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Comparative study on seed germination characteristics of two species of Australia saltbush under salt stress

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ABSTRACT

In order to examine the response characteristics and possible reasons of *Atriplex lentiformis* and *Atriplex undulata* under salt stress at stage of seed germination, the seeds were treated with different concentrations of NaCl (0, 50, 100, 200 and 300 mmol·L^{-1}), 20 mmol·L^{-1} LiCl or mannitol whose iso-osmotic concentrations corresponding to 200 mmol·L^{-1} NaCl. The results showed that the germination rate of two species of saltbush was depressed with the increase of NaCl concentration, and *A. lentiformis* showed greater salt tolerance compared with *A. undulata*. After removal of salt stress, the final germination ratio of *A. lentiformis* was over 93%, while that of *A. undulata* was only 56%. Evans blue staining revealed that 200 mmol·L^{-1} NaCl did not damage membrane permeability of *A. lentiformis* seed embryos, but significantly increased the membrane permeability of *A. undulata* seed embryos and caused irreversible damage to them, especially radicles. The results on water uptake indicated that the inhibition of NaCl on seed germination was mainly due to osmotic stress instead of ionic toxicity, and *A. lentiformis* exhibited higher salt tolerance due to its greater resistance to osmotic stress.

1. Introduction

Atriplex is a typical secretohalophyte in arid and semi-arid regions of the whole world. *Atriplex* species play an important role in vegetation reconstruction and soil conservancy for saline and arid regions. There are 17 species and 2 varieties of *Atriplex* in China [1], among which *A. tatarica* and *A. centralasiatica* had high salt tolerance [2–4]. Australian saltbush *A. semibaccata* R. Br. and *A. Canescens* were introduced in Qinghai, Xinjiang in the late 1990s, which showed great potential in enriching the biodiversity and improving the ecological environment in the saline and arid regions [5]. *A. lentiformis* and *A. undulata* are saltbushes which were introduced from Australia. The previous studies on *A. lentiformis* and *A. undulata* focused on the later stage of seedlings and little is known about response characteristics of the seeds under salt stress [6–10]. The adaptation to the salinity during seed germination and seedling growth is very important for halophyte population growth and construction in saline–alkaline condition [11]. In the present study, the response characteristics and possible reasons of seed germination for *A. lentiformis* and *A. undulata* under salt stress were determined to screen salt tolerant saltbushes being used in saline soil.

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2. Materials and methods

2.1. Materials

Two species of Australian saltbush seeds (*Atriplex lentiformis* and *Atriplex undulata*) collected in 2010 were stored at 2 °C in refrigerator for further use.

2.2. Methods

2.2.1. Determination of salt tolerance of seeds

Uninformal and full seeds were washed with 0.1% $HgCl_2$ for 5 minutes and rinsed with distilled water. Four NaCl concentrations (50, 100, 200 and 300 mmol·L^{-1}) were used to determine the salt tolerance of seeds. NaCl was dissolved in distilled water and distilled water was as control (0).

Three replicates of 20 seeds each were germinated on two layers of filter paper in Petri dishes (9 cm in diameter) containing 5 mL each of the test solution respectively and the NaCl solutions were changed completely every 2 days. The seeds were allowed to germinate at 25/15 °C day/night temperatures in the dark [12,13]. A seed was considered to have germinated when the emerging radicle elongated to 5 mm. The number of seeds germinated was counted daily for 10 days, then some data were determined by the following formulas: Germination rate = the total number of seeds germinated in saline solution/the total number of sown seeds × 100%. Relative germination rate = the germination rate of each salt treatments/the germination rate of control × 100%. Germination

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energy = the total number of seeds when seed germination reached its peak/the total number of sown seeds × 100%. Germination index (Gi) = Σ(Gt/Dt) and Vigor index (Vi) = S × Gi, where Gt is the number of seeds germinated at t day, Dt is corresponding days of germination, S is the mean weight of five seedlings after 5 day germination [14,15].

After 10 days, all ungerminated seeds were placed in distilled water and under the same condition for 7 days to determine their ability to recover from salt stress. The final germination rate was determined by the following formula: Final germination rate = the total number of seeds germinated after being transferred to distilled water/the total number of sown seeds × 100%.

The membrane permeability of *A. lentiformis* and *A. undulata* seed embryos treated with 200 mmol·L^{-1} NaCl was detected with Evans blue staining [16].

2.2.2. Determination of ionic effect or osmotic effect of salt stress on seed germination

Salt-induced inhibition of seed germination could be attributed to osmotic stress or to specific ion toxicity [17,18]. The toxicity of Li^+ is 10 times as that of Na^+, and 20 mmol·L^{-1} LiCl has no osmotic effect [19–23]. Three replicates of 20 seeds each were sown in Petri dishes (9 cm in diameter) on two layers of filter paper moistened with 5 mL distilled water (control), 20 mmol·L^{-1} LiCl, 200 mmol·L^{-1} NaCl or isotonic mannitol, and each solution was changed completely every 2 days. Germinated seeds were recorded daily. Seeds were considered to have germinated when the emerging radicle was at least 5 mm.

