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Scientific Paper

Tolerance to salinity in seedlings of *Elymus scabrifolius* and *Thinopyron ponticum*

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Abstract

Elymus scabrifolius and *Thinopyron ponticum* are two winter native and exotic, respectively, forage plants, which stand allomorphic sites. These species, through adequate management strategies, can propitiate the restoration and forage increase in zones with salinity stress. The objective of this work was to determine the seedling germination capacity and growth, under controlled laboratory conditions with increasing saline concentrations. Two varieties of *E. scabrifolius* from La Pampa (Argentina) and three commercial varieties of *T. ponticum* were evaluated. Twenty five seeds of each population were distributed in rectangular plastic trays, and the treatments consisted in applying 20 mL of NaCl solution with osmotic potentials (ψ_o) of 0; -0,5; -0,8; -1; -1,5 MPa. Afterwards, they were incubated in germination chamber (20-30 ºC) fulfilling light and darkness cycles. During the study the germination capacity, length of the aerial part and biomass were determined with regards to a control (0 MPa); besides, the root length: aerial part ratio was calculated. The results showed that *T. ponticum* was more tolerant than *E. scabrifolius* under salinity stress conditions in the seedling germination and growth stage; the latter is not adequate for environments with high salinity (more than -0,8 MPa). Differences were found among varieties within each species under the same saline conditions. It is concluded that the varieties can be used as forage plants for the restoration of zones with different salinity degree.

Keywords: biomass, germination, forage plants

Introduction

The Argentinean territory comprises many ecoregions, with varied climate conditions that provide a very particular soil and ecophysiology characteristic for each site. According to FAO-UNESCO, Argentina is the third country with larger surface of halomorphism-affected soils in the world, after Russia and Australia (Lavado, 2008). This situation occurs in arid and semi-arid as well as humid and sub-humid regions, with different problems in each zone (Cisneros *et al*., 2008; Taleisnik and López-Launestein, 2011).

On the other hand, agricultural expansion has generated a territorial rearrangement of livestock production, with an increase of livestock in marginal regions that support a lower stocking rate (Rearte, 2007; Viglizzo *et al*., 2010; Demaría and Aguado Suárez, 2013; Estelrlich and Castaldo, 2014) and accentuate the fragility of these sites (Lavado, 2008).

In general, the soil salinization problem is bound to oscillations in the depth of the water table, with salt-loaded water, due to the climate variability (Casas, 2013). At the end of the rainy seasons, the desiccation of the top soil layers starts, which along with the temperature increase, originates the capillary rise of the water table enriching with salts the whole profile and mainly the topsoil, with which its osmotic potential decreases (Zamolinski, 2000; Casas, 2013).

Seedling planting in marginal environments is a key element to increase the productivity of these systems (Borrajo and Alonso, 2004; García *et al*.,2011; Bolaño *et al*., 2015). Under salinity stress conditions a decrease of water availability occurs for the seed in its germination stage as well as for the later growth of the plant, and also ions can cause toxic effects. Along with this, in general, perennial species have slow initial growth and little reserves in the seed, which makes the seedling establishment a crucial stage (Ruiz and Terenti, 2012).

A convenient and sustainable alternative is the use of forage plants adapted to salinity, which show good establishment and restore the unfavorable soil conditions through a slower and more gradual but less costly and more efficient process (Casas, 2014). Among the different forage species adapted to extreme conditions are *Thinopyron ponticum* (Podp.)

Bartworth et Dewey (Ferrari and Maddaloni, 2001; Bazzigalupi *et al*., 2008; Di Marco *et al*., 2013; Fernández Grecco, 2013) and *Elymus scabrifolius* (Döll) J. H. Hunz (Ferrari, 2001; Rúgolo de Agrasar *et al*., 2005). In both species there are commercial varieties and locally adapted native or naturalized populations; it is important to know the limitations of both species and their varieties, in order to consider them for marginal environments. The objective of this work was to evaluate germination under laboratory conditions and the initial growth of seedlings of two *E. scabrifolius* varieties and three *T. ponticum* varieties with different salinity concentrations.

