions: 1) What are the similarities and differences in the germination characteristics of two seed morphs of test species, and 2) Are there differences in sensitivity of two seed morphs to variations in salinity, temperature and photoperiod?

MATERIALS AND METHODS

Seed collection site and collection

Mature brown and black seeds (caryopses) of *Halopyrum mucronatum* (L.) Stapf. were collected from the Hawks bay coast, Karachi during successive winter (November/ December, 2014) and summer (June/July 2015) seasons, respectively (Fig. 1). Seeds were handpicked randomly from large number of plants and later scrub-cleaned in the laboratory to get rid of inflorescence husk. Cleaned seeds were then surface-sterilized using 1% (v/v) commercial bleach solution as detailed in Rasool *et al.* (2017). Sterilized seeds were used in experiments within six weeks of their collection.

Seed Germination Experiment

Germination was carried out in sterilized clear-lid plastic petri-plates filled with 5-mL of the test solutions. Three concentrations (0, 150 and 300 mM) of NaCl were used as salinity treatments with four replicates of 25 seeds each for every treatment. Germination experiment was conducted in programmed germination chambers set at four different 12h night/12h day thermoperiods (10/20, 15/25, 20/30 and 25/35 °C). Light source in the chambers were Phillips cool-white fluorescent tubes (~25 µmol Intensity). Percent germination (i.e. embryo protrusion; *sensu* Bewley and Black, 1994) across different salinity treatments from all 12h night/12h day thermoperiods was noted on every alternate day for 20 days. A modified Timson Index of Germination Velocity (Khan and Ungar, 1984) given below was used to calculate germination rate:

$$\text{Rate of Germination (Timson Index)} = \Sigma G/t$$

Where *G* is the seed germination percentage on alternate days and *t* is the total germination period. Mean germination time (MGT) (Ellis and Roberts, 1981) was also calculated by using following formula:

$$\text{MGT (Days)} = \sum Dn / \sum n$$

Where, *D* is number of days counted from the beginning of germination and *n* is the number of germinated seeds on day D. In addition, sensitivity index (SI) was calculated according to the following expression:

$$SI = [\,100 \times (MFG_{NaCl} - MFG_{Control})] \,/\, MFG_{Control}$$

Where, MFG is the mean final germination either in any NaCl solution or non-saline control. This index is more negative when sensitivity to salinity increases (Ghars *et al.*, 2008).

One additional set of petri-plates was wrapped in dark-photographic envelops to impose 24h darkness under above mentioned salinity and thermoperiod conditions. Germination of this set was noted once on 20th day.

Statistical analysis

Analyses of variance (ANOVA) was used to assess if grouping factors such as morphs, salinity, temperatures, photoperiods affected germination parameters significantly. A Bonferroni test ($p < 0.05$) was used to compare

individual means. SPSS (version 16) and Sigma Plot (version 11) were used for all statistical analyses and graph preparation respectively.

RESULTS AND DISCUSSION

A four-way ANOVA indicated significant effects of seed morphology, thermoperiod, salinity but not of photoperiod on mean final germination (MFG) of *H. mucronatum* seeds (Table **1**). Under 12 h photoperiod, both seed morphs displayed maximum germination (~ 100%) at all temperatures except at 10/20 ºC which was inhibitory (Fig. **2**). In contrast, Khan and Ungar (2001) reported that optimal thermoperiod for the germination of winter seeds of *H. mucronatum* was 20/30 ˚C, while that for summer seeds was 25/35 ˚C. Whereas, similar to our finding heteromorphic seeds of *Atriplex centralasiatica* (Li *et al*., 2008) and *Suaeda splendens* (Redondo-Gomez *et al*., 2008) also showed similar thermoperiod optimum for the germination.

Under moderate to warmer thermoperiods germination of both seed types of *H. mucronatum* decreased only in high (300 mM NaCl) salinity (Fig. **2**), while at cooler thermoperiod salinity increment led to a gradual decline in germination of both summer and winter seeds. However, summer seeds of *H. mucronatum* were generally more tolerant to salinity than the winter seeds, as they showed relatively higher MFG and germination rate with lower Sensitivity Index (Fig. **3**). Likewise, a number of studies have showed differential salinity tolerance in heteromorphic seeds such as those of *Arthrocnemum macrostachyum* (Nisar *et al*., 2019), *Suaeda salsa* (Li *et al*., 2005) and *S. moquinii* (Khan *et al*., 2001). In contrast, heteromorphic seeds of *Atriplex rosea* (Khan *et al*., 2004) showed similar salinity tolerance. Hence, responses of heteromorphic seeds to salinity increments could be species-specific.

