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Effect of seed weight and salinity on the germination of Garden orache (*Atriplex hortensis* L.)

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Garden orache (*Atriplex hortensis* L.), a halophytic forb in the family Chenopodiaceae, is well adapted to dry saline habitats. Here, we present the results of the effect of salinity and seed weight on seed germination, radicle emergence of *Atriplex hortensis*. Highest germination percentages were obtained under non-saline conditions and increases in salinity inhibited seed germination, with less than 50% of the seeds germinating at 260 mM NaCl. Seeds of *Atriplex hortensis* exhibited a very pronounced morphological and physiological seed polymorphism. Seed weight varied from 0.11 to 0.19 g. Large seeds had a mean dry weight of 0.19 mg and a mean length of 2.6 mm; medium seeds, mean dry weight of 0.15 mg and mean length of 1.9 mm; small seeds, mean dry weight of 0.11 mg and mean length of 1.1 mm. Seedling dry weight was related to initial seed size. The degree of salt tolerance increased progressively with increasing seed dry weight. Radicle elongation was increased by low salinity and it decreased with an increase in salinity. Based on the observations, it is inferred that the germination percentage of large seed size class of *A. hortensis* was highest compared to small seed size class. the seed size is an important factor that influences the germination under normal as well as salinity stress conditions.

Keywords: Annual *Atriplex*, salinity, germination, seed weight.

INTRODUCTION

Halophytes are potentially useful for ecological applications, such as landscaping, or rehabilitation of damaged ecosystems. Halophyte species vary in their tolerance to salinity during seed germination (Khan et al., 2002; Debez et al., 2011). Seeds of halophytes can recover the capacity to germinate after exposure to salt stress that inhibits their germination (Woodell 1985; Khan et al., 2002). Population level effects of salinity stress may be particularly likely to arise at the seed germination stage, particularly for annual species, because salinity has strong impacts on germination in many plants including halophytes (Li et al., 2010). Annual halophytes vary in their upper limit of salt tolerance and increases in salinity usually delay germination (Ungar 1996). *Atriplex* species are frequent in many arid and semi-arid regions of the world, particularly in habitats that combine relatively high soil

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salinity with aridity (Redondo-Gómez et al., 2007) and therefore constitute a useful material for the identification of physiological mechanisms involved in salt stress resistance.

Seed polymorphism can enable halophytes to adapt to varying salt-marsh or dry salt desert environments. It also enhances chances for seedling establishment and survival in a saline environment (Imbert 2002). The presence of different kinds of seed/fruit morphs appears to be related to the ecological strategies of *Atriplex* species to maximize survival under variable environmental stresses.

Although many halophytes are salt tolerant as adults, seed germination is often impaired by salinity (Atia et al., 2010a). Salt impact on the germination of halophytes is influenced by several factors among which the salt type, light, temperature, but also the provenance, i.e., geographical origin, of the species investigated Sosa et al., 2005; Atia et al., 2010a; Baker 1974), found that *A. triangularis* plants arising from large seeds had faster root and shoot growth than seedlings from small seeds that germinated at the same time.

In this context, our objectives were to evaluate the effect of seed size (weight) and salinity on seed germination recovery and radicle elongation using seeds of *A. hortensis*.

MATERIALS AND METHODS

Plant material

Atriplex hortensis (L.), a C_3 xero-halophyte of the family Chenopodiaceae, is an annual species that is well adapted to saline and drought conditions. Seeds of *A. hortensis* were obtained from CN Seeds Ltd. (Ely, UK). Effects of salinity on the germination of various size classes of *Atriplex hortensis* seeds and subsequent seedling growth were studied in light.

The effects of seed weight on seed germination, seedling survival and growth of *A. hortensis* were studied and seeds of each of the 2 weight classes were separately soaked for 24 h in distilled water under laboratory conditions.

The seeds were categorized into two size classes, small (1.1 - 1.9 mm) and large size class (2 – 2.6 mm). On the basis of diameter, the standard of the weight classes is the medium sized seed class and 50 seeds were used for each class.

Germination tests were carried out in Petri dishes with 5 ml of test solution, and each dish was placed in a 10 cm diameter plastic Petri dish as an added precaution to avoid water loss from dishes by evaporation. Six replicates of 25 seeds each were used for each of the treatments and we considered seeds to have germinated after radicle emergence.

