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Sodium chloride improves seed vigour of the euhalophyte *Suaeda salsa*

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Abstract

Suaeda salsa is an annual herbaceous euhalophyte in the family Chenopodiaceae that produces dimorphic seeds on the same plant under natural conditions. In order to determine the effect of salinity on seed quality traits during seed formation, seeds from plants grown under control conditions and on 200 mM NaCl were used to investigate the effect of NaCl on seed production and seed germination. Results showed that size and weight of both black and brown seeds generated from 200 mM NaCl-treated plants were markedly greater than those from controls. The germination percentage of brown seeds from both control and NaCl-treated plants was higher than that of black seeds. Furthermore, the germination percentage of the black seeds generated from 200 mM NaCl-treated plants was significantly higher than that of the control at different concentrations of NaCl, although germination percentage declined with the increase NaCl concentration. Surprisingly, NaCl did not affect germination of the brown seeds. The germination index and vigour index of both black and brown seeds from the control plants were significantly lower than those of seeds from the different NaCl treatments. Seed starch, soluble sugar, protein and lipid content of both black and brown seeds generated from the 200 mM NaCl-treated plants were significantly higher than those from the control. These results suggest that a certain concentration of NaCl plays a pivotal role in seed vitality of the euhalophyte *S. salsa* through increasing seed weight and contents of storage compounds such as protein, starch and fatty acids.

Keywords: euhalophyte, germination, seed vigour, sodium chloride, *Suaeda salsa*

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Introduction

Salinity is an increasing problem in many regions worldwide. Halophytes are plants that can adapt to the high salt concentrations of saline environments (Flowers *et al.*, 1977; Flowers and Yeo, 1986; Munns and Tester, 2008). These environments are normally dominated by NaCl, and the optimal NaCl concentration for the growth of halophytes in culture solutions ranges from 20 to 500 mM (Flowers *et al.*, 1977).

Salinity concentration at the surface of the soil changes over time due to continuous evaporation and occasional precipitation in natural saline environments (Tobe *et al.*, 2000). Particularly in the saline soil areas of northern China, salinity and drought stress, due to high evaporation and low precipitation, markedly affect the seed germination of annual halophytes (Jin *et al.*, 1999).

Germination is a crucial stage in the life cycle of halophytes as it determines whether a plant can establish itself successfully in a given condition (Bajji *et al.*, 2002). Soil salinity inhibits the germination of seeds by creating an unfavourable osmotic potential external to the seed, which prevents water uptake (Welbaum *et al.*, 1990), and through the toxic effects of Na^+ and Cl^- ions on the germinating seeds (Khajeh-Hosseini *et al.*, 2003). Although halophytes are salt tolerant, germination of their seeds is often inhibited by increased salinity; the best germination rate of halophytes is obtained under non-saline conditions and their germination rate decreases with an increase in salinity (Ungar, 1991; Khan and Gul, 1998). For successful establishment of plants in saline environments, seeds must remain viable at high salinity and germinate when salinity in the growth medium decreases (Ungar, 1978, 1995).

Seeds can be classified as having low or high vigour, depending on the degree of field emergence, particularly under unfavourable conditions. When seeds are exposed to stress, plant reproduction and crop yield are severely affected (Kranner *et al.*, 2010), and seed characteristics such as size and mass are positively correlated with seed vigour. Germination tests provide essential information on the best conditions for germination in order to exploit full seed potential.

Seed vigour is an important factor that affects seedling establishment and crop growth and, ultimately, production rate, which provides valuable information for assessing seed quality. Many factors affect seed vigour. Seed size is an important characteristic for determining seed germination and seedling growth (Temme, 1986; Wulff, 1986; Seiwa, 2000). Variability in seed size may contribute to variability in seed germination. With increased seed size, higher germination and emergence rates were obtained in oat (*Avena sativa*; Willenborg *et al.*, 2005); furthermore, larger seeds resulted in improved stand establishment and faster germination in field bindweed (*Convolvulus arvensis;* Tanveer *et al.*, 2013).

Seed storage compounds are the initial raw materials to support seed germination. Wheat seeds (*Triticum aestivum* L.) with high protein content will produce more vigorous seedlings and sometimes higher yields (Ries and Everson, 1973). Seed proteins form nitrogen stores to supply the growing seedlings. Evidently, storage in seeds is related to the nutrient quality of the seeds (Shewry and Halford, 2002). Reduction in the accumulation of rice seed storage protein leads to changes in nutrient quality and storage organelle formation (Kawakatsu *et al.*, 2010). Lipids have also been shown to be important for seed germination or seedling establishment in Arabidopsis seeds (Kelly *et al.*, 2011).

