Germination and Emergence of Populations of *Lotus tenuis* (Waldst.) Under Saline Conditions and in Different Soils.

O.R. Vignolio[1,2] and O.N. Fernández[1]

ABSTRACT

The effects of NaCl on the germination of five populations of *Lotus tenuis* (Waldst.) and their emergence in soils which supported halophytic and non-halophytic plant communities were studied. The germination of all populations decreased from over 90% to less than 20% as salinity increased from 0 to 200 mM NaCl, but populations differed in their germination recovery after being transferred to distilled water and there was a significant salinity x population interaction. Emergence of the populations did not differ between soils, averaging >70% for four of the five populations. The poor performance (<40% emergence) of the fifth population was ascribed to poor seed vigour after ageing during storage. The populations were classified for their responses to seed tolerance, and the possible adaptive value of these responses for the establishment and growth of *L. tenuis* in halophytic communities is discussed.

Additional index words: emergence, germination, *Lotus tenuis* populations, salinity tolerance.

INTRODUCTION

The Flooding Pampa (Buenos Aires Province, Argentina) occupies about 9,000,000 ha. More than 1,000,000 ha of this grassland has developed on halomorphic soils, where sodium is the predominant cation (Lavado, Rubio and Alconada, 1992; Lavado and Alconada, 1994). Plant communities on this type of soil are halophytic (and named "D"), and are dominated by *Distichis scoparia, D. spicata, Paspalum vaginatum* and *Chaetotropis elongata* (León, 1975).

Lotus tenuis is a perennial herb that propagates by seeds and is naturally established in "D" communities of the Flooding Pampa. The potential of *L. tenuis* for use in saline soils has been recognised by several authors (Ayers, 1948; Grant and Martens, 1985; Schachtman and Kelman, 1991; Rogers, Noble and Pederick, 1997). Farmers sow *L. tenuis* to improve the production and quality of the forage required for beef cattle production (Miñón and Colabelli, 1993; Sevilla, Fernández, Miñón and Montes, 1996). However, oversowing of *L. tenuis* has not been satisfactory in "D" communities (Miñón and Colabelli, 1993).

The deleterious effects of salinity on germination and plant growth are accounted for by a decrease in the osmotic potential, toxicity and nutrient ion deficiency. The response of seed germination to salinity varies among populations and cultivars of the same species (Ryan, Miyamoto and Stroehlein, 1975; West and Taylor, 1981; Rogers *et al.*, 1997; Khan and Ungar, 1998). Intraspecific differentiation in salinity tolerance in *L. tenuis* accessions makes this species a very valuable resource in revegetating saline soils (Ayers, 1948; Schachtman and Kelman, 1991). However *L. tenuis* seedlings often have low vigour and are very vulnerable to environmental hazards (Sevilla and Fernández, 1991; Sevilla *et al.*, 1996). Salt tolerance at the germinating stage and during seedling growth contributes to the understanding of the success of some forage species in saline soil, because these stages are critical for plant survival and growth (Allen, Dobrenz and Bartels, 1986; Rogers and Noble, 1991).

The objectives of this study were a) to describe the effects of salinity on the germination of five local populations of *L. tenuis*, and b) to compare the emergence, survival, growth and nodulation of the seedlings in soils from halophytic ("D") and non-halophytic ("B") plant communities.

MATERIALS AND METHODS

L. tenuis populations

Of the five *L. tenuis* populations, three were harvested in the Flooding Pampa: Ayacucho (Ay), on a "D" community from Ayacucho county at San Ignacio; Napaleofú (N), near route 29, close to the Napaleofú brook in Balcarce county, and Reserve 8 (R8), from a "D" community in a grassland of the EEA-INTA, in Balcarce county. The other two were Tresur Chaja (TS), a commercial population from Balcarce and Late Flowering (LF), an accession from Europe. This last one was selected for tolerance to sodic-saline soil conditions (González Garcia, 1995) and collected during the summer of 1996 after two cycles of recurrent selection. Seeds of population R8 had been collected in 1991, while seeds of populations Ay, N and TC were collected in 1997. All were stored at 4°C until the experiment was started in June 1997.