Table 1. Analysis of Variance (ANOVA) indicating effects of morph, temperature, salinity and photoperiod on mean final germination (MFG), germination rate (GR), mean germination time (MGT), sensitivity index in light (SI_L) and dark (SI_D) of brown and black seed morphs of *Halopyrum mucronatum*.

Factors	MFG	GR	MGT	SI_L	SI_D
Morph	7.523**	1.263[ns]	4.002*	72.844***	23.778***
Temperature	2557.395***	465.365***	4.597**	57.492***	17.397***
Salinity	8344.922***	2535.915***	9.444***	2854.689***	204.638***
Photoperiod	34.617***	-	-	-	-
Morph * Temperature	7.437***	16.515***	28.098***	29.248***	8.896***
Morph * Salinity	53.138***	37.968***	2.732*	.922[ns]	4.026*
Morph * Photoperiod	.421[ns]	-	-	-	-
Temperature * Salinity	523.568***	107.969***	10.801***	64.835**	55.799***
Temperature * Photoperiod	127.103***	-	-	-	-
Salinity * Photoperiod	138.579***	-	-	-	-
Morph * Temperature * Salinity	15.294***	6.573***	1.247[ns]	16.794**	4.501**
Morph * Temperature * Photoperiod	1.672[ns]	-	-	-	-
Morph * Salinity * Photoperiod	12.161***	-	-	-	-
Temperature * Salinity * Photoperiod	44.724***	-	-	-	-
Morph * Temperature * Salinity * Photoperiod	10.639***	-	-	-	-

Numbers are *F*- values. Where, ns = Non-significant, * = $p<0.05$, ** = $p<0.01$ and *** = $p<0.001$

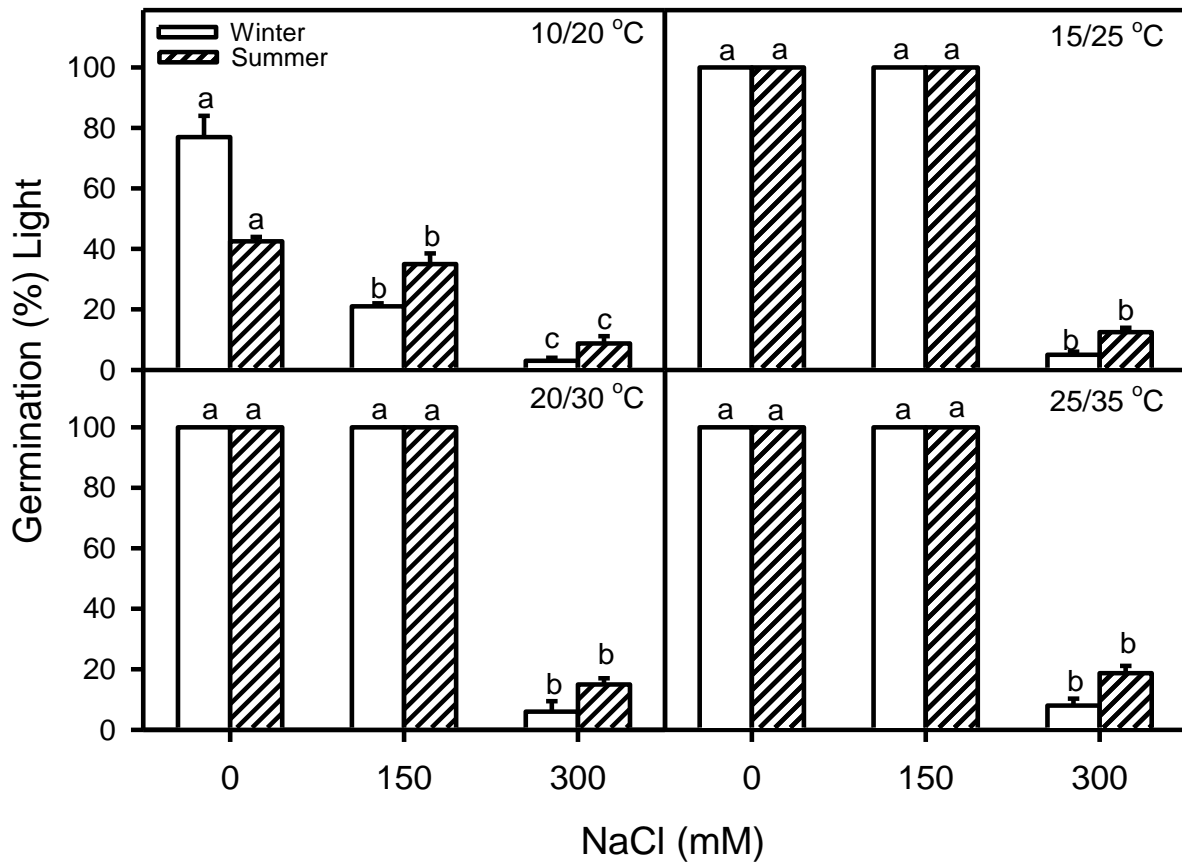Fig. 1. Summer (black) and Winter (brown) seeds of *Halopyrum mucronatum*.