Seed heteromorphism or dimorphism is an adaptive mechanism in many xerophyte and halophyte species to an unfavourable environment during germination (Imbert, 2002). These adaptations are commonly associated with environments that are highly variable, either in time or space. Previous studies have shown that brown seeds of *Suaeda salsa* germinated faster and appeared more tolerant than black seeds under salinity or drought stress (Zhao *et al.*, 2004; Song *et al.*, 2008). As an adaptation strategy, this may indicate that the dimorphic seeds of *S. salsa* have different responses to salt and drought, or combined stresses. However, there is little information on germination of dimorphic seeds of *S. salsa* grown under culture conditions of low and optimum sodium chloride (NaCl) concentrations.

Besides inherent factors, seed vigour is also influenced by environmental factors. For non-halophytes, salinity greatly affects seed germination of green gram cultivars (Misra and Dwivedi, 2004), and induces a reduction in germination rate and a delay in the initiation of germination and seedling establishment of durum wheat (*Triticum durum*; Almansouri *et al.*, 2001). Environmental conditions may affect seed quality during seed formation of cotton (*Gossypium barbadense* cv. Giza 86). High application of nitrogen fertilizer significantly increased seed weight, seed viability, seedling vigour and cold germination test performance, whereas application of foliar potassium and the plant growth retardant mepiquat chloride improved seedling vigour in the next growing season (Sawan *et al.*, 2009). In hybrid maize, the use of 165 kg ha^{-1} nitrogen fertilizer and seven-day irrigation intervals during growth of the mother plant resulted in improved seed germination and seedling vigour indices (Farhadi *et al.*, 2014). However, little is known about effect of environmental factors on the seed quality of halophytes.

S. salsa is a leaf succulent annual herb belonging to the family Chenopodiaceae; it is one of the main halophytic species distributed widely in the saline soils of northern China (Zhao *et al.*, 2002). As its seeds contain approximately 30–40% edible oil, are rich in unsaturated fatty acids (Bai *et al.*, 2003) and its fresh branches are edible, this species has economic potential as a source of oil, food or fodder (Wang *et al.*, 2001; Zhao *et al.*, 2002). *S. salsa* is adapted to, and can grow well in, saline soils, through its ability to hyper-accumulate Na^+ and Cl^- in its succulent leaves (Zhao *et al.*, 2005). For example, the optimum salt concentration for vegetative growth and photosynthesis of the euhalophyte *S. salsa* is 200 mM NaCl (Lu *et al.*, 2003; Qiu *et al.*, 2007; Song *et al.*, 2009). To our knowledge, very few studies have been carried out on the effect of NaCl on seed quality of the euhalophyte *S. salsa* grown in the absence and presence of NaCl for its complete life cycle. Whether non-saline growth conditions affect seed germination and seed vigour of the halophyte *S. salsa* is still unknown.

In order to determine the seed quality generated from *S. salsa* plants under low and optimum NaCl concentration, *S. salsa* plants were grown under sand culture conditions, from seed germination to seed harvest. The main objective of the research was to evaluate the effects of low and optimum concentrations of NaCl application to the mother plant on seed production and seed quality of the euhalophyte *S. salsa* through observing the germination and vigour of seeds under the different levels of NaCl.

Materials and methods

Seed material

Seeds of *S. salsa* were collected from the Yellow River Delta (37°20′N; 118°36′E) in Shandong, the middle-eastern province of China. The saline land where *S. salsa* grows has Na^+ and Cl^- concentrations of 2.4 and 2.0 g (kg dry soil)$^{-1}$, respectively (Liu, 2006). After 6 months of refrigerated storage (<4°C), seeds were sown in plastic buckets (26 cm diameter, 30 cm height) with drainage holes and filled with rinsed river sand. The plants were irrigated twice a day, in the morning and late afternoon, with 1 mM NaCl (control) or 200 mM NaCl (six pots for each concentration of NaCl) dissolved in Hoagland nutrient solution. In the 200 mM NaCl treatments, the chance of osmotic shock was reduced by stepping up in 50 mM increments every 12 h until final concentrations (200 mM) were achieved. The pH of all solutions was adjusted to 6.2 ± 0.1 with 1 M potassium hydroxide (KOH) and sulphuric acid (H_2SO_4). NaCl treatments were carried out throughout the whole plant life cycle. The plants were grown in a greenhouse under natural light conditions at Shandong Normal University; the temperature was 28 ± 3/23 ± 3°C (day/night) with a relative humidity of 60/80% (day/night).

Seeds were hand-harvested from plants cultured with 1 or 200 mM NaCl and then cultured under the same conditions as the mother plant. That is, the seeds from the 1 mM NaCl (control) continued treatment at 1 mM NaCl (control); the seeds from the 200 mM NaCl treatment continued at 200 mM NaCl. The third generation of seeds was obtained as the seed source for future germination and other experiments, as described below.