Effects of salinity on germination

Three levels of salinity stress were produced through three concentrations of NaCl (90, 180 and 260 mM NaCl /l) against distilled water (0 mM NaCl /l) being run as control. The experiment used a randomized complete block design, and seeds were considered as germinated when their emerging radicals were visible. Germinated seeds were counted and removed daily for a period of 15 days, after which no more germination was observed.

Radicle elongation percentage

Seeds for 15 d were incubated initially in deionized water at 25 °C in the light. When the radicles had barely emerged (<1.0 mm), 20 of these young seedlings were transferred into petri dishes containing deionized water control. Seedling incubation was terminated after 15 d and mean radicle length recorded.

Final germination percentage

Final germination percentage (GP) was calculated as the cumulative number of germinated seeds with normal radicles (Larsen and Andreasen 2004):

$GP = \sum n$, where n is number of seeds that had germinated at each counting.

1.1. Germination rate

The rate of germination was estimated using a modified Timson index germination velocity (TIGV)

$TIGV = \sum G/t$

where G is the percentage of seed germination at 2 day intervals and t is the total germination period (Khan and Ungar 1984).

Data analysis

Seed germination percentage and radicle length were expressed as mean ±s.e. One-way analysis of variance (ANOVA; $P < 0{\cdot}05$) was used to compare treatment effects.

RESULTS

Effects of seed weight on germination

Germination was rapid with almost all seeds germinating in control conditions. The frequency distribution indicated a bimodal distribution (figure 1). Large seeds had a mean dry weight of 0.19 mg and a mean length of 2.6 mm; small seeds, mean dry weight of 0.11 mg and mean length of 1.1 mm. Seeds of all sizes in all treatments recovered very quickly. Final germination percentages for all seed sizes