Materials and Methods

E. scabrifolius seeds from two populations of the La Pampa province (Argentina) were used. A population of *E. scabrifolius* was selected at the Farming Research Station of Anguil, belonging to the National Institute of Farming Technology (INTA EEA Anguil), Don Alberto, and the other was from the School of Agronomy (FA) of the National University of La Pampa (UNLPam). The seeds were harvested and preserved in paper envelopes at ambient temperature (20-25 ºC) during ten months, moment at which the trials were started. For *T. ponticum*, commercial seeds were used: Pucará INTA, Hulk Gentos and Barpiro Barenbrug. Before performing the salinity essays, the germination capacity of each variety was determined at temperature of 20-30 ºC during 21 days (ISTA, 2013).

The experimental design was completely randomized, with factorial arrangement (5 x 5), five salinity levels x five populations or varieties, with four repetitions.

Twenty five seeds of each population were distributed in plastic rectangular trays of 5 x 10 cm and 5 cm of height, on filter paper (Muntkel 1700). The treatments consisted in applying 20 ml of NaCl solution, with osmotic potentials (ψ_o) of 0; -0,5; -0,8; -1; -1,5 Megapascals (MPa) which were obtained following the empirical relation of Van´t Hoff (Salisbury and Ross, 2010). For the control distilled water was used with an electrical conductivity of 0 $dS.m^{-1}$.

The trays were introduced in transparent polyethylene bags ((20 mµ of thickness), to reduce evaporation and maintain the concentration of the saline solution. Afterwards, they were incubated in germination chamber (Pasti Ingeniería, Pergamino, Argentina), with a daily cycle of 8 h of light at a temperature of 25 ± 3 ºC and 16 h of darkness at a temperature of 20 ± 3 ºC (ISTA, 2013). Each experimental unit was changed from one place to another every two days, to prevent difference because of site within the chamber. Follow-up took place every 72 h, during a 15-day period, to add distilled water or saline solution to those trays that required it. Thus the germination capacity and rate were also evaluated, being expressed in percentage with regards to the control. The seeds were considered germinated with the appearance of the radicle and the ones that did not germinate after that period were washed and put to germinate in substrate with distilled water to evaluate their regeneration capacity once the stress was over. After one week, those which had not germinated were subject to tetrazolium test and were observed in the stereoscope to determine their viability (Ruiz, 2009; ISTA, 2013).

Thus, a count was made and they were classified into: normally germinated those that germinated with a good morphological development of their parts; abnormally germinated the ones that showed some deformation (ISTA, 2013) and normal and abnormal recovered the ones that grew after being conditioned with distilled water.

From each seedling and experimental unit, five normal seedlings were randomly extracted, of which the radicle and aerial part length were measured; the results were expressed in length relative to the control (percentage) and the ratio of the root on the aerial part (R/AP; %) was calculated.

Afterwards, they were maintained in stove at 60 ºC until constant weight to obtain the absolute biomass per plant (mg) which was expressed in percentage with regards to the control, in order to evaluate the effect generated by salinity.

Variance analysis was performed according to a factorial salinity x variety (5 x 5), and when the interaction was significant, the means of the varieties within each salinity level were compared through the protected least significant difference (FLSD) ($p<0,05$) using the statistical pack InfoStat version 1.1 (Di Rienzo *et al*., 2016). Previously, the normality of variables was tested with the Shapiro-Wilks test and the homoscedasticity of the variances, with Levene's test. In the necessary cases the data were transformed according to the recommendation made by the bibliography (Zar, 1996).

Results

Germination capacity with regards to the control. Significant differences were found among varieties ($p<0,01$) and among treatments ($p<0,01$), and the interaction among factors was significant ($p<0,01$). For such reason, the varieties were independently analyzed for each of the salinity levels.

The effect of salinity was remarkable in both varieties of *E. scabrifolius*, which started to decrease abruptly their germination percentage from the potential -1 MPa; however, the *T. ponticum* varieties were not substantially affected by salinity in any of the treatments (fig. 1).

The Barpiro variety was more sensitive to high salinity (-1,5 MPa), being significantly different from the other two varieties within this species (fig. 1).

