# Research Note

# Seed germination of *Atriplex undulata* under saline and alkaline conditions

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## Summary

In this study the germination response of seeds of *Atriplex undulata* (Chenopodiaceae), a native of Argentina, to saline and alkaline conditions was evaluated. This species has potential as a rehabilitation crop for saline soils and is used as salt land pasture. The study involved three aspects: (1) germination response to three temperature regimes (20, 25 and 30°C); (2) the effects of saline (NaCl) and alkaline ($Na_2CO_3$) stresses, osmotic potential (0, -1, -2 MPa) and the removal of the bracteole on germination; (3) the recovery of germination with and without the bracteole following exposure to a high level of alkalinity and salinity stress. The best germination temperature was 20°C. There were no significant differences in the response to salt and alkali treatments. Seeds failed to germinate at -2 MPa, while -1 MPa reduced germination, more so for the seeds with the bracteole removed. After a pre-treatment with salt or alkali stress, seeds exposed to alkali showed lower germination percentage if bracteoles were removed from the fruit.

## Experimental and discussion

*Atriplex undulata* (Moq.) D. Dietr. (wavy-leafed saltbush) is a perennial shrub from the Chenopodiaceae family, native to arid and semi-arid rangelands of central Argentina (100–500 mm annual rainfall). Seedlings of *A. undulata* are reported to tolerate extremely high salinities (2500-5000 mS m^{-1}) on alkaline duplex soils (Barson *et al*., 1995) and the species is therefore regarded as a promising crop for rehabilitation of bare saline environments (Bennett *et al*., 2009).

Germination is a critical stage in the life cycle of halophytes (Ungar, 1978), which is affected by many environmental factors, such as temperature, salt, light, water, oxygen concentration and alkalinity (Wang *et al*., 2013). Saline soils contain multiple types of soluble salts, and where a saline soil becomes alkaline as a result of hydrolysis of carbonates ($NaHCO_3$ or $Na_2CO_3$), it causes injury to plants not only through salt stress

but also through alkali stress (Tobe *et al.*, 2004). While neutral salts (NaCl and $Na_2SO_4$) in a soil generally produce osmotic stress and ion-induced injury (Munns, 2002), there is an additional effect of high pH leading to an alkali stress. Although salinity and alkalinity may have different effects on seed germination, this difference has received little attention (Wang *et al.*, 2013).

The structure of fruits or other dispersal units may play a significant role in the timing of germination. For example, winged perianths of *Salsola komarovii* Iljin contain abscisic acid which inhibits germination (Takeno and Yamaguchi, 1991). For some species salt accumulation (chloride ions) has been detected in bracteoles (Beadle, 1952), whilst in other species, dispersal units mechanically inhibit or delay germination (Wei *et al.*, 2008). Removal of bracteoles was found to improve seed germination of several *Atriplex* species (Beadle, 1952; Stevens *et al.*, 2006), although *A. prostrata* Boucher germination was not affected by their bracteoles (Ungar and Khan, 2001).

Dispersal and germination strategies of halophytes determine their establishment and survival in harsh environments. The capacity to preserve seed viability during exposure to high salinity or alkalinity, and to trigger germination once stress is removed, is one of the mechanisms that allow tolerant species to persist. It was hypothesised here that temperature, bracteoles, salinity and alkalinity affect seed germination of *A. undulata*. This study therefore analysed (1) seed germination response of *A. undulata* under three different temperature regimes; (2) the effects of saline and alkaline stresses on the germination of intact and scarified fruits; and (3) the recovery of germination following exposure to a high level of alkalinity and salinity stress of intact and scarified fruits.

Fruits (hereafter referred to as seeds), of *A. undulata* were collected from autochthonous populations in the coastal zone of Bahía Blanca, Argentina (38°52'23 S, 62°27'7 W). Seed collection was carried out during seed ripening in May 2011 and seeds were stored in paper bags at room temperature (18-22°C) until used (within a year after collection). To test the effects of temperature on germination, bracteoles were manually detached from the utricles and surface-sterilised in 5% sodium hypochloride followed by washing in distilled water. Treated seeds were incubated in six Petri dishes containing two layers of filter paper wetted with 10 mL distilled water with 20 seeds in each dish. Germination was carried out at constant 20, 25 and 30°C, under continuous darkness, in growth chambers. Seed germination was monitored daily for 17 days. The seeds were considered to have germinated after radicle emergence and discarded after counting. A chi-square test and Tukey's multiple comparisons among proportions (Zar, 1999) were performed to establish the optimal germination temperature to be used subsequently.