Germination percentage and germination potential

Five NaCl concentrations (0, 25, 50,100 and 150 mM) were used to determine the salt tolerance of the various seed samples. NaCl was dissolved in 1/5 Hoagland solution and 0 mM NaCl was used as the control. Hoagland solution (1/5) had the following composition: 1 mM $Ca(NO_3)_2$, 1 mM KNO_3, 0.4 mM $MgSO_4$, 0.2 mM KH_2PO_4, 10 μM Fe-EDTA, 23 μM H_3BO_3, 4.55 μM $MnCl_2$, 0.16 μM $CuSO_4$, 0.38 μM $ZnSO_4$ and 0.06 μM Na_2MoO_4. The pH was adjusted to 6.2 ± 0.1 with 1 M KOH and H_2SO_4.

Each treatment consisted of four replicates of 80 seeds. The criterion for germination was visible radicle protrusion (Bewley and Black, 1994). First, seeds of *S. salsa* were surface sterilized by soaking in 6% sodium hypochlorite for 15 min, followed by five rinses with distilled water, before the start of each germination trial. Twenty uniformly sized and undamaged seeds were placed on two layers of filter paper, which was moistened with 5 ml of NaCl solution in a Petri dish (90 mm in diameter). The experiment was carried out in darkness in a growth chamber (RXZ-500, Ningbo, China). The temperature was maintained at 20 and 25°C during night and day, respectively, with a 12-h photoperiod. Daily germination rate was measured and NaCl solutions were replaced every 2 d to keep the NaCl concentration unchanged. Final germination rate and germination potential were calculated after 9 d of incubation using the following formulas:

$$\text{Germination percentage (\%)} = (\text{sum of the number of seeds germinated/the total number of seeds tested}) \times 100$$

$$\text{Germination potential (\%)} = (\text{sum of the number of seeds germinated when seed germination reached its peak/the total number of seeds tested}) \times 100$$

For the germination index and seed vigour index, five normal seedlings from each replicate were selected at random, and root length was measured. Germination index and seed vigour index were then calculated using the following formulas:

$$\text{Germination index (GI)} = \sum (G_t/D_t)$$

where G_t is the number of seeds germinated at day t and D_t is the corresponding days of germination.

$$\text{Seed vigour index (VI)} = \text{GI} \times (S/100)$$

where GI is the germination index and S is the mean root length of seedlings after 9 d of germination (Corchete and Guerra, 1986).

After 9 d, ungerminated seeds in each treatment were rinsed three times with distilled water and then incubated in new Petri dishes with distilled water for 6 d to determine total germination percentage, using the following formula:

$$\text{Total germination percentage (\%)} = (A/C) \times 100$$

where A is the sum of the number of seeds germinated in NaCl solutions plus those that were recovered to germinate in distilled water, and C is the total number of seeds tested (Khan and Ungar, 1984).

Measurement of seed protein content

The seeds of *S. salsa* were washed three times using purified water, after which proteins were extracted using a modified protocol described by Shen *et al.* (2003). Four replicates of dried seeds (0.1 g samples) were ground with a pestle and mortar to a powder under liquid nitrogen. The powder was transferred to a 2-ml centrifuge tube containing 1 ml extraction buffer [20 mM Tris–HCl, pH 7.5, 250 mM mannitol, 10 mM EDTA, 1 mM phenylmethanesulphonyl fluoride (PMSF), 1 mM dithiothreitol (DTT) and 1% Triton X-100]. After mixing and a 30-min incubation, the mixture was centrifuged (15,000 *g*, 4°C, 20 min) twice. The supernatant was collected and the protein concentration measured using the Bradford method with bovine serum albumin as the standard (Bradford, 1976).

Measurement of soluble sugar and starch content

Four dried-seed replicates (0.1 g samples) were ground with a pestle and mortar to a powder under liquid nitrogen. Each powder was transferred to a 10-ml glass tube and extracted with 5 ml of 80% ethanol at 80°C for 30 min, followed by two extractions with 2 ml of 80% ethanol. The supernatants were combined and soluble sugar content was determined with anthrone reagent, using glucose as the standard (Yemm and Willis, 1954).

The remaining pellets were dried at 45°C to remove the ethanol, and boiled for 10 min with 3 ml double-distilled water in 10-ml centrifuge tubes. Subsequently, the samples were cooled to room temperature, and 4 ml perchloric acid ($HClO_4$) was added to decompose the starch. Starch in the paste was hydrolysed for 15 min, and the glucose content was determined at 630 nm as described previously (Yemm and Willis, 1954). Starch content was calculated using the formula:

$$\text{Starch content } (\%) = G \times 0.9/\text{DW} \times 100$$

where G is the glucose content of the samples after acid hydrolysis, the constant 0.9 is the theoretical factor to convert glucose to starch, and DW is the dry weight (g).

