

Responses of mycorrhizal infection in the drought resistance and growth of *Lotus glaber*

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Introduction

The drought stress is considered the main factor that imposes limitations to yield crops. The effects of water deficiencies depend of several factors such as its intensity, duration, phenological phase of growth and genetic resistance capacity of plants (Panozzo and Eagles, 1999).

In the pampas lowlands, water limitation is usually an environmental restriction and in spite of natural *Lotus glaber* drought tolerance, this factor affects its persistence and forage production in grassland community.

The arbuscular mycorrhizal are fungus that live in roots of a great number of plant species, enhancing host drought resistance modifying soil-plants water relations and increasing water absorption capacity (Ruiz-Lozano et al. 1995; Augé et al 1987; Subramanian et al. 1995). Moreover it is known that plants with mycorrhizal roots infection recover faster compared with plants without infection, in water stress conditions (Al-Karaki 1998).

In severe drought stress conditions, several reports demonstrated that leaf numbers, chlorophyll contents, stomata conductance and rate of transpiration were greater in plants with mycorrhizal roots infection compared with plants without mycorrhizal infection (Beltrano *et al*, 2003).

The response of *Lotus glaber* to mycorrhizal infection is practically unknown. Therefore, the objective of this work was to study the mycorrhizal infection response of *Lotus glaber* in different water stress conditions with the purpose of incorporate these adaptative mechanism to reach great potential forage yield in *Lotus glaber*, in abiotic stress conditions.

Materials and methods

Seeds of *Lotus glaber* were disinfected with Ca hypochlorite, washed thoroughly and sown in 0,5 L pots filled with substrate composed of a mixture of cultured soil (Argiudol vértic soil, containing 50 ppm Phosphorus and pH 6,8)-vermiculite and perlite (2:1:1), tinalized previously. Pots were placed under natural conditions in a greenhouse at La Plata, Argentina (34° S.L., 54° W.L.). Inoculum (1 g) composed by a mixed of soil, spores (50 spores/g inoculum), mycelia and root fragments of *Trifolium sp.* plants, colonized by *Glomus mosseae* was incorporated to the substrate of half plants at sowing time. The rest of plants were not inoculated. Inoculated and not inoculated plants were subjected to the following treatments 45 days after sowing (DAS): a) Control (C) watered daily, to maintain solute potential to field capacity values (-0.03 Mpa) along all trial; b) Stress (S) plants subjected to water stress by withholding watering, reaching substrate solute potential approximately -1.3 Mpa, and maintain this value during all treatments; c) ABA plants were sprayed with 10^{-5} M ABA solutions. The experimental design was a randomized blocks with 3 replicates. Seventy days after sowing and at 10 days intervals, 5 plants of each treatment were collected to determine morphological parameter such as plant height (H), leaf area (LA), roots volume (RV) and roots length (RL). Fresh and dry weight, oven drying at 60 °C until constant weight, were determined in leaves (LFW/LDW), shoots (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 Trouvelot et al. (1986) method 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 ratio of infected roots over total root fragments. ANOVA was applied for all parameters studied, and Tukey test was used to separate means with significant differences.

Results and discussion

All treatments had significant higher and uniform percent of mycorrhizal infection. These results are in agreement with Bryla and Duniway (1997) and Morte *et al.* (2001) for mycorrhizal inoculated plants, since water stress did not affected the proportion of mycorrhizal infection (Table 1).

Table 1. Intensity of root colonization (M %) inoculated with *Glomus mosseae* evaluated at 80, 100 and 120 days after sowing (DAS), in control, stressed and ABA treated plants.

Treatments	80 DAS	100 DAS	120 DAS
C	81.73	91.1	91.06
S	76.5	90.53	85.4
ABA	82.1	91.2	86.6

Inoculated plants growing to field capacity had a significant increase in aerial parameters as SDW and a significant reduction in LA and LDW (Table 2). In roots parameters inoculated plants growing to field capacity had a significant increase in RV, RFW and RDW and a significant reduction in RL compared with not inoculated plants at 80 DAS (Table 3). Inoculated plants subjected to water stress treatments had a significant increment in aerial parameters as LA, LFW and SFW (Table 2) and in roots parameters as RV and RDW (Table 3) meanwhile a significant reduction in RL (Table 3) compared with not inoculated plants for same treatment. Inoculated plants subjected to ABA treatments showed an significant reduction in aerial parameters as LA, LFW and LDW (Table 2), and roots parameters as RV and RFW (Table 3) compared with not inoculated plants for same treatment.

Table 2. Height (H), leaf area (LA), fresh and dry weight of leaves (LFW/LDW) and shoots (SFW/SDW), in not inoculated (NI) and inoculated (I) *Lotus glaber* plants with *Glomus mosseae*, and treated with ABA or subjected to water stress (S), after 80 days after sowing.

Treatments	H (cm)	LA (cm ²)	LFW (g)	SFW (g)	LDW (g)	SDW (g)
NI-C	42.42a	167.42a	4.23a	3.90a	0.91a	0.84b
NI-S	29.08b	37.11d	0.84d	1.29c	0.62b	0.77b
NI-ABA	38.17a	170.85a	4.59a	3.96a	1.02a	1.10a
I-C	37.00a	127.03b	3.80a	3.93a	0.78b	1.01a
I-S	30.58b	64.06c	1.58c	1.90b	0.66b	0.66b
I-ABA	37.42a	124.61b	3.03ab	3.81a	0.75b	1.08a

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

Table 3. Roots volume (RV) and roots length (RL). Fresh and dry weight of roots (RFW/RDW), in not inoculated (NI) and inoculated (I) *Lotus glaber* plants with *Glomus mosseae*, and treated with ABA or subjected to water stress (S), after 80 days after sowing.

Treatments	RV (cm ³)	RL (cm)	RFW (g)	RDW (g)
NI-C	6.33b	9.17a	5.91b	0.30c
NI-S	5.67b	7.75b	4.94b	0.66b
NI-ABA	8.50a	6.17c	8.30a	0.87a
I-C	9.33a	7.50b	7.40a	0.96a
I-S	9.08a	4.17c	4.22b	0.99a
I-ABA	5.70b	4.75c	4.63b	0.88a

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

Similar results were obtained at 90 and 100 DAS, where there was a significant reduction in the number of inflorescences (approximately 50 %) comparing ABA-inoculated and ABA-not inoculated plants (data not shown).

These results showed *Lotus glaber* as a mycotrophic specie reaching a high mycorrhizal infection percent (90 % around), for all treatments. Moreover there are evidence that the mycorrhizal infection is supported for host photosynthates, in first steps, according to lower

values found in parameters analyzed, when inoculated vs. not inoculated plants at 70 DAS, were compared (Data not shown). This effect was reverted in the following determinations, particularly for plants subjected to water stress treatments. These results confirmed others research, respect to mycorrhizal infection is more favorable to hosts when water stress affected plants. Likewise ABA treatments plants did not show significant differences between inoculated and not inoculated plants, except for inflorescences number.

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