Fig. 2. Effects of salinity (0, 150 and 300 mM NaCl) on final germination percentages of summer (black) and winter (brown) seeds of *Halopyrum mucronatum* under different thermoperiods (10/20, 15/25, 20/30 and 25/35˚C) and 12-h photoperiod. Bars represent mean ± standard error. Same letters across similar bars are not significantly different from each other (Bonferroni test; p<0.05).
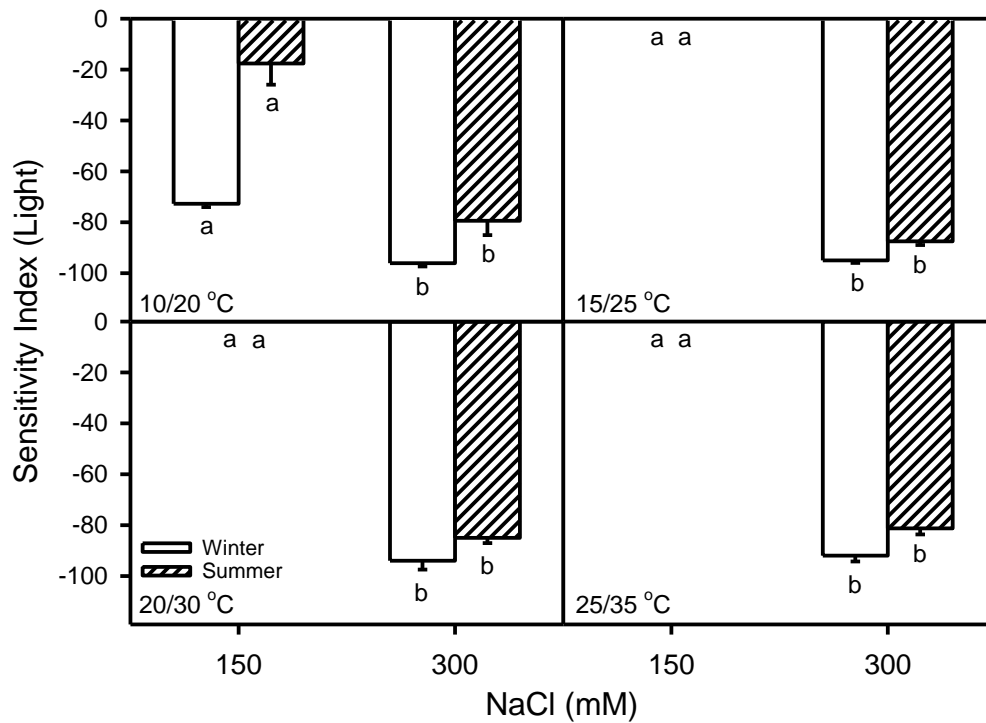
Fig. 3. Effects of salinity (150 and 300 mM NaCl) on sensitivity index of summer (black) and winter (brown) seeds of *Halopyrum mucronatum* under different thermoperiods (10/20, 15/25, 20/30 and 25/35°C) and 12-h photoperiod. Bars represent mean ± standard error. Same letters across similar bars are not significantly different from each other (Bonferroni test; p<0.05).
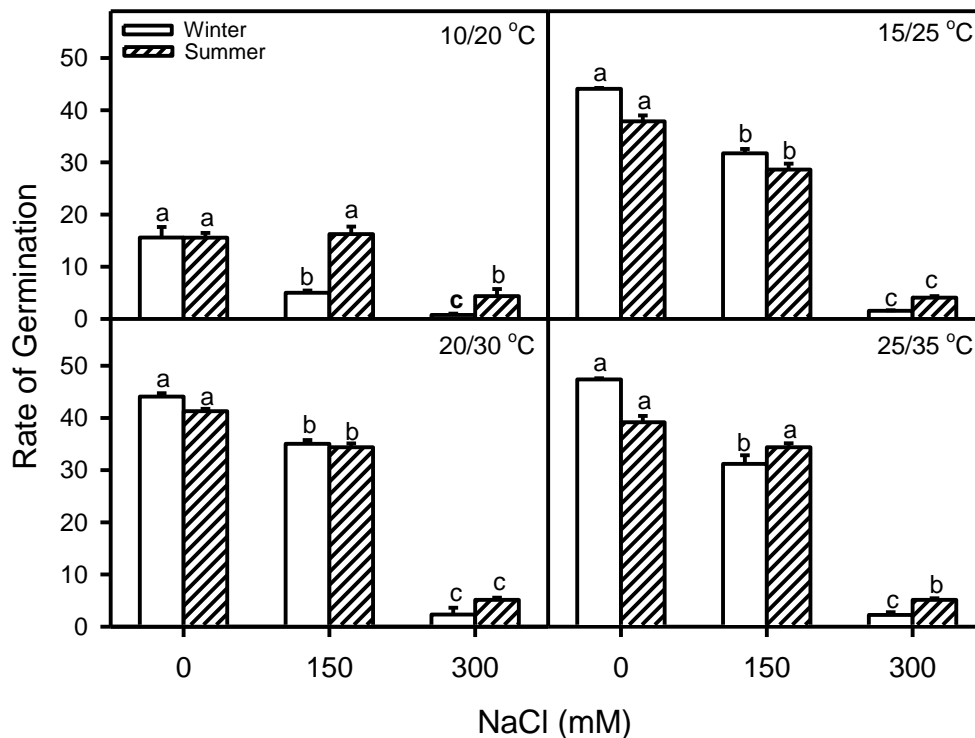