Measurement of total lipid content

For lipid extraction, four replicate samples of dried seeds (0.1 g) were weighed accurately and frozen in liquid nitrogen for a few minutes; the frozen samples were then ground to a fine powder in a mortar, and the powder was transferred to 10-ml glass tubes for lipid extraction. Lipids were extracted using a mixture of hexane and isopropanol (3:2, v:v) to determine total lipids gravimetrically (Hara and Radin, 1978).

Statistical analysis

Each experiment described above was performed randomly with four replicates, the statistical results were presented as means ± SD. Data were analysed using SPSS, version 17 one-way analysis of variance (ANOVA) statistical software packages (SPSS Inc., Chicago, Illinois, USA). Different letters indicate significant differences in the mean (at $P \leq 0.05$) from Duncan's tests.

Results

NaCl markedly increases seed size and weight

To investigate the effect of NaCl on the seeds of *S. salsa*, seed size and thousand-seed weight of the black and brown seeds were examined. Compared with the control, the seed size and seed weight were significantly augmented by salt (200 mM NaCl). The latter treatment resulted in significantly bigger seeds than those of the control (Fig. 1A); the length and thickness of black seeds and brown seeds were 125.96%, 128.00% and 151.26%, 129.33% of the control, respectively (Fig. 1B), and individual black seed and brown seed weights were 228.19% and 173.73% of the control, respectively (Fig. 1C).

NaCl stress reduces germination of black seeds but does not affect the brown seeds

Seed size plays a major role in germination and seedling establishment, particularly under environmental stress, such as salinity. Both black and brown seeds of *S. salsa* grown under the 200 mM NaCl treatment were significantly bigger than those grown under the 1 mM NaCl treatment. We examined the response of germination percentage and germination potential of black and brown seeds to different concentrations of NaCl and the total germination percentage after NaCl removal. For the black seeds, both germination percentage and germination potential decreased progressively as the level of salinity increased. However, at different NaCl concentrations (0, 25, 50, 100 and 150 mM), the germination percentage and germination potential of the black seeds from the 200 mM NaCl treatment was significantly higher than those of the control; these were 126.67%, 154.16%, 155.31%, 140.43%, 233.33% and 135.18%, 137.50%, 160.46%, 156.41%, 335.29% of the control, respectively (Fig. 2A, B). Surprisingly, for the brown seeds, there was no significant difference in the germination rate and germination potential between the seeds from the mother plants of the 1 and 200 mM NaCl treatments, except for germination potential in the 150 mM NaCl treatment. When the ungerminated seeds were transferred to distilled water after the 9-day exposure to salinity, the total germination percentage of the *S. salsa* seeds was obtained (Fig. 2C). The result showed nearly 100% germination of the ungerminated black and brown seeds from the mother plants of the 200 mM NaCl treatment and a slight reduction in germination of the ungerminated black seeds from the maternal control plants (Fig. 2C).

NaCl improves germination and seed vigour indices

The germination index reflects the speed and uniformity of germination. The germination index of both black and brown seeds from the mother plants of the control was significantly lower than that of the seeds from the mother plants of the 200 mM NaCl treatment. Furthermore, the germination index of brown seeds from the mother plants of both the control and the 200 mM NaCl treatment was significantly