[1] *Cátedra de Ecología. Unidad Integrada Balcarce. EEA Balcarce, Instituto Nacional de Tecnología Agropecuaria-Facultad de Ciencias Agrarias de la Universidad Nacional de Mar del Plata. C.C. 276 (7620) Balcarce, Buenos Aires, Argentina.*

[2] *Present address: Crta. de Cabrils s/n, Dept. de Tecnologia Horticola, 08348 Cabrils, Barcelona, Spain.* *Accepted for publication 21 September 1999.*

Germination in saline solutions

Germination studies were carried out in 9 cm diameter plastic petri dishes. Two Whatman N97 filter paper disks were moistened with 4 ml of water saline solutions (0, 30, 90, 120, 150, 230 and 320 mM NaCl). Salt concentrations were calculated by means of a previous assay, in order to include the maximum electrical conductivity of the upper layer of a "D" community (25 μmhos cm^{-1}) reported by other authors (Berasategui and Barberis, 1982). The electrical conductivity was determined with an Orion Research meter(model 101). Seeds were scarified with sand paper and evenly distributed on the top filter paper. The petri dishes were sealed with transparent plastic film to prevent moisture loss. The experimental design was a randomised complete block with three replicates, each block being one sample of 50 seeds for each combination of population (5) and salt concentration (7). Petri dishes were placed in a growth chamber with an 8h/16h photoperiod (29μES^{-1} m^{-2}) and alternating temperatures 30°C/20°C , respectively (ISTA, 1996).

Counts were recorded at 2 day intervals for 10 consecutive days. A seed was considered germinated when the radicle was at least 2 mm long. A seed was considered abnormally germinated when the radicle did not develop and presented a callus. Germinated seeds were removed from the dishes. On day 10 any ungerminated seeds were transferred for four days to petri dishes with two filter papers moistened with distilled water to germinate. Those that did not germinate were analysed through a viability test with tetrazolium chloride at 0.5% (ISTA, 1996).

Emergence in different soils

The same five populations were sown in soils from "D" and "B" plant communities. Emergence and growth of *L. tenuis* is very satisfactory in soils of "B" communities. Community "B" is integrated with among other species, *Paspalum dilatatum, Sporobolus indicus, Sipa* spp, *Cyperus* spp. and *Stenotaphrum secunadatum.* Details on the soils' chemical characteristics are given in Table 1.

The soil from community B was taken from the 20 cm upper layer in Reserve 7 (EEA INTA, in Balcarce county). The soil from community "D" was taken from the 2 cm upper layer in the Ayacucho grassland, where the Ay population was harvested. Both soils were air dried, homogenised and sieved to remove roots and other organic materials before filling plastic trays (14 cm long x 9.5 cm wide x 5 cm deep, and without drainage), with 200 g of soil per tray. Seeds were scarified with sand paper and inoculated with the selected strain 733 of *Rhizobium loti.* Fifty seeds of each population were sown in each tray. The seeds were sown at a regular distance and 3 mm deep in 5 rows of 10 seeds each. A 5 x 2 factorial experiment with 3 replicates (trays) was conducted over two weeks in order to observe seedling emergence of *L. tenuis.* To determine the presence of *L. tenuis* in the seed bank, unsown samples of both soils were also incubated under the same glasshouse conditions. The trays were arranged at

Table 1. Chemical properties of the soils of plant communities B and D.