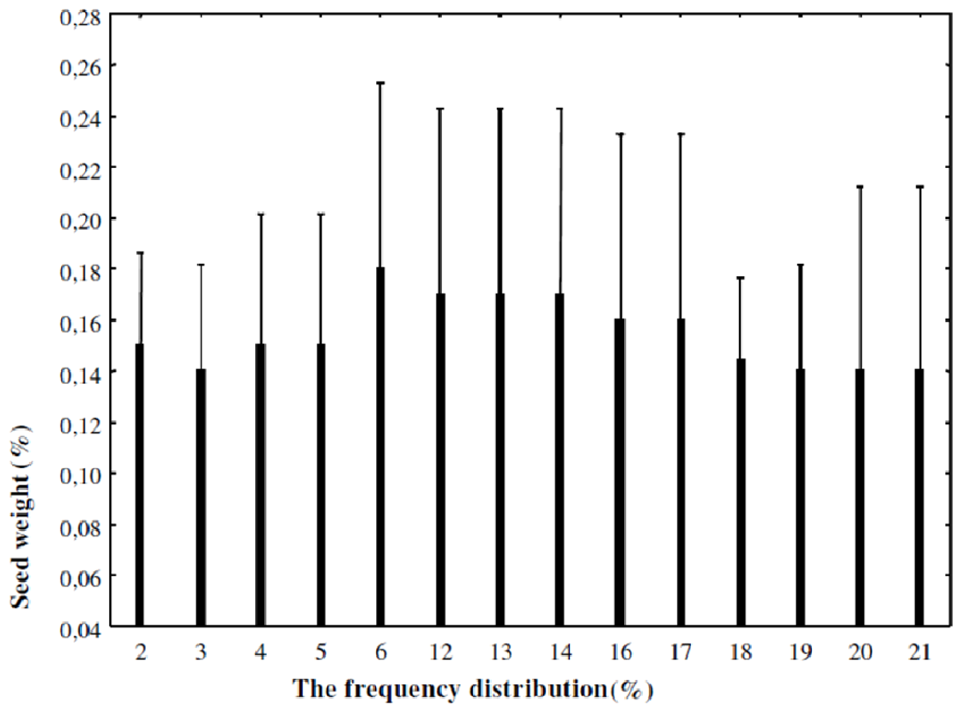

Figure 1. Variation in seed weight of Atriplex hortensis

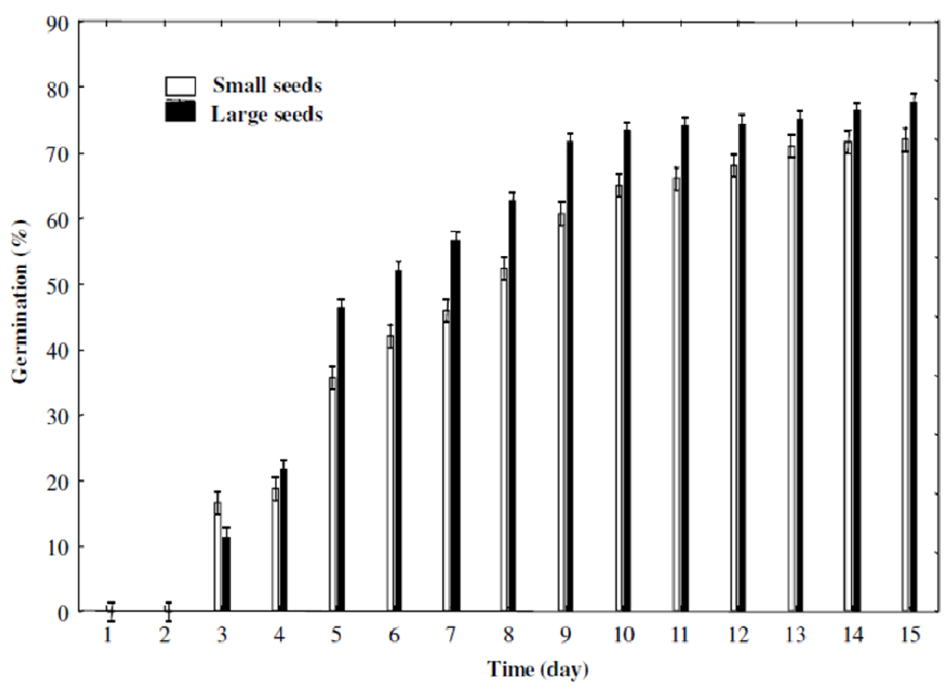

Figure 2: Effect of seed polymorphism of Atriplex hortensis on germination after 15 d of incubation in deionized water. Values are means ± s.e.

in all treatments were different from each other. Presoaking increased the final germination percentage of large seeds compared with small seeds (figure 2). However, radicle elongation progressively increased for all seed sizes (figure 3). Radicle growth of large seeds was much higher than that of small seeds.

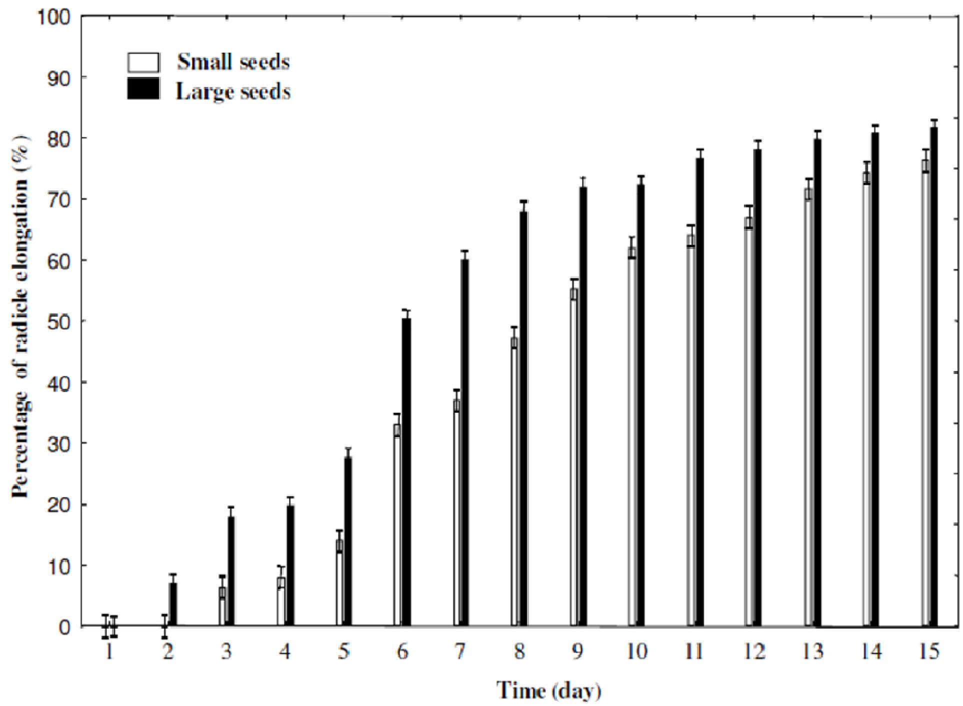

Figure 3. Radicle elongation percentage in Atriplex hortensis after transfer to deionized water. Radicle elongation was recorded after 15 d of incubation in deionized water at 25 °C in the light. Values are means ± s.e.