The seeds of *E. scabrifolius* that remained without germinating in the treatment of -1,5 MPa, once the stress was over, were recovered in a high percentage. The ones which were not recovered mostly preserved their viability.

Length of the aerial part of the seedlings with regards to the control. In general, at moderate as well as high salinity levels, *T. ponticum* showed higher length of the aerial biomass than *E. scabrifolius* ($p<0,05$).

The growth of the aerial part of *E. scabrifolius* was very low (10 % with regards to the control) at -1 MPa and there was no germination at -1,5 MPa. However, *T. ponticum* at moderate salinity levels (-0,5 and -0,8 MPa) showed a reduction of 20 % and at high salinity levels (-1 and -1,5 MPa), of 27 and 74 %, respectively, as average for the different varieties (fig. 2). At -1,5 MPa Pucará was the *T. ponticum* variety which showed higher growth of the aerial part, although without significant differences from Hulk.

Root length with regards to the control. At moderate salinity levels (-0,5 and -0,8 MPa) the variety Hulk was different from the others ($p<0,05$) and showed higher root length (fig. 2), which was practically not affected by salinity.

At high salinity levels (-1 and -1,5 MPa) the two species were different. *E. scabrifolius* showed lower root growth than *T. ponticum* ($p<0,05$) and, in turn, within *T. ponticum* Hulk had a higher root length, although it was not different from Barpiro at -1 MPa or from Pucará at -1,5 MPa (fig. 2).

Ratio of root length with regards to the aerial part (R/AP). The salinity x variety interaction was significant ($p<0,05$), and differences were found among salinity levels and varieties. At -0,5 MPa the R/AP was higher in *T. ponticum*, especially in Hulk and Pucará, while Barpiro behaved as the *E. scabrifolius* variety Don Alberto, with lower R/AP, and the selection FA was the one with the lowest R/AP ratio. In the seedlings germinated with distilled

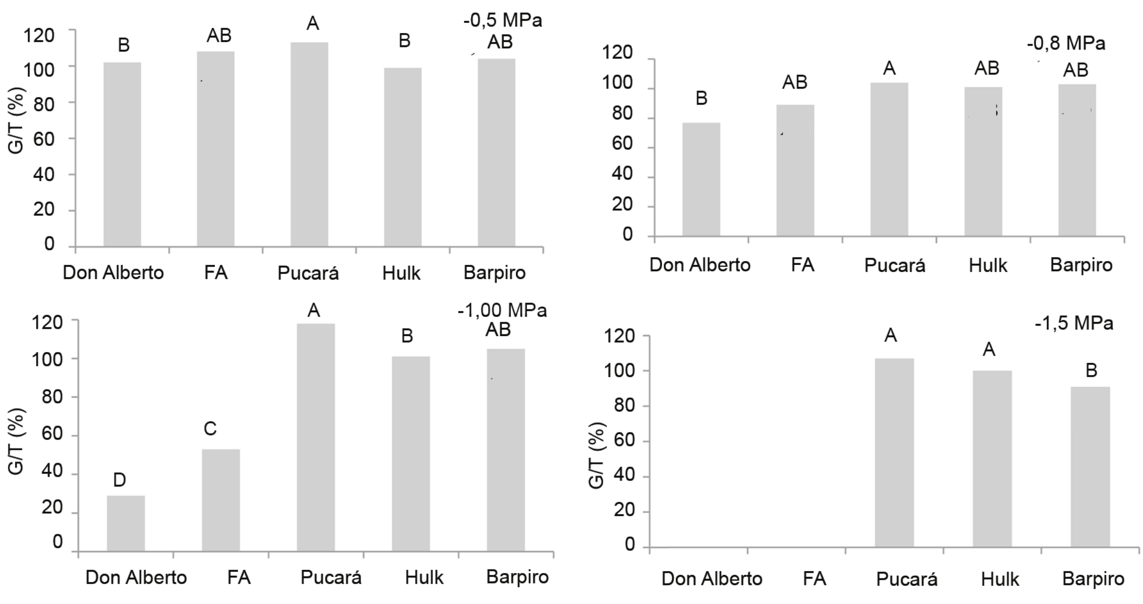

Figure 1. Germination capacity with regards to the control according to the different salt concentrations. Different letters indicate significant differences among varieties within each osmotic potential (FLSD, $p < 0,05$).