Temperature significantly affected final germination percentage of *A. undulata* ($\chi^2$ = 26.0476, $P < 0.01$; data not shown). According to Tukey's multiple comparisons among proportions, germination at 30°C was significantly lower (22.5%), but no significant difference was found between 20 and 25°C (77.5 and 62.5%, respectively). Since seeds germinated faster at 20°C than at 25°C, this was considered the optimum germination temperature to be used in further assays.

Early studies on the *Atriplex* genus in Australia state that seeds of different species could germinate in the range of 0-35°C, with an optimum temperature between 20 and 25°C (Beadle, 1952). Similarly, *A. obovata* Moq. was reported to germinate better at

20°C, although a room temperature of 23°C allowed good germination (Edgard and Springfield, 1977), and for *A. semibaccata* R.Br., the highest germination percentages occurred at 25°C (De Villiers *et al*., 1994). In the coastal zone of the Bahía Blanca Estuary, where seeds were collected, these temperatures are within the range of those observed during autumn, the season when seeds of *A. undulata* have been observed on adult plants. Rain in the study area is concentrated in autumn and spring, but seedlings do not appear in the field until late spring (Pratonlogo, personal observation), commonly after heavy rains in December, suggesting that some mechanism of dormancy may prevent germination soon after seed ripening. Similar behaviour was described for *A. prostrata* at Rittman salt marsh (Ohio, USA) (Ungar and Khan, 2001), where the presence of bracteoles may inhibit germination in late autumn, protecting seedlings from freezing temperatures in winter.

For the second experiment, seeds were divided into two groups: intact utricles and bracteoles removed. To test the effects of salinity and alkalinity, seeds were germinated in neutral salt (NaCl) and alkali ($Na_2CO_3$) solutions with equivalent osmotic potentials: $\Psi = 0$ (distilled water control), $\Psi = -1MPa$ and $\Psi = -2MPa$. The experiment was carried out in a full factorial design, with three factors: bracteoles (present or removed), salt type (neutral or alkali) and osmotic potential (0, -1 or -2 MPa). Sterilised seeds were germinated in Petri dishes, as described previously, with 10 ml of the corresponding test solution, in complete darkness, at the optimum germination temperature. To avoid changes in salinity throughout the germination period, Petri dishes were weighed on a precision balance at the beginning of the experiment and water level was adjusted at 2-day intervals with distilled water to compensate for losses due to evaporation (Vicente *et al*., 2007). For each treatment, 10 replicates of 20 seeds each were used. The germinated seeds were counted and removed every second day for 60 days. Differences in final germination percentage were tested by means of a three-way ANOVA and Tukey's multiple comparisons, after arcsine transformation of proportion data.

*A. undulata* seeds did not germinate at $\Psi = -2$ MPa and all treatments at this osmotic potential were eliminated from the analysis. Three-way ANOVA showed that salt type had no significant effect on germination percentage ($P > 0.05$ for salt type factor and all its interactions). Osmotic potential, bracteole presence/absence and their interaction significantly affected germination ($P < 0.01$ in all cases), with higher values obtained for distilled water and seeds without bracteoles (figure 1). Tukey's multiple comparisons revealed that an osmotic potential of -1 MPa significantly reduced germination percentage of seeds with and without bracteoles, but the negative effect was more pronounced for naked seeds (figure 1).