Effects of salinity on germination

Analysis of figure 4 revealed that at NaCl concentrations of 90-260 mM, germination rate decreased. Seed germination percentage did not reach 50 % at salinities exceeding 260 mM until after 15 d of incubation (Figure 4). As the pre-treatment concentration of salinity increased, the percentage and rate of germination decreased, and for all salinities germination recovery percentages were higher than that of the control (distilled water). Germination recovery did not differ among seeds transferred from 0–90 mM NaCl solutions to deionized water.

To evaluate the effects of salinity on germination of *A. hortensis*, germination percentage (GP) and germination rate (GR) were examined (Figure 6). Germination was affected by salt stress. As salinity increased from 0 to 260 mM NaCl, the rate and percentage of germination decreased, and they were inhibited significantly by increased severity of salinity (Figure 6).

Effects of salinity on radicle growth

Radicle growth recovered after seedlings were transferred to deionized water. Concentrations of 90–260 mM NaCl decreased radicle elongation. No radicle elongation occurred at NaCl concentrations of ≥260 mM (Figure 5). However, elongation recovery decreased with the increase in pre-treatment salinities. Seedlings from solutions ≥260 mM NaCl showed no capacity for recovery (Figure 5).

Furthermore, the radicle elongation was severely influenced by NaCl, the radicles from seedling under non-stress conditions were the longest, whereas those under the highest NaCl concentration were the shortest. Increasing the NaCl concentration resulted in a decrease in the radicle length, but no difference between 90 mM NaCl and the control was observed. The radicle growth was noticeably inhibited by 260 mM NaCl but not by 90 mM.

DISCUSSION

Germination is a principal component of seedling establishment and survival, and is considered the most critical phase of the plant life cycle (Rajjou et al., 2012). Germination is affected by several environmental and plant conditions including salinity, temperature, genotype, seed size, and soil conditions (Bewley and Black 2012). The study presented here reveals how seed weight and salinity can significantly affect germination of Annual *Atriplex*. Our study demonstrated that germination was significantly enhanced when salinities were increased from 90 to 260 mM. The research presented here indicated differences in

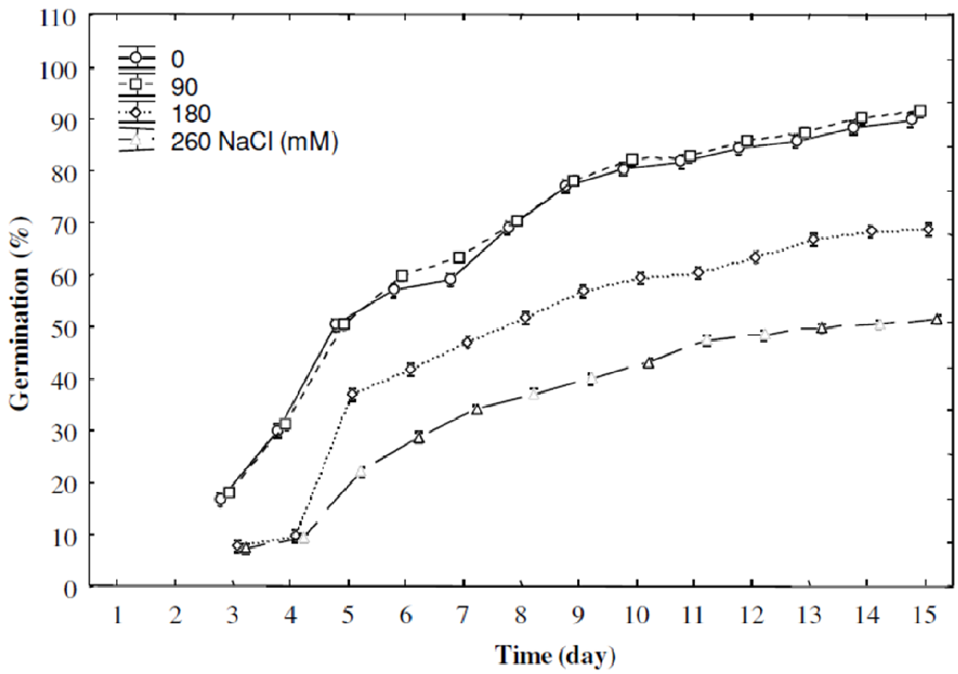

Figure 4. Effect of NaCl concentration on germination of Atriplex hortensis seeds incubated at 25 °C in the light for 15 d. Values are means ± s.e.