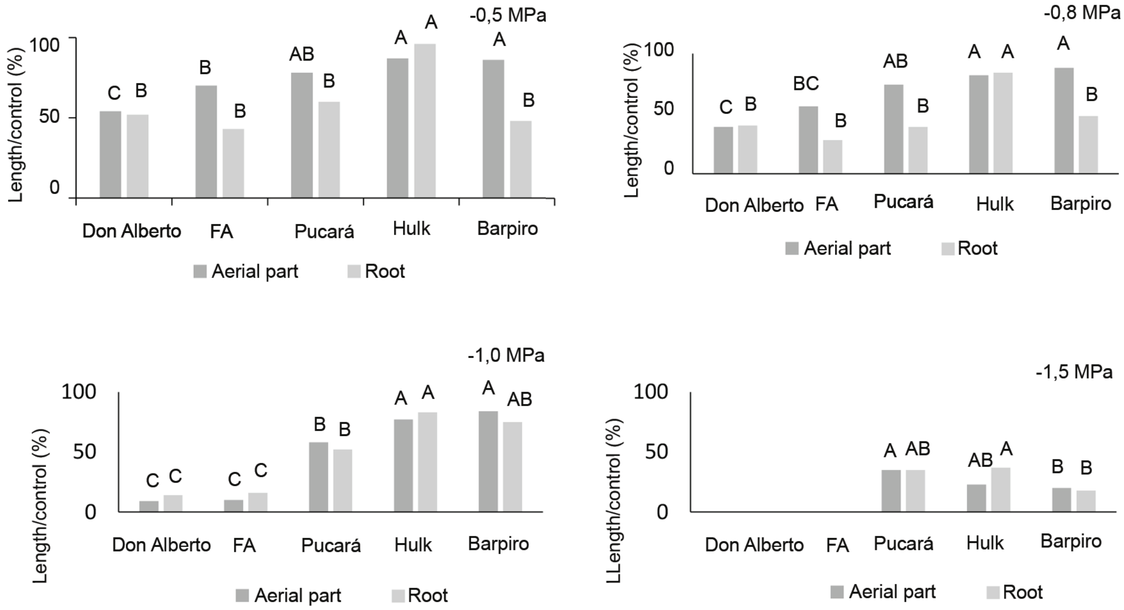

Figure 2. Length of the aerial part and root with regards to the control (%) according to the different salt concentrations. Different letters indicate significant differences among varieties (FLSD, p < 0,05).

water, the R/AP ratio was higher in the *T. ponticum* varieties (fig. 3).

In the presence of higher salinity, in general, there was a trend to increase the R/AP in the *E. scabrifolius* varieties, mainly due to the decrease of the aerial growth. At -1,5 MPa this variable could not be calculated because of the absence of aerial growth in *E. scabrifolius*; in the *T. ponticum* varieties R/AP was higher in Barpiro than in Hulk and Pucará as there was also higher reduction of the aerial growth in the first one.

Seedling biomass. The salinity x variety interaction was significant (p<0,01), just like the effect of salinity (p<0,01). Significant differences were also observed among varieties. Under the salinity condition of -0,5 and -0,8 MPa, the variety *E. scabrifolius* Don Alberto was more affected than the others (p<0,05), while *E. scabrifolius* FA was not significantly different from the *T. ponticum* varieties. Meanwhile *T. ponticum* with these concentrations practically did not show reduction of its biomass, especially the variety Hulk. Nevertheless, with high salinity (-1 MPa) the biomass of *E. scabrifolius* seedlings was much reduced, without significant differences between the varieties.

In the concentration of -1,5 MPa there was no growth of the aerial part in Don Alberto or in FA. Within the *T. ponticum* varieties Hulk and Pucará reduced their biomass in 30 % with regards to the control and Barpiro showed the highest sensitivity within the species and was significantly different (p<0,05) from the other two varieties (fig. 4).