2.2.3. Determination of the water uptake

Water uptake was studied to validate whether the inhibition of salt stress on seed germination was mainly caused by osmotic effect. Seeds sown in Petri dishes (9 cm in diameter) on two layers of filter paper moistened with 10 mL of distilled water (control), 20 mmol·L^{-1} LiCl, 200 mmol·L^{-1} NaCl or isotonic mannitol were weighed after incubation for 10 h at constant temperature (25 °C) in the dark. The relative increase in fresh weight (W_r) was calculated as $W_r = (W_f - W_i)/W_i \times 100\%$, where W_i is the initial weight of seeds, and W_f is the weight after 10 h [24,25].

2.3. Data analysis

Data were subjected to one-way analysis of variance (ANOVA) using Duncan's multiple range test ($P < 0.05$).

3. Results

3.1. The effect of salt stress on seed germination

3.1.1. Germination rate and germination energy

For two saltbush seeds, both germination rate and germination energy decreased progressively as the level of salinity increased (Figs. 1 and 2). Low NaCl concentration did not significantly affect the germination of *A. lentiformis* and *A. undulata*. However, at 200 mmol·L^{-1} NaCl, the germination rate and germination energy of *A. lentiformis* were about 76.7% and 82.2% respectively while that of *A. undulata* were 15% and 13.6% respectively. The trend of relative germination rate was coincident with that of germination rate and germination energy (Fig. 3). These results suggested that *A. lentiformis* had higher salt tolerance than *A. undulata.*

3.1.2. Germination index

Germination index can reflect the speed and uniformity of the germination. The germination index of *A. lentiformis* and *A. undulata* decreased progressively as the level of salinity increased and differed significantly under different NaCl concentrations (Fig. 4).

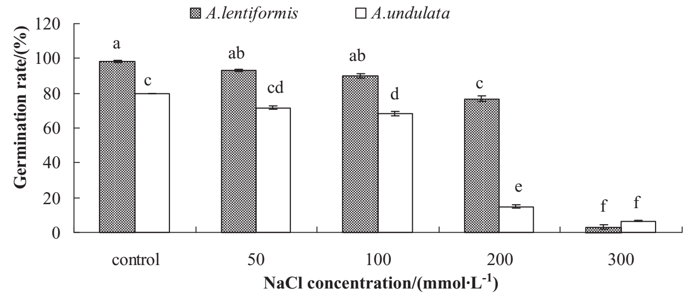

Fig. 1. The effect of different concentrations of NaCl on the germination rate of *A. lentiformis* and *A. undulata*. Data are means ± SD, and each treatment has three replicates. Different letters (a, b, c, d, e, f) represent significant difference at $P < 0.05$ according to Duncan's multiple range test. The same notes are for the following figures.

However, at 200 mmol·L^{-1} NaCl, the germination index of *A. lentiformis* was 48.5% of control while *A. undulata* was only 13.9%, which indicated *A. lentiformis* had higher salt tolerance than *A. undulata.*

3.1.3. Vigor Index

Vigor index represents the germination capacity and growing tendency of seedling. The vigor indexes of *A. lentiformis* and *A. undulata* were significantly reduced as salinity increased (Fig. 5). At 100 mmol·L^{-1} NaCl, the vigor index of *A. lentiformis* decreased about 43.4% of control in comparison to *A. undulata* decreasing 51.7%. At 200 mmol·L^{-1} NaCl, the vigor index of *A. lentiformis* decreased 61.7% of control while *A. undulata* decreased 90.4%. These results illustrated that *A. undulata* was more sensitive to NaCl than *A. lentiformis.*

3.1.4. Final germination rate

When the ungerminated seeds were transferred to distilled water after 10-day exposure to salinity, the differences in final germina-

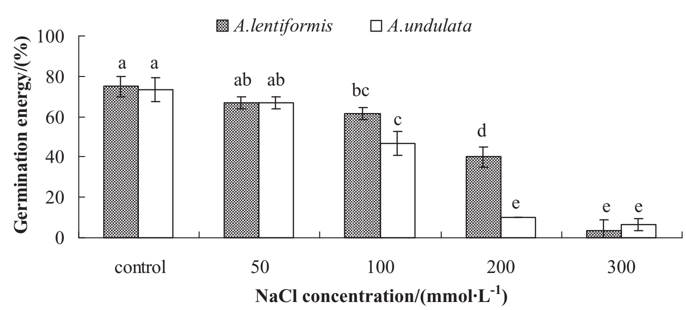

Fig. 2. The effect of different concentrations of NaCl on the germination energy of *A. lentiformis* and *A. undulata*.

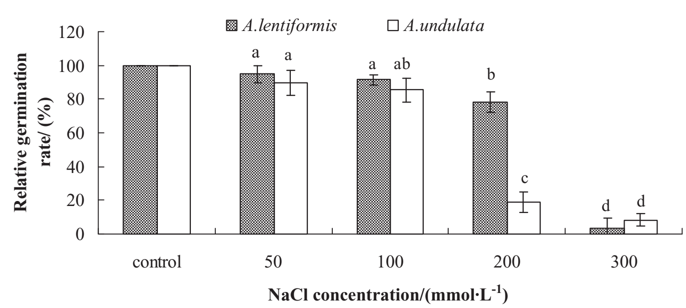

Fig. 3. The effect of different concentrations of NaCl on the relative germination rate of *A. lentiformis* and *A. undulata*.