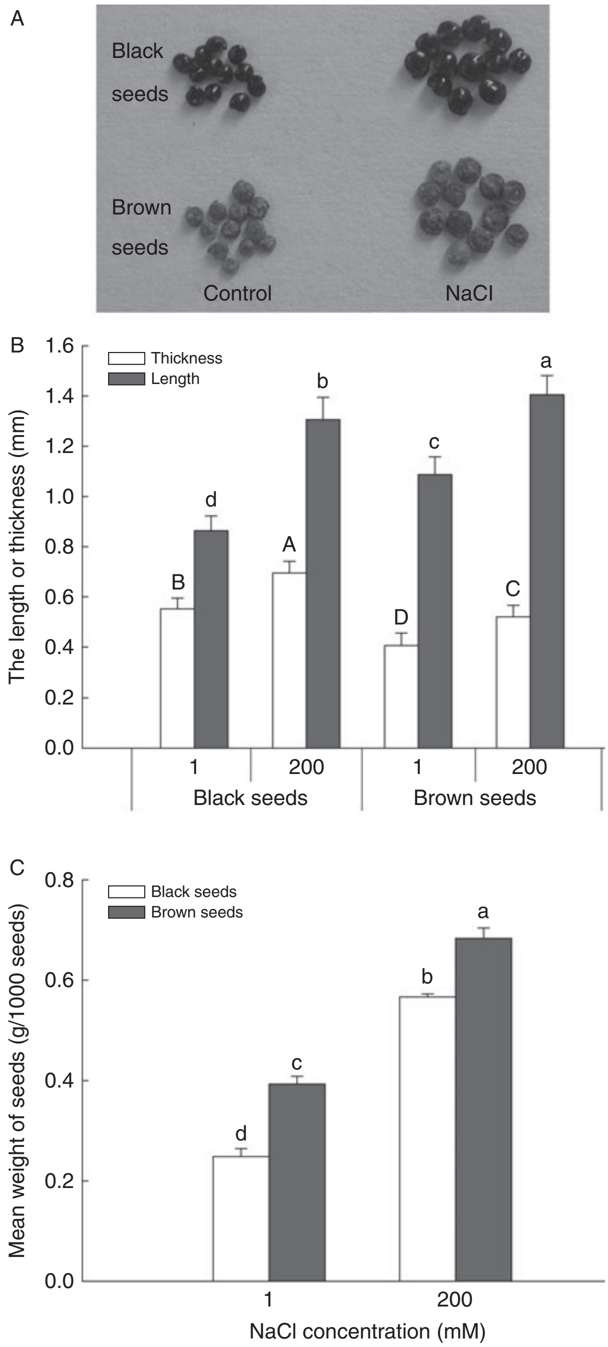

Figure 1. Size (A, B) and 1000-seed weight (C) of black and brown seeds of *Suaeda salsa* from mother plants grown under conditions with 1 and 200 mM NaCl. Values of seed size are means ± SD ($n = 100$) and values of seed weight are means ± SD ($n = 5$). Different letters indicate significant differences at the level of 0.05 according to Duncan's test.

higher than that of the black seeds (Fig. 2D). At 150 mM NaCl, the germination indexes of black seeds and brown seeds from the mother plants of the 200 mM NaCl treatment were 311.32% and 176.13% of the control, respectively. At 0 mM NaCl the germination indexes of black seeds and brown seeds from the mother plants of the 200 mM NaCl treatment were 154.86% and 154.30% of the control, respectively (Fig. 2D). These results indicate that the seeds from the mother plants of the 200 mM NaCl treatment had a higher germination index and salt tolerance as compared to the seeds from the control plants.

The seed vigour index represents the germination capacity and growing tendency of seedlings. Similar results in vigour index of the seeds from the mother plants of the control and the 200 mM NaCl treatment were obtained under different concentrations of NaCl (Fig. 2E). At 150 mM NaCl, the vigour indexes of black seeds and brown seeds from the mother plants of the 200 mM NaCl treatment were 993.50% and 234.63% of the control, respectively. At 0 mM NaCl, the vigour indexes of black seeds and brown seeds from the mother plants of the 200 mM NaCl treatment were 360.50% and 275.83% of the control, respectively (Fig. 2E). These findings further indicate that the seeds from the mother plants of the 200 mM NaCl treatment had a higher seed vigour index than those from the control plants.

Total seed storage compounds

Seed proteins are stored nitrogen sources for germinating seedlings. As shown in Fig. 3A, the total seed protein content in the brown seeds was much higher than in the black seeds. Furthermore, the protein content of the black and brown seeds from the mother plants of the 200 mM NaCl treatment was significantly higher than that from the control (111.46% and 113.06% of the control, respectively; Fig. 3A). The total soluble sugar content of the brown seeds was also higher than that of the black seeds, whereas the starch content of the brown seeds was lower than that of the black seeds. However, both the soluble sugar and starch contents of the seeds from the mother plants of the 200 mM NaCl treatment were significantly higher than those from the control; this was 130.50% and 155.28% (black seeds) and 104.06% and 149.66% (brown seeds) of the control, respectively (Fig. 3B, D). The total lipid content of the brown seeds was significantly higher than that of the black seeds from the mother plants at the same growth condition of 1 or 200 mM NaCl; moreover, the lipid content in both the black and brown seeds from the mother plants of the 200 mM NaCl treatment was significantly higher than that of the seeds from the control (128.74% and 127.76% of the control, respectively; Fig. 3C).

Discussion

Environmental conditions of the parental plant during reproductive growth affect the performance of the seeds. In hybrid sweet pepper (*Capsicum annuum* L. cv.