the germination percentage and radicle lengths of the *Atriplex* specie studied. Seeds of most halophytes germinate better in distilled water than in saline solutions, but they differ from glycophytes in their ability to germinate at higher salinities (Khan and Ungar 1999). Halophytic grasses have been reported to have different levels of salt tolerance, ranging from 300 mM to 430 mM (Khan and Ungar 1999). Seeds of some halophytes are reported to tolerate high salinity during the period when they are dormant in the soil and subsequently germinate when soil salinities are reduced (Khan and Ungar 1997).

Other investigations here shown that *Atriplex* spp. produce polymorphic/dimorphic dispersal units or polymorphic/dimorphic seeds (Mandák and Pyšek 2001a). Seed size also affected seed germination in salt or drought stress in such *Atriplex* spp. as *A. nummularia* Lindl, *A. prostrata* and *A. patula* (Katembe et al., 1998). Recent studies showed that seed size has strong effects on *A. sagittata* entire life cycle and it has an important impact on population regeneration in successive years (Mandák and Pyšek 2005; Nobs and Hagar 1974), showed that, during the first 5 h, the large brown seeds of *A. hortensis* had a very rapid rate of water imbibition and reached saturation at 48 h. The small black seeds showed a considerably slower rate of water uptake. (Osmond et al., 1980) reported that, under similar conditions, uptake was 100 times faster in brown than in black seeds of *A. hortensis.* The absorption of water by black seeds was more limited by the conductance of the seed coat than by the addition of NaCl to the solution (Osmond et al., 1980). A similar insensitivity to NaCl was observed with the black seeds of *A. holocarpa* (Osmond et al., 1980). Increase in salinity progressively inhibited seed germination and 50% seeds germinated in the 260 mM NaCl treatment (figure 4). Dimorphic seeds of the halophytes, *Atriplex prostrata* and *Atriplex patula* were, treated with various solutions of NaCl have suggested that the influence of NaCl is a combination of an osmotic effect and a specific ion effect (Katembe et al., 1998). In the present study, the radicle length was reduced by NaCl, and the shortest length measured was under 260 mM NaCl (figure 5). Seedlings of halophytes can develop in solutions with low concentrations of salts; however, radicle growth may be greatly retarded by high salinity (Malcolm et al., 2003).

The germination percentage and rate of germination were decreased with salt stress in *Atriplex* seeds (figure 6). Germination rate was decreased by salinity in *Atriplex hortensis*. This agreed with the results of (Gholamin and Khayatnezhad 2010), in wheat; (Mostafavi 2011), in safflower and (Yildirim et al., 2011), in Physalis. Due to this fact more studies with salt stressed seeds germination are necessary for the complete elucidation its effect on Orache development.

CONCLUSION

The overall results of this experiment showed the inhibitory effects of salt stress on seed germination parameters of *Atriplex* seeds. In conclusion, salt stress inhibited the