The observed decrease in germination percentage as osmotic potential decreased is regarded as the usual response of halophytes (Ungar, 1978), and was also observed for other *Atriplex* species (Ungar, 1996; Katembe *et al*., 1998). Stevens *et al*. (2006) reported that seeds of *A. undulata* without bracteoles, showed a 50% decrease in germination percentage at -0.7 MPa, compared with germination in distilled water. In our study, at -1 MPa, naked seeds showed a 61% reduction while germination of intact fruits decreased by 24%. Several studies have demonstrated that alkaline soils may have particularly harmful effects due to the high pH stress (Yang *et al*., 2009). In *A. undulata*, we did
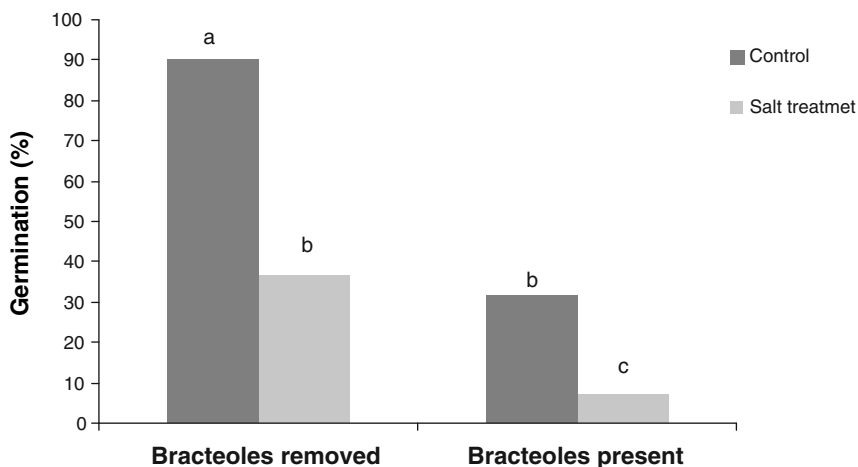
Figure 1. Final germination of *Atriplex undulata* seeds with bracteoles present and bracteoles removed, incubated using solutions with osmotic potentials of 0 MPa (control) and -1 MPa (salt treatment). Different letters indicate significant differences ($P < 0.01$, Tukey's multiple comparisons).

not find significant differences between $Na_2CO_3$ and NaCl treatments, suggesting that, at least at the germination stage, the species has some level of tolerance to alkaline soil conditions.

To determine whether salt type and bracteoles affect germination recovery after exposure to a very low osmotic potential, seeds with and without bracteoles were incubated in Petri dishes containing two layers of filter paper with 10 ml NaCl or $Na_2CO_3$ solutions with osmotic potential of -2 MPa. After a week, ungerminated seeds were removed, washed in distilled water several times and incubated again in Petri dishes with 10 ml distilled water. The recovery percentages (RP) were determined by the following formula:

$$RP = [(a-b)/(c-b)] * 100$$

where $a$ is the total number of seeds germinated after being transferred to distilled water, $b$ is the total number of seeds germinated in the saline solution and $c$ is the total number of seeds (Timson, 1965). In control treatments, seeds with intact bracteoles and with bracteoles removed were directly germinated in distilled water, with no previous incubation in salt solutions. Main factors for this full factorial experiment were salt pre-treatment (NaCl, $Na_2CO_3$ and control) and bracteoles (present or removed). For each treatment 10 replicates of 20 seeds each were used. Growth chamber setting and germinated seed counts were as described in the previous section. Differences in final germination percentage were tested by means of a two-way ANOVA and Tukey's multiple comparisons test, after arcsine transformation of proportion data.

As observed in the former experiment, no seeds germinated during incubation at $\Psi = -2$ MPa and the recovery percentages (RP) were equivalent to the final germination percentages obtained after incubation of ungerminated and control seeds in distilled water. The main effects of bracteoles and salt pre-treatment were highly significant as