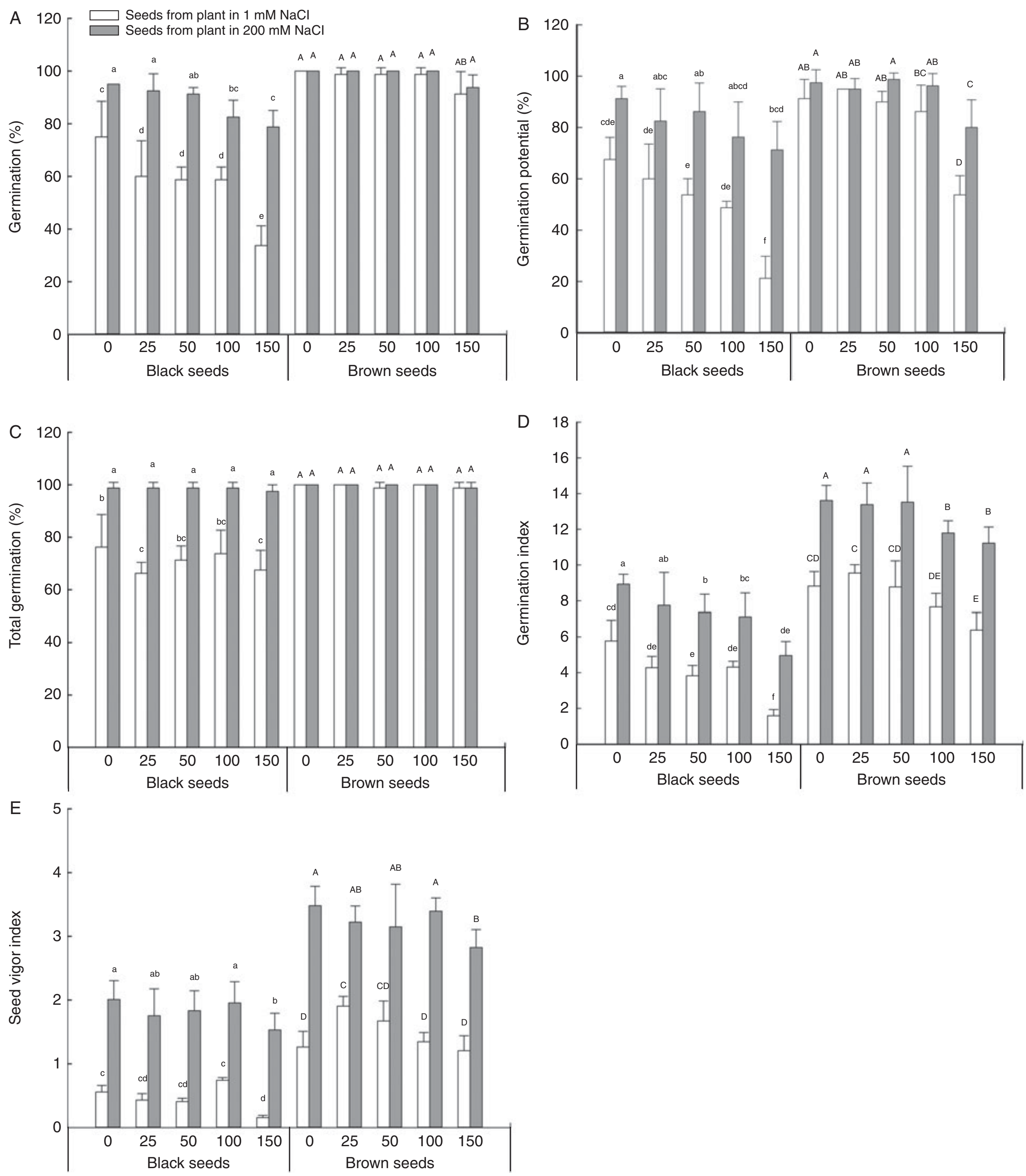

Figure 2. Effect of different concentrations of NaCl on the germination percentage (A), germination potential (B), total germination percentage (C), germination index (D) and seed vigour index (E) of the black and brown seeds of *Suaeda salsa* from mother plants grown under conditions with 1 and 200 mM NaCl. Values are means ± SD (n = 4). Different letters represent significant differences at the level of 0.05 according to Duncan's test.