Discussion

In general, forage perennial grasses show slow initial growth and little reserves in the seed, which makes the seedling establishment a crucial stage for crop growth. If the adverse conditions in the environment, such as lack of water or presence of salts in the soil profile, are added to this, the pasture establishment is even more difficult (Ruiz and Terenti, 2012). In essays conducted by García *et al.* (2011) a reduction of germination was found with the increase of salinity stress; caused by the physiological drought induced through the decrease of the osmotic potential. This provokes lower water availability, which generates water stress in the seed and delays its growth as consequence of the energetic cost for the plant; besides, NaCl can generate, in turn, an additional toxic effect.

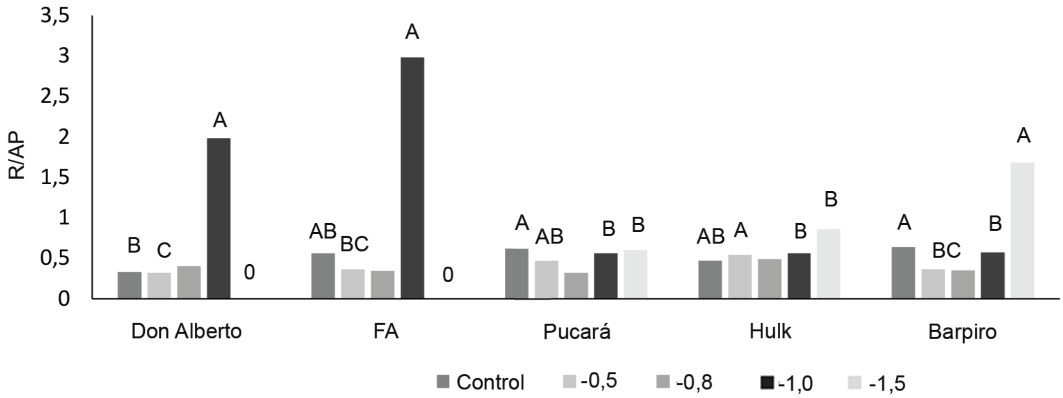

Figure 3. Root/aerial part (R/AP) ratio according to the different salt concentrations.
Different letters indicate significant differences (FLSD, p<0,05).