Properties	Community "D"	Community "B"
P (ppm, Bray 1 method)	17.4	13.2
pH (1:2.5 soil/H_2O	9.1	7.4
Organic C (%, Walkley-Black)	1.6	4.0
Organic matter (%. Walkley-Black)	2.7	5.2
Electrical conductivity (mmhos cm^{-1})	2.5	5.2
Exchangeable cations (meq 100 g^{-1})		
Ca^{+}	11.3	12.0
Mg^{++}	4.5	8.0
Na^{+}	6.9	5.1
K^{+}	1.5	1.1
Cation exchange capacity (ammonium acetate method)	14.9	23.6
Exchangeable sodium percentage	46.7	21.9
Soluble cations (meq 1^{-1})		
Ca^{+}	1.5	11.4
Mg^{++}	1.6	10.0
Na^{+}	18.2	57.1
K^{+}	0.3	1.2
Sodium adsorption ratio	14.5	17.4

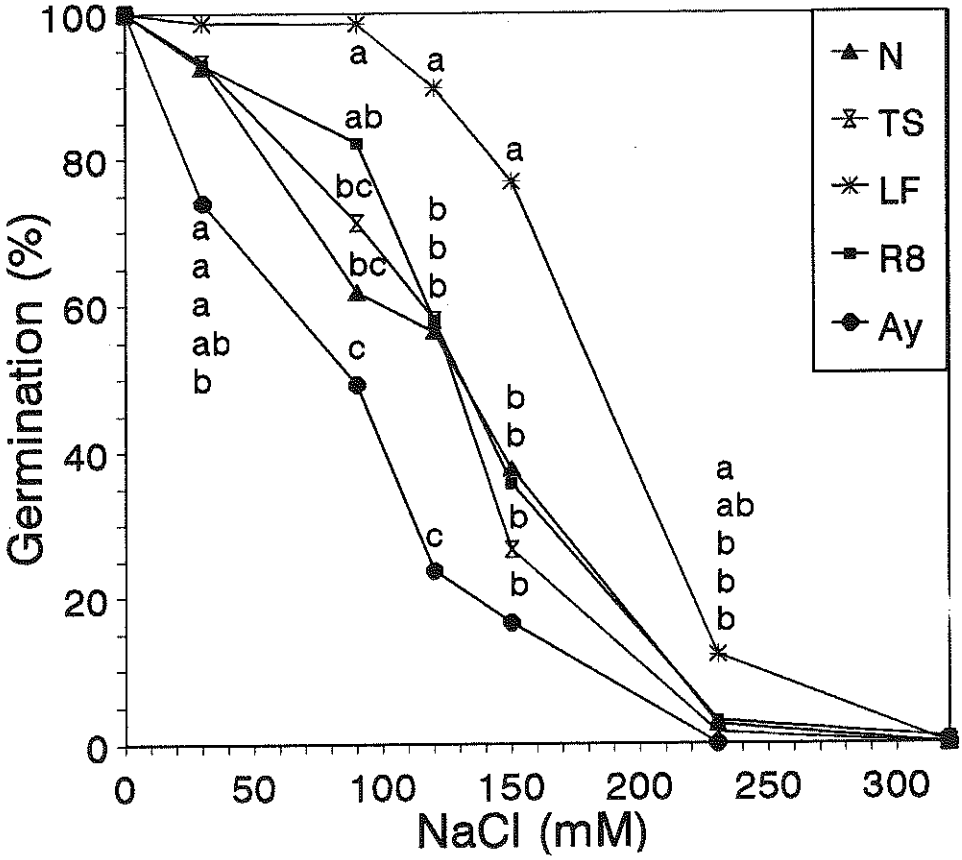

Figure 1. The effect of increasing NaCl concentration on the germination of *Lotus tenuis* populations: (N) Napaleofú; (TS) Tresur Chaja; (LF) Late Flowering; (R8) Reserve 8; and (Ay) Ayacucho. For comparison for the same salinity, percentage germination without a common letter represents significant differences among populations at P<0.05.

Table 2. Percentage of dead (D) and abnormally germinated seeds (A) of the populations of *Lotus tenuis* at different NaCl concentrations.