'Hazera' 1195), seeds developed at higher temperatures (in summer) displayed a lower percentage of seedling emergence than seeds developed under lower temperatures (in winter) (Xu and Kafkafi, 2003). However, *Arabidopsis thaliana* seeds from the warm parental environment showed faster germination rates than those from the cold parental environment (Blödner *et al.*, 2007). But for *Leymus chinensis* the increased temperature significantly reduced the number of flowering plants and seed number per square meter, and reduced the number of germinating seeds per unit area (Gao *et al.*, 2012). Water during

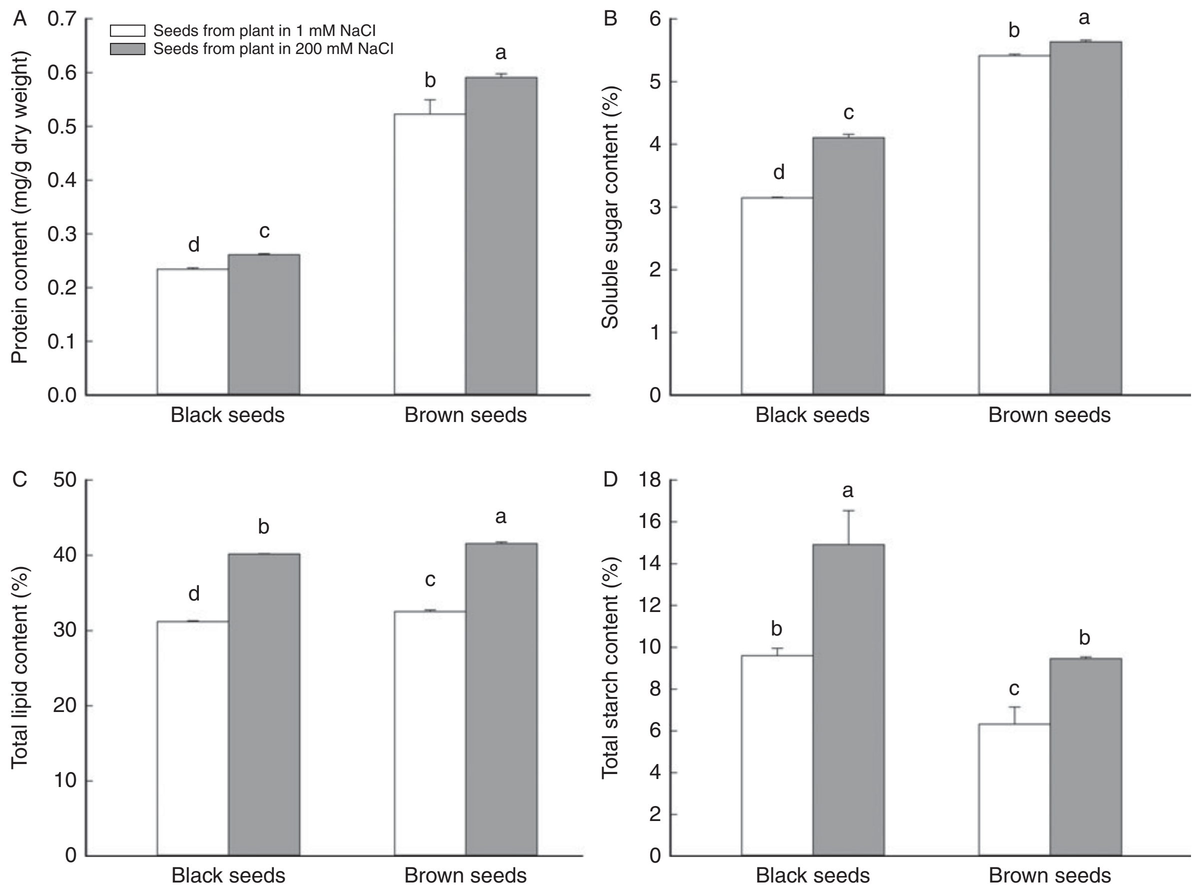

Figure 3. Total seed protein (A), soluble sugar (B), seed lipid (C) and seed starch (D) content of black and brown seeds of *Suaeda salsa* from mother plants grown under conditions with 1 and 200 mM NaCl. Values are means ± SD ($n = 4$). Different letters represent significant differences at the level of 0.05 according to Duncan's test.

reproductive growth also influenced seed yield. Plants of the alpine perennial *Ranunculus adoneus*, growing at sites where snow melts earlier, produce larger seeds with higher survival and greater probability of emergence than seeds from plants at later-melting sites (Santon and Galen, 1997). Water stress during reproductive growth resulted in a drastic reduction of seed yields of rapeseed (*Brassica napus* L.) (Ahmadi and Bahrani 2009) whereas in *Plantago ovata* and *Nigella sativa*, the lowest seed yield was observed when irrigation was stopped at the blooming stage; however, the oil content of the seeds was not reduced (Bannayan *et al.*, 2008).