Fig. 4. Effects of salinity (0, 150 and 300 mM NaCl) on rate of germination of summer (black) and winter (brown) seeds of *Halopyrum mucronatum* under different thermoperiods (10/20, 15/25, 20/30 and 25/35°C) and 12-h photoperiod. Bars represent mean ± standard error. Same letters across similar bars are not significantly different from each other (Bonferroni test; p<0.05).
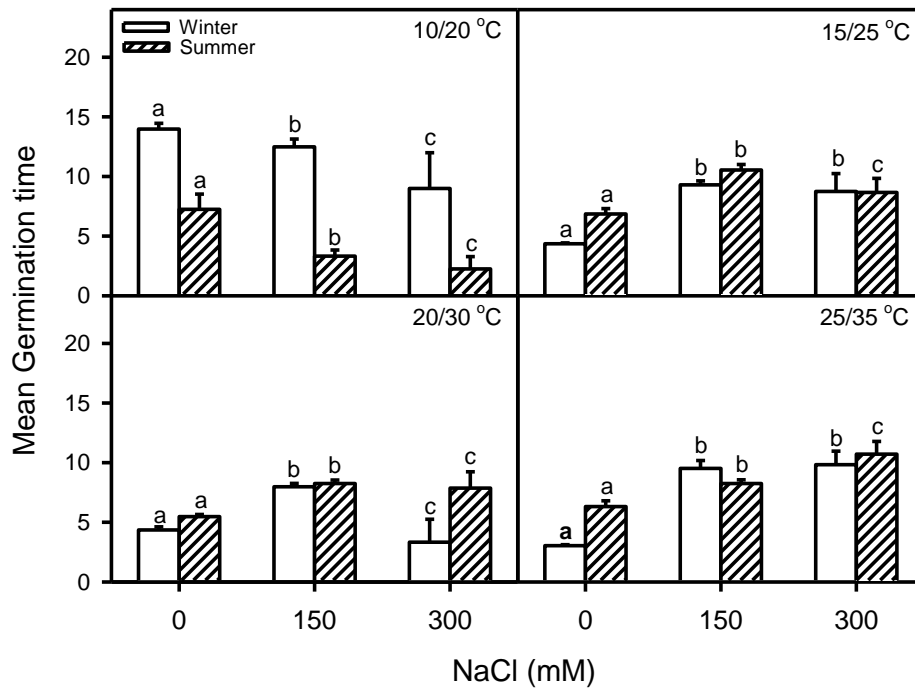
Fig. 5. Effects of salinity (0, 150 and 300 mM NaCl) on mean germination time of summer (black) and winter (brown) seeds of *Halopyrum mucronatum* under different thermoperiods (10/20, 15/25, 20/30 and 25/35˚C) and 12-h photoperiod. Bars represent mean ± standard error. Same letters across similar bars are not significantly different from each other (Bonferroni test; p<0.05).
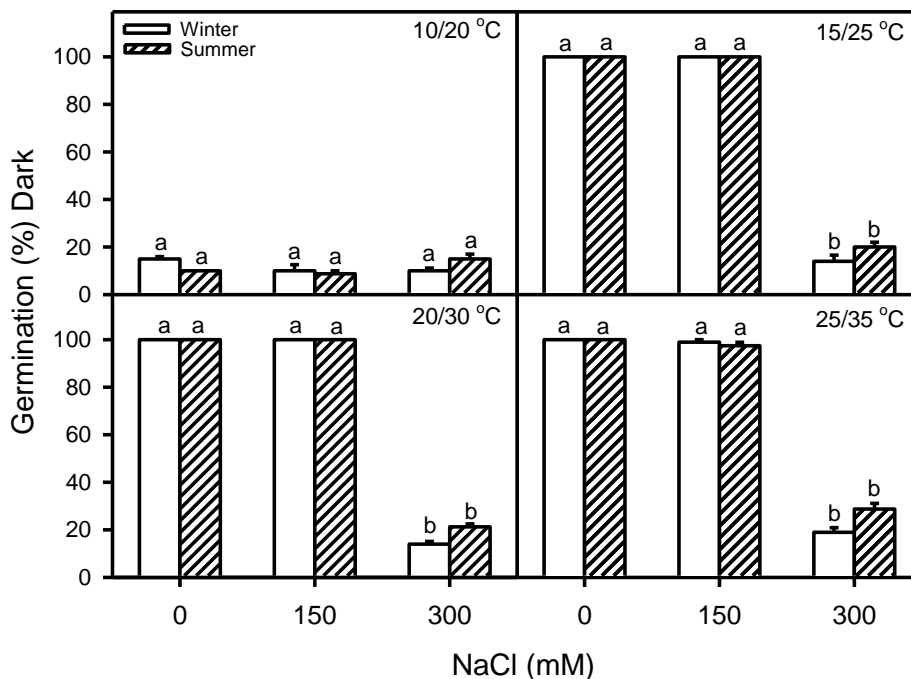