		NaCl (mM)					
Population	Category	30	90	120	150	230	320
Ay	D	8	9	12	6	1	0
	A	0	2	1	0	0	1
LF	D	1	0	1	1	19	2
	A	0	0	2	11	14	14
N	D	2	4	2	3	1	2
	A	1	6	2	5	7	7
TS	D	0	5	4	1	1	2
	A	2	6	11	11	14	14
R8	D	1	2	4	8	4	9
	A	5	2	5	13	38	25

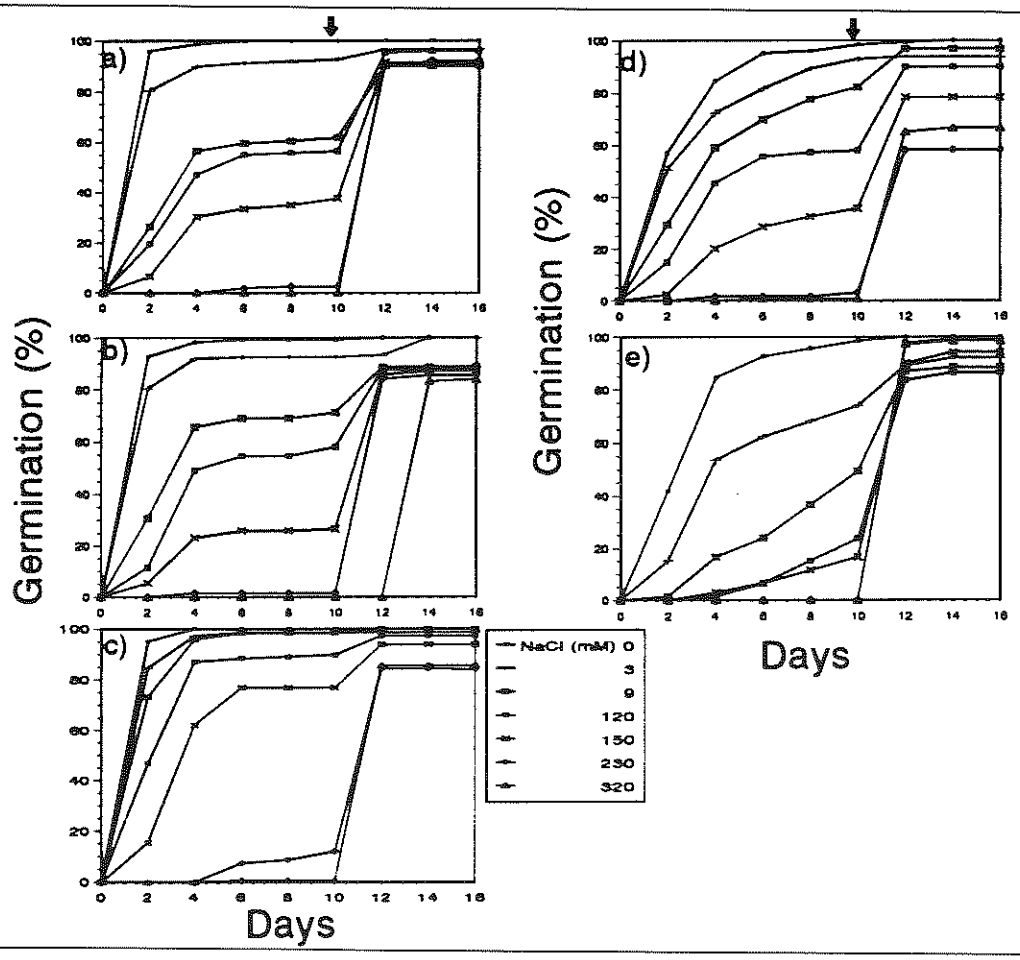

Figure 2. Germination responses to different NaCl concentrations of *Lotus tenuis* populations (a) Napaleofú: (b) Tresur Chaja: (c) Late Flowering: (d) Reserve *: (c) Ayacucho, over 10 days. The arrow indicates the date on which ungerminated seeds in NaCl saline solutions were transferred to distilled water.

random on benches and watered to field capacity with distilled water. Cumulated emergence at the end of the experimental period was expressed as percentage of viable seeds. During the experimental period the air temperature in the glasshouse was recorded (mean maximum temperature 21.9°C, SE = 3.6. and mean minimum temperature 11.9°C. SE = 2.4).