Soil salinity is a major constraint to the economic exploitation of land for agriculture and forestry, and an increase in arid and semi-arid regions is one of the most severe environmental factors limiting the productivity of agricultural crops (McWilliam, 1986). The saline environment of the parental plant may affect seed quality. For example, in the salt-tolerant species *Iris hexagona,* seeds produced on maternal plants growing at high salinity germinated earlier and in greater numbers than seeds from low-salinity plants (Van Zandt and Mopper, 2004). In the present study, the saline concentrations in which the euhalophyte *S. salsa* grows were shown to markedly affect their seed quality. Compared with control plants, the black and brown seeds from mother plants grown in the 200 mM NaCl treatment displayed significantly higher germination parameters, such as germination percentage, germination potential, germination index and seed vigour index, except for the brown seed germination percentage and germination potential in the range of 0–100 mM NaCl. What may be the reasons for the difference between the germination ability of *S. salsa* from the control and 200 mM NaCl-treated plants? Our results indicate that a certain concentration of NaCl enhances seed vitality and seed quality during seed formation, that is to say, NaCl is a beneficial factor in seed development of the euhalophyte *S. salsa*.

Seed size and weight affect seed quality (Willenborg *et al.*, 2005; Tanveer *et al.*, 2013), and many factors affect seed size and weight on the maternal plant. As demonstrated by Sawan *et al.* (2009) for cotton, an increase in nitrogen and potassium application significantly increased the seed weight. In non-halophytes, the reproductive growth is limited in saline soils (Munns and Termaat, 1986; Munns *et al.*, 2006). Salt stress significantly decreased the seed weight per head and 1000-achene weight of sunflower hybrids (Di Caterina *et al.*, 2007). As in salt-tolerant species, the individual seed weight and quality decreased with increasing levels of salinity in rye (*Secale cereale* L.) (Francois *et al.*, 1989). However, in the present experiment, 200 mM NaCl in the growth medium markedly improved the seed quality, including seed size and seed weight, which possibly contributes to a good germination performance under high salinity. As described for *Convolvulus arvensis*, larger seeds result in a higher germination percentage and germination index than small seeds (Tanveer *et al.*, 2013), and in sunflower (*Helianthus annuus* L.) seed size had a significant effect ($P \leq 0.01$) on germination percentage (Farahani *et al.*, 2011).

Seed dimorphism and polymorphism are well known for several halophytes (Khan and Ungar, 1984; Khan *et al.*, 2001; Li *et al.*, 2005). Heavy seeds of *Primula* species, for example, germinated in greater numbers and more quickly than light seeds (Tremayne and Richards, 2000). In the natural habitat of *S. salsa*, brown seeds are more salt resistant than black seeds, and germinate more rapidly than black seeds (Song *et al.*, 2008). In the present study, the brown seeds were bigger and heavier than the black ones and this may be one of the reasons why the germination percentage for brown seeds was greater than that for the black seeds at all NaCl concentrations. Besides seed size and weight, seed vigour was also related to seed storage compounds. The bigger seeds of wheat cultivars had higher levels of protein than the smaller seeds (Ries and Everson, 1973; Ries *et al.*, 1976) and reduced seed storage protein contents led to altered storage organelle formation in rice (Kawakatsu *et al.*, 2010). Salinity (50 mM NaCl) markedly reduced the protein and starch contents of chickpea seeds (*Cicer arietinum* L.) (Murumkar and Chavan, 1986). However, in the present study the bigger seeds displayed higher contents of storage compounds, as well as better germination parameters. These results suggest that the higher vigour of seeds from the mother plants of the 200 mM NaCl treatment is due to the higher content of seed storage compounds.

Seed tolerance to salinity should be considered at two levels (Prado *et al.*, 2000): (1) the ability to germinate under high salinity, and (2) the ability to recover and germinate following the removal of the saline conditions. In this study, the *S. salsa* seeds from the mother plants grown at 200 mM NaCl showed a favourable performance, both in germination parameters and recovery at 0–150 mM NaCl concentrations, as compared to seeds from the 1 mM NaCl treatment. As suggested by Ungar (1978, 1995), for successful establishment of plants in saline environments seeds must remain viable at high salinity and germinate when salinity in the growth medium decreases. Taking our results together, euhalophytes such as *S. salsa* need a high level of salt in their growth environment to produce high-quality seeds for faster germination and population establishment in high saline soils, and in this sense, low levels of salt represent a 'stress' condition for halophytes.

The present study investigated, for the first time, the effect of very low and optimum levels of NaCl on seed production and seed quality of the euhalophyte *S. salsa*. Seed germination characteristics, seed quality and seed storage compound contents from mother plants grown under 200 mM NaCl throughout a whole life cycle were markedly higher than those from the control plants (grown in 1 mM NaCl). These results suggest that a certain concentration of NaCl in the growth environment is favourable for seed development in euhalophytes, which possibly contributes to high seed germination and population establishment in high-salt environments.

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Conflict of interest

None.

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