Fig. 6. Effects of salinity (0, 150 and 300 mM NaCl) on final germination percentages of summer (black) and winter (brown) seeds of *Halopyrum mucronatum* under different thermoperiods (10/20, 15/25, 20/30 and 25/35˚C) and 24-h dark period. Bars represent mean ± standard error. Same letters across similar bars are not significantly different from each other (Bonferroni test; p<0.05).
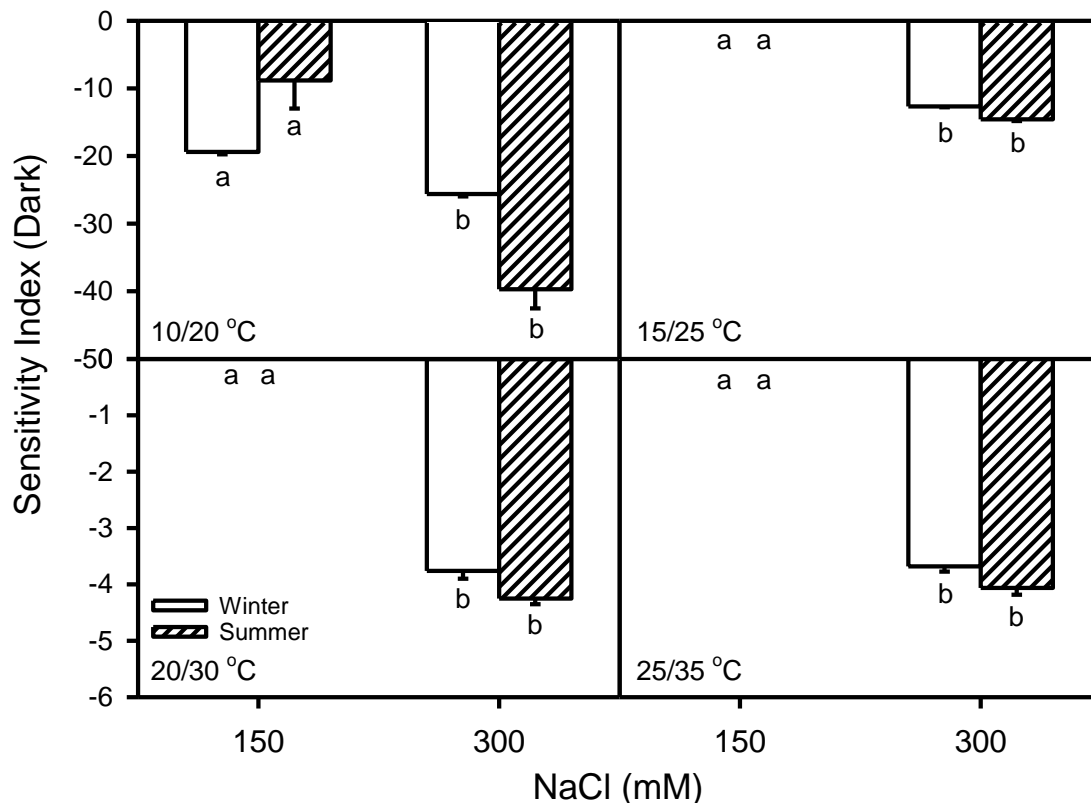
Fig. 7. Effects of salinity (150 and 300 mM NaCl) on sensitivity index of summer (black) and winter (brown) seeds of *Halopyrum mucronatum* under different thermoperiods (10/20, 15/25, 20/30 and 25/35˚C) and 24-h dark period. Bars represent mean ± standard error. Same letters across similar bars are not significantly different from each other (Bonferroni test; p<0.05).