RESULTS

Germination in saline solutions and emergence

The germination of all the *L. tenuis* populations decreased with increased salinity (Fig. 1). Significant differences occurred among populations (P<0.0001), salinity (P<0.0001) and their interaction (P<0.001). Germination of the LF population showed little change up to 120 mM NaCl (Fig. 1) and in the range of 150 mM NaCl to 230 mM NaCl it was higher than for the other populations (Fig. 1). Even though at 230 mM NaCl the seeds of all the populations had imbibed, the germination was very low. However, when ungerminated seeds were transferred to distilled water, they exhibited high germination (Fig. 2). The germination of the Ay population was slow and showed a marked difference in relation to the other populations with the increased salinity (Fig. 2).

The percentage of dead seeds was lower (0.0% to 12.5%) than that of abnormally germinated seeds (0.0% to 24.8%). In all populations but Ay, abnormally germinated seeds increased with salinity, mainly between 150 mM NaCl and 320 mM NaCl (Table 2).

Emergence varied among populations (P<0.0001) but no significant differences between soils (P<0.764) and population x soil interaction (P<0.385) were detected (Fig. 3). The R8 population showed very low emergence in both soils.

DISCUSSION

The results from the germination experiment in saline solutions suggest an inhibitory effect of salt concentration on the germination of *L. tenuis* rather than ion toxicity, as seeds that did not germinate in saline solutions did so when transferred to a distilled water medium. This is in close agreement with previous work in *Medicago sativa* (Redman, 1974), *Lolium* spp. (Marcar, 1987) and *Polygonum aviculare* (Khan and Ungar, 1998).

The significant interaction of population x salt concentration on germination indicates intraspecific variation. This variation was most evident at intermediate salt concentrations (90 mM NaCl to 150 mM NaCl) where the selected population LF showed higher germination. That response and the capacity for germination and growth in community "D" soil are consistent with selection for tolerance to sodic-saline soil

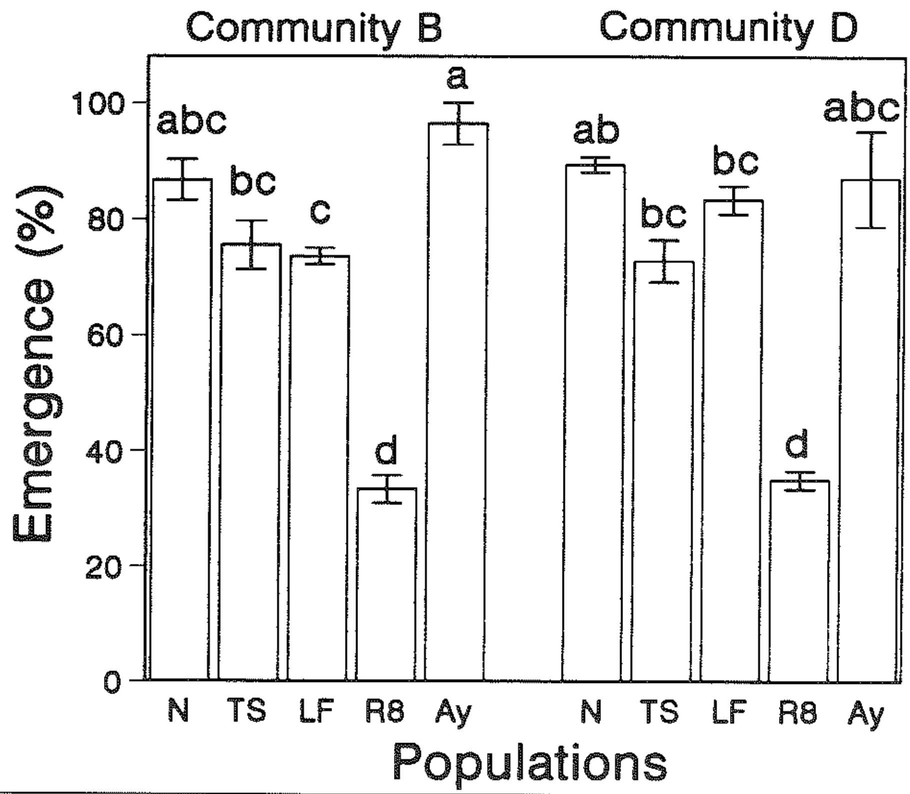

Figure 3. Emergence of *Lotus tenuis* in soils of communities B and D, over two weeks. Bars indicate standard errors of means and bars capped without a common letter represent significant differences among populations and soils at P<0.05. For population names see Figure 1.