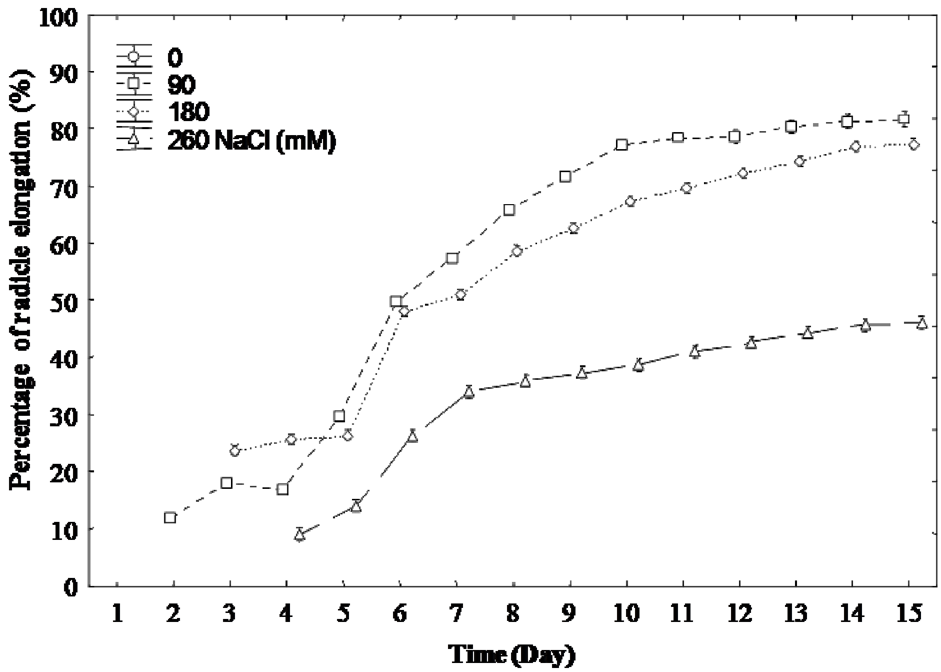

Figure 5. Effect of NaCl concentration on radicle elongation of Atriplex hortensis at 25 °C in the light after 15 d of incubation. Values are means ± s.e.

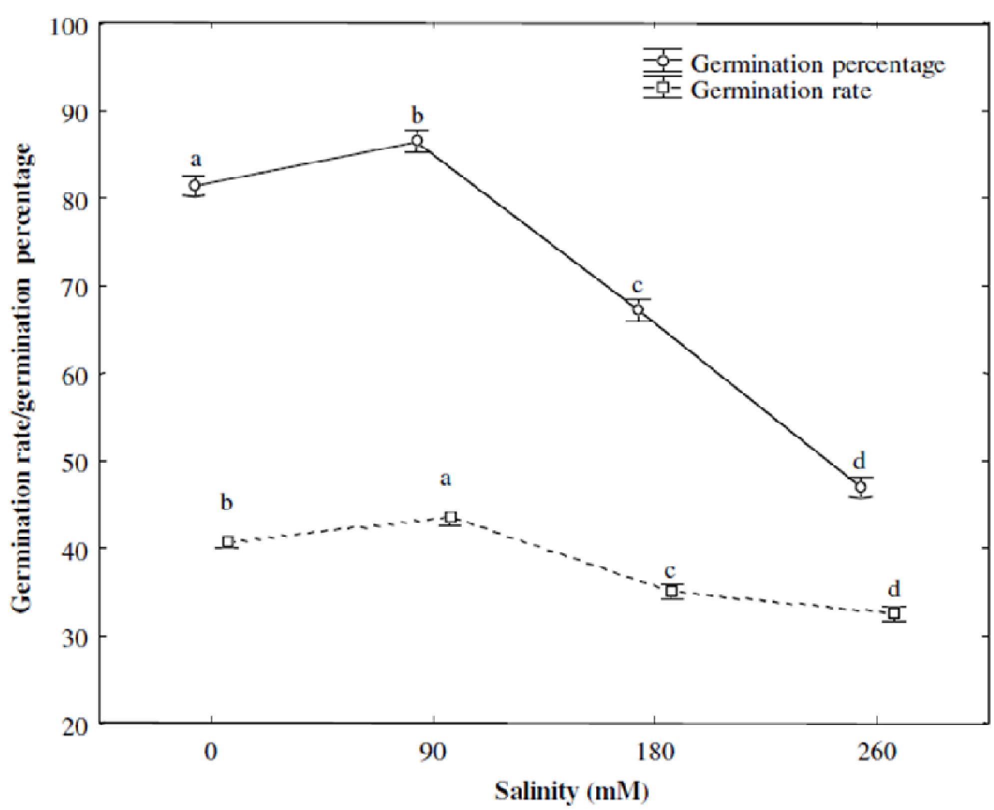

Figure 6. Effects of salinity on final percentage germination (GP) and the rate of germination (TIGV). Each point represents the mean of six replications.

germination of *A. hortensis* seeds and decreased the germination percentage and radicle lengths. Thus, understanding of the mechanics of seedling regeneration and seed bank formation is crucial to the successful management and restoration in habitats with high soil salinity.

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