Under non-saline controls, rate of germination of summer seeds of *H. mucronatum* was lesser than the winter seeds at moderate to warm thermoperiods (Fig. **4**), as they required relatively higher mean germination time (MGT) than the later described morph (Fig. **5**). Similarly, heteromorphic seeds of *Arthrocnemum macrostachyum* and *A. indicum* also showed differences in rate of germination (Nisar *et al.*, 2019). In addition, despite comparable germination in control and 150 mM NaCl at most thermoperiods, germination rate of heteromorphic seeds of *H. mucronatum* was lower (Fig. **4**) alongside higher MGT (Fig. **5**) in 150 mM NaCl compared to controls. (Zia and Khan (2004) also highlighted that rate of germination is more sensitive to salinity increments compared to MFG. They showed that despite comparable to control germination of *Limonium stocksii* seeds in up to 200 mM NaCl, there was a gradual decline in rate germination under salinity increments.

Under moderate to warmer thermoperiods, germination of either seed type of *H. mucronatum* in dark was comparable to that under 12h photoperiod (Fig. **6**). In contrary, seed germination of most other co-occurring monocot species such as *Cyperus conglomeratus* (Jeelani *et al.*, 2018), *Phragmites karka*, *Dichanthium annulatum* and *Eragrostis ciliaris* (Zehra *et al.*, 2012) is substantially inhibited in dark compared to light. This contrasting germination response of *H. mucronatum* to dark might be linked to the relatively larger seed size compared to aforementioned species. A number of researchers have shown that the seedlings of large-seeded species can emerge after burial in deeper soil or litter layers due to large amount of seed reserves (Seiwa *et al.*, 2002; Xia *et al.*, 2016), hence their germination is not or weakly dependent on light. In many cases, interaction of a factor with others may intensify its effects on germination (Gul *et al.*, 2013). For example, at 10/20 ˚C dark led to a substantial reduction in germination of both seed morphs of *H. mucronatum* across all salinity treatments including distilled water control compared to 12-h photoperiod (Fig. **6**). Furthermore, summer seeds of *H. mucronatum* germinated to relatively higher percentage in dark under high salinity compared to winter seeds (Fig. **6**), which was supported by SI values (Fig. **7**). Likewise, black but not brown seeds of *Suaeda corniculata* required light for germination (Cao *et al.*, 2012).

Conclusions

Both summer and winter seeds of *H. mucronatum* were non-dormant and germinated maximally in distilled water. Cooler thermoperiod (10/20 ˚C) was most inhibitory and optimal germination of two seed morphs occurred at warmer thermoperiod (25/35 ˚C). Under moderate to warmer thermoperiods germination of either seed type decreased only in high (300 mM NaCl) salinity. In contrast, at cooler thermoperiod, increases in salinity resulted in a gradual decline in germination of both seed morphs. Summer seeds were generally more tolerant to salinity compared to winter seeds. Summer seeds also germinated better in dark under high salinity in comparison to winter seeds. Hence, summer seeds appear to be more tolerant to salinity and dark compared to brown seeds, whereas both seed morphs share similar thermoperiod niche during germination.

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