conditions. This further emphasises the potential to breed salt tolerant *L. tenuis* cultivars (Ayers, 1948; Schachtman and Kelman, 1991; Rogers *et al.*, 1997). That potential has been also assessed for other legumes such as *Medicago sativa*, and significant progress for NaCl tolerance during seed germination in *M. sativa* by recurrent selection has been reported (Allen *et al.*, 1986).

All the populations, except Ay, germinated at high salinity levels, but the abnormal seedlings developed under these stressing conditions appear to have very low probability of surviving and growing in halophytic grasslands. The response of the Ay population resembles that described for halophyte species (Khan and Ungar, 1998) which "escape" from salinity stress by delaying germination as salt concentrations in the soil increase. Their seeds remain in the bank under induced dormancy and begin to germinate when salinity stress is reduced (Khan and Ungar, 1998). Woodell (1985, cited by Khan and Ungar, 1998) proposed a classification of seeds based on their responses to saline stress and their subsequent germination in distilled water. According to their classification, the Ay population could be placed into the type 2 category, because seeds were inhibited at high salt concentration, but the recovery was equal to the control when transferred to distilled water. The other populations, with a low recovery in distilled water, could be placed in the type 1 germination category.

In the grasslands of the Flooding Pampa, top-soil salinization varies, with maximum values in spring and summer (Lavado and Taboada, 1987). The persistence of *L. tenuis* in grassland is dependent on annual recruitments from the soil seed bank in autumn and spring (Sevilla *et al.*, 1996). Given the temporal coincidence of the germination with the fluctuations of soil salinity, a delayed germination induced by salt concentration, as observed in the Ay population, could be of significant adaptive value (Khan and Ungar, 1998).

The poor germination in saline solutions and emergence of the R8 population in the different soils could be attributed to low seed vigour, related to the seed ageing during a dry storage period of more than 6 years. This evidence is also in agreement with previous work by Allen *et al.* (1986) with 5 year old seeds of *Medicago sativa*. The germination of those seeds was slower and less vigorous both in distilled water and NaCl, resulting in a decreased ability to express potential tolerance in saline media. Many emerged seedlings of R8 had early falling cotyledons.

Although emergence was not affected by soil type, Miñón and Colabelli (1993) found that growth and the survival of *L. tenuis* seedlings in soil of community "D" was low. Salt stress decreases the growth of most plant species and in legumes such as *Trifolium repens* also decreases the number of root nodules and nitrogen fixation (Ikeda, 1994). Similarly, the higher soil pH in community "D" is another very important determinant of plant growth in saline - alkaline soils. This factor can affect the uptake of water, absorption of nutrients, nodule formation and root growth (Tang and Robson, 1993). The early association *L. tenuis* - *Rhizobium loti* and the development of root nodules promote growth in this legume (Quadrelli, Laich, Andreoli and Echeverria, 1997). Poor nodulation by *Rhizobium loti* in roots of *L. tenuis* plants growing in soils of community "D" was shown in another study (Vignolio unpubl. data) and is another aspect that may explain the low growth in that soil (Miñón and Colabelli, 1993).

The results of our studies show that seed germination of *L. tenuis* populations differed with salinity and that populations could be classified in different categories based on their salt tolerance and recovery. The emergence of seedlings was not affected by type of soil. In further studies, whether population selection for tolerance to salinity in NaCl solution is related to the capacity for growth and reproduction in soils of plant community "D" should be investigated.

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