

Responses of *Lotus glaber* to mycorrhizal infection in salinity environment

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Introduction

High salinity naturally occurring in soils or through irrigation water is a common environmental problem that affects around 7 % of crop areas in earth surface. Salinity factor imposes two principal kinds of stress on plants: an osmotic and a toxicity stress. In the first case there is a water limitation through osmotic potential reduction, affecting plant growth and productivity. In second case an excess of Cl⁻ and Na⁺ ions produce membrane integrity alterations. The most typical symptom of salinity injury in higher plants is a retarded growth due to an inhibition of cell wall extension and cell elongation by water limitation (Nieman, 1965; Staple and Toenniessen, 1984).

In the pampas lowlands, high salts contents in soils is the usual environmental condition and in spite of natural *Lotus glaber* drought and salinity resistance, these factors affect its persistence in grassland community and forage production.

The arbuscular mycorrhizal are funguses that usually live in saline soils. Though salinity affects growth and physiology of fungus too, several studies showed that mycorrhizal inoculation enhance growth plants in high salinity conditions, representing a biological improve of these soils (Al-Karaki, 2000).

The response of *Lotus glaber* to mycorrhizal infection is practically unknown. Therefore the purpose of this research was to analyze this response, searching adaptative mechanism to abiotic stress and incorporate it to a great potential forage crops as *Lotus glaber*, through the study of the mycorrhizal infection response of *Lotus glaber* growing in high salt contents substrates.

Materials and methods

Seeds of *Lotus glaber*, obtained from naturalized population (Saladillo, Buenos Aires Province), were disinfected with Ca hypochlorite, washed thoroughly and sown in 0,5 L pots filled with two different substrates. Substrate 1 (S1) was a mixed of cultured soil (Argiudol vertic soil, containing 50 ppm Phosphorus and pH 6.8), vermiculite and perlite (2:1:1).

Substrate 2 (S2) was cultured soil (Argiudol vertic soil, containing 50 ppm Phosphorus and pH 6.8) with a high conductivity (5.5 dsm). Both substrates were tinalized previously to sowing. Pots were placed under natural conditions in a greenhouse in La Plata, Argentine (34° S.L., 54° W.L.). The inoculum was incorporated at sowing time to half plants of each substrate: 1 g of inoculum composed by a mixed of soil, spores (50 spores/g inoculum), mycelia and root fragments of *Trifolium sp.* plants, colonized by *Glomus mosseae*. The remaining plants were not inoculated. Inoculated and not inoculated plants of each substrate were watered daily, to maintain water potential to field capacity values (-0.03 Mpa), along all trial. The experimental design was randomized blocks with 3 replicates. 5 plants of each treatment were collected 70 days after sowing and at 10 days intervals to determine morphological parameter such as plant height (H), leaf area (LA), roots volume (RV) and roots length (RL). Fresh and dry weight, oven dried at 60 °C until constant weight, were determined in leaves (LFW/LDW), stems (SFW/SDW) and roots (RFW/RDW). Colonization development by *Glomus mosseae* was determined in roots at 80, 100 and 120 days after sowing. It was evaluated according to the method developed by Trouvelot *et al.* (1986) and expressed as intensity of colonization (M%). The roots were cleared with 10% KOH and stained with trypan blue in lacto-phenol (Phillips and Hayman, 1970). Thirty randomly chosen root fragments of 1 cm long were mounted on slides and examined microscopically. M% was calculated as the proportion of infected roots over total root fragments. ANOVA was applied for all parameters studied, and Tukey test was used to separate the means with significant differences.

Results and discussion

Plants growth in S1 showed percents of mycorrhizal infection significant higher compared with plants growth in S2, reaching S1 plants more than 90 % (Table 1).

Table 1. Percent of roots infected after inoculation with *Glomus mosseae*, at 80, 100 and 120 days after sowing (DAS), in S1 and S2 plants.

| Treatments | 80 DAS | 100 DAS | 120 DAS |
|------------|---------|---------|---------|
| S1 | 81.73 a | 91.1 a | 91.06 a |
| S2 | 52.70 b | 62.73 b | 63.3 b |

Inoculated and non inoculated plants growing in S1 substrate showed a significant increase in all parameters at 80 DAS, compared with S2 plants (Table 2). S2 inoculated plants had non significant effect on H, SDW and RDW, and significant increment of RV, compared with S2 not inoculated plants. S1 inoculated growing plants had significant enhance of RFW, RV, SDW and RDW compared with S1 not inoculated plants.

Similar results were found in all treatments at 90 and 100 DAS. S2 plants had approximately 50% reduction in the inflorescences number reduction in both inoculated and not inoculated plants (data not showed) compared with S1.

Table 2. Height (H), leaf area (LA), roots volume (RV) and roots length (RL). Fresh and dry weight of leaves (LFW/LDW), shoots (SFW/SDW) and roots (RFW/RDW), of *Lotus glaber* plants growing in S1 and S2 substrates and not inoculated (NI) and inoculated (I) with *Glomus mosseae*.

| Treatment | S1-NI | S2-NI | S1-I | S2-I |
|-----------------------|-------|-------|-------|-------|
| H (cm) | 42.4a | 27.8b | 37.0a | 28.9b |
| LA (cm ²) | 167a | 100c | 127b | 91c |
| LFW (g) | 4.2a | 3.0b | 3.8a | 2.0c |
| SFW (g) | 3.9a | 1.7b | 3.9a | 1.4b |
| RFW (g) | 5.9a | 3.3b | 7.4a | 2.8c |
| RL (cm) | 9.2a | 8.7a | 7.5a | 4.2b |
| RV (cm ³) | 6.3b | 3.2c | 9.3a | 6.1b |
| LDW (g) | 0.9a | 0.5bc | 0.8b | 0.4c |
| SDW (g) | 0.8b | 0.4c | 1.0a | 0.4c |
| RDW (g) | 0.3b | 0.3b | 0.6a | 0.3b |

(*) values with different letters within the same row are significantly different (P>0.05)

Results of this research confirm *Lotus glaber* as a mycotrophic plant, but the mycorrhizal infection was dependent of the substratum, according to the higher proportion of S1 roots infected plants compared with S2 roots infected plants. There are evidences that *Lotus glaber* has a great variability in stress saline tolerance (Sannazzaro *et al.*, 2005; Clúa, unpublished data).

Different responses between substrates could be explained by differential radical systems growth of plants in S1 and S2, confirming the results obtained by Bressan and Vasconcellos (2002), in the sense that mycorrhization could modify roots architecture. The results of the present study had shown that roots volume was significantly modified by treatments, although roots length was neither affected by the substratum or the mycorrhization.

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