was their interaction ($P < 0.01$ in all cases). Recovery percentages in treatments including seeds with their bracteoles attached were always lower than the naked seed treatments ($P < 0.01$, Tukey's multiple comparisons test; figure 2). For intact utricles, neither NaCl nor $Na_2CO_3$ pre-treatments had a significant effect, and the final germination percentages did not differ from those observed for seeds that had not been exposed to high salinity ($P > 0.05$, Tukey's multiple comparisons test). On the other hand, all treatments differed from each other where bracteoles had been removed. Thus naked seeds in the control treatment showed a higher germination percentage than those seeds previously exposed to NaCl, and the lowest recovery percentage was observed for seeds without bracteoles, exposed to $Na_2CO_3$ ($P < 0.01$, Tukey's multiple comparisons test).
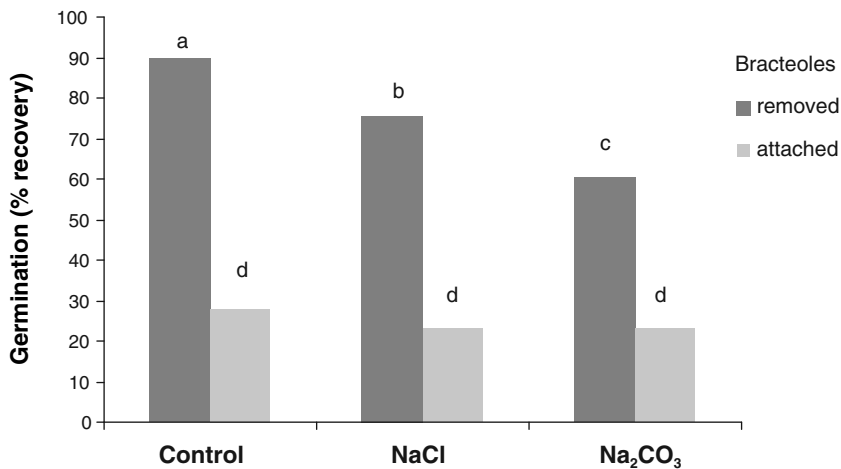


Figure 2. Recovery percentage of *Atriplex undulata* seeds with bracteoles present and bracteoles removed, incubated in distilled water after exposure to NaCl and $Na_2CO_3$ solutions with osmotic potential of -2 MPa. In control incubations, seeds had no pre-treatment. Different letters indicate significant differences in the recovery percentage ($P < 0.01$, Tukey's multiple comparisons).

Katembe (1998) tested germination recovery in *A. prostrata* and *A. patula* L. seeds that had been exposed to high salinity. After long periods of exposure to an extremely high salinity or osmotic stress (20 days in NaCl or PEG at -2 MPa) seeds remained viable, and germination was 90% two days after seeds were transferred to distilled water. For some halophyte species, the osmotic priming of seeds may even have a stimulatory effect (Ungar, 1978, 1991). In this study, salt treatments did not permanently inhibit seed germinability. However, recovery of seeds without bracteoles was significantly lower than control, for both types of salt. In the alkali treatment *A. undulata* seeds turned a blackish colour (Piovan, personal observation) and the lower recovery could indicate some permanent damage to the seed.

When bracteoles were attached, germination percentage was lower, but no significant differences were observed between exposure to high salinity, alkalinity or distilled water. Several mechanisms have been proposed to explain the inhibitory effect of bracteoles: induction of light requirements, mechanical inhibition, osmotic stress or specific ion

effects, negative effect of growth regulatory substances, or presence of allellopathic compounds (Ungar and Khan, 2001). For some species, salt accumulation (chloride ions) has been detected in bracteoles, which could have some ecological role in the protection of the fruit (Beadle, 1952). In this study, bracteole removal enhanced germination and promoted a shorter germination time. Bracteoles of different *Atriplex* species may serve to control the timing of seed germination and also assist the dispersal of seeds (Osmond *et al*., 1980). For other species of the genus, these effects have also been described (Ungar and Khan, 2001). In unpredictable salt desert environments, preventing all seeds from germinating simultaneously may provide an adaptive advantage, given that salt stress could unexpectedly increase during seedling development (Egan and Ungar, 1999).

Salt stress is a widespread environmental problem in the arid zones. Saline soils contain multiple types of soluble salt components and soil salinisation and alkalinisation frequently co-occur in nature. Different *Atriplex* species are widely grown as salt land pastures and fodder shrubs. Our results suggest that *A. undulata* has a good potential for saline-alkaline soils rehabilitation. Further work should be directed to the study of seed dormancy and germination recovery from salt and alkali stress, as well as understanding the ecological role of bracteoles on field emergence of this species.

## Acknowledgements

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