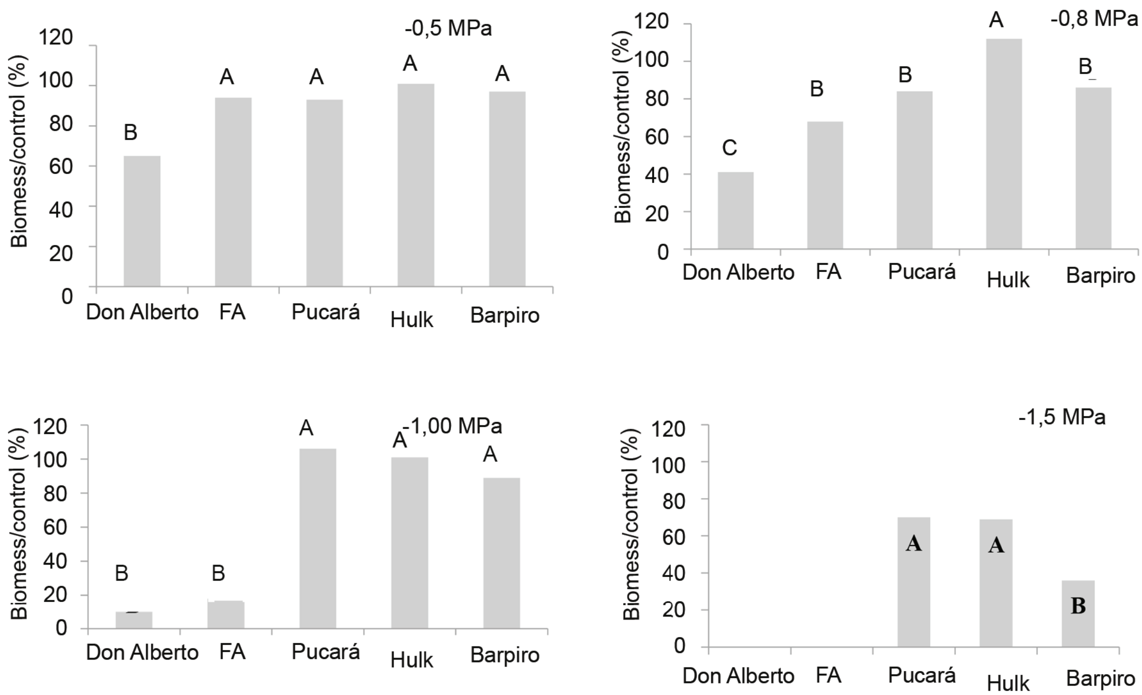

Figure 4. Biomass with regards to the control according to the different salt concentrations.
Different letters indicate significant differences among varieties within each osmotic potential (FLSD, p<0,05).

The results of this work showed that in the two *E. scabrifolius* varieties, germination was reduced as the salt concentration was increased to the point of being completely inhibited in the potential of -1,5 MPa (350 mM). In general, *T. ponticum* showed higher tolerance to salinity and a significant decrease of the germination percentage was shown only for the variety Barpiro with the highest salinity (-1,5 MPa). This explains the report by Borrajo and Alonso (2004) regarding the fact that plant growth is proportional to the salt concentration and varies between and within the species.

This variability in response to saline conditions, even within the same species, may include genetic and environmental components (linked to the site where the seeds were collected). In several trials with forage species the existence of inheritable genetic variability with regards to the salinity tolerance in populations of sites with contrasting conditions has been proven (Bazzigalupi *et al.*, 2008). The populations from saline sites were the most tolerant to salinity; however, those of non-saline sites showed variability for such trait, which means that it is possible that this condition is hidden in such populations due to a lack of natural selection that favors the adaptation and persistence under those environmental conditions (Bazzigalupi *et al.*, 2008; Abbott *et al.*, 2009). This behavior could be proven in this study, because the results of seedling biomass and length, under salinity treatments, showed significant differences among varieties belonging to the same species.

For the case of *T. ponticum* varieties, in the highest saline treatment (-1,5 MPa) a sudden decrease in the total seedling length and biomass was detected. Nevertheless, in *E. scabrifolius* the decrease was marked for a lower salinity level [-1,0 MPa (230 mM)].

These results are similar to the ones found by Bazzigalupi *et al.* (2008), who found that the only solution that allowed to discriminate among populations of *T. ponticum* was -1,0 MPA (18 $dS.m^{-1}$) and it was considered adequate to work with high selection pressure in this species. In this work, the *T. ponticum* varities with concentration of -1,0 MPa showed a low decrease of growth, which did not exceed 10 % in total length and was between 10 and 20 % in biomass. However, *E. scabrifolius* proved to be more sensitive. Yet, Zabala (2007) has stated that this latter species shows genetic adaptations to grow and develop in different saline environments, through tolerance mechanisms related to the differential accumulation of sodium in its leaf tissues. In turn, the three *T. ponticum* varieties have previously undergone genetic modifications and adaptations to marginal environmental conditions; in the case of Hulk, the establishment vigor increased (Gentos, 2011) and Pucará has been selected for its adaptation to saline-sodic sites (Andrés, 2014), showing good response to stress treatments.

Both species can provide, with time, the possibility of improving the soil structural conditions and generating more feedstuffs with good nutritional quality to achieve the development of sustainable livestock production activities in those sites (Harkes, 2011; Vecchio, 2014).

Conclusions

In general, the two species showed difference in the tolerance to saline conditions, in seedling germination as well as initial growth. Notoriously, *T. ponticum* turned out to be more resistant to salinity stress than *E. scabrifolius*. In turn, it was proven that the evaluated varieties in each species also responded differently when they were subject to equal saline treatments. With regards to the varieties there was a better response in *E. scabrifolius* FA and in *T. ponticum* Hulk and Pucará were superior.

Although this study was conducted under controlled conditions, allowed to deduce that the varieties of *T. ponticum* and *E. scabrifolius* can be used as forage plants to carry out management and restoration activities in areas with salinity stress conditions. Particularly, the two *E. scabrifolius* varieties should be used in less restrictive environments (lower than -0,8 MPa, 180 mM) than the *T. ponticum* ones.

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