

Research article

Modulation of polyamine balance in *Lotus glaber* by salinity and arbuscular mycorrhiza

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Abstract

In this work we investigated the involvement of *Glomus intraradices* in the regulation of plant growth, polyamines and proline levels of two *Lotus glaber* genotypes differing in salt tolerance, after longterm exposure to saline stress.

The experiment consisted of a randomized block design with three factors: (1) mycorrhizal treatments (with or without AM fungus); (2) two salinity levels of 0 and 200 mM NaCl; and (3) *L. glaber* genotype. Experiments were performed using stem cuttings derived from *L. glaber* individuals representing a natural population from saline lowlands.

One of the most relevant results was the higher content of total free polyamines in mycorrhized plants compared to non-AM ones. Since polyamines have been proposed as candidates for the regulation of root development under saline situations, it is possible that AM plants (which contained higher polyamine levels and showed improved root growth) were better shaped to cope with salt stress.

Colonization by *G. intraradices* also increased (Spd + Spm)/Put ratio in *L. glaber* roots. Interestingly, such increment in salt stressed AM plants of the sensitive genotype, was even higher than that produced by salinization or AM symbiosis separately. On the other hand, salinity but not mycorrhizal colonization influenced proline levels in both *L. glaber* genotypes since high proline accumulation was observed in both genotypes under salt stress conditions.

Our results suggest that modulation of polyamine pools can be one of the mechanisms used by AM fungi to improve *L. glaber* adaptation to saline soils. Proline accumulation in response to salt stress is a good indicator of stress perception and our results suggest that it could be used as such among *L. glaber* genotypes differing in salt stress tolerance.

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1. Introduction

Lotus glaber Mill. (narrow-leaf trefoil; syn *L. tenuis* Waldst et Kit. Ex Wild.) is a glycophytic, perennial legume of European origin that occurs frequently in saline habitats. Previous observations indicate the occurrence of genotypes highly

tolerant to salinity in different populations of *L. glaber* [34], which is the best-adapted forage species used for cattle production in the Salado River basin (9×10^6 Ha). Mendoza and Pagani [33] showed this species has a high dependence of arbuscular mycorrhizal (AM) colonization in P-deficient soils. More recently, highly diverse AM fungal colonization patterns in roots of *L. glaber* were found in saline fields [43].

Several researchers have shown AM fungi can protect the plant against adverse effects of salinity. For instance, salt tolerance of banana plants was enhanced in the presence of *Glomus* isolates [52]. In cotton, improvement of plant growth,

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biomass accumulation and P-nutrition varied depending on both the AM fungal isolate and severity of salt stress.

Free polyamines are small organic cations that are absolutely required for eukaryotic cell growth. The three main polyamines found in plants are putrescine (Put), spermidine (Spd) and spermine (Spm). These compounds are thought to play an important role in plant responses to a wide array of environmental stresses such as low and high temperatures [6,21,40,49], salinity [3,25,44], high osmolarity [4], hypoxia [36], and oxidative stress [26,28]. For instance, exogenously added Spd and Spm protected rice plants from saline stress [8] whereas transgenic plants overexpressing enzymes intervening in Spd and Spm biosynthesis were more tolerant to saline and hyperosmotic stress [23,41,51]. In plants and bacteria, the diamine Put can be synthesized directly from ornithine via ornithine decarboxylase or indirectly following decarboxylation of arginine by arginine decarboxylase. In turn, Spd and Spm are synthesized from Put by successive additions of aminopropyl groups.

Information regarding polyamines in mycorrhizal fungi or in plant-fungal symbiotic interactions is limited to few records. Similar pathways for Put synthesis to those described in plants and bacteria have been found in ectomycorrhizae [16] and in an AM fungus [42]. Free polyamines have been suggested to play an important role in the initial stages of the infection of pea roots by *Glomus intraradices* (Schenck and Smith) [14]. In addition, mycorrhization may change the polyamine balance of plants. Kytöviita and Sarjala [27] showed that ectomycorrhizal symbiosis increased free Put levels in mycorrhizal roots of Scots pine seedlings.

On another hand, proline is one of the most common compatible osmolytes in plants [20]. It is believed to increase plant adaptation to drought and salinity [13,19] and it may also be related with survival of non-halophytes upon salt stress

[9,46,47]. This molecule serves as an osmolyte and as a protectant for enzymes and cellular structures [24].

Proline accumulation in mycorrhizal plants subjected to drought has been reported [2,18] and variable effects of mycorrhizal colonization on proline levels of plants under salt stress have been observed. Proline accumulation was greater in mycorrhized moong plants at 12.5 mM and 25 mM NaCl at 40 and 62 days after sowing [22], whereas non-AM faba bean plants accumulated much more proline than AM plants at various salinity ranges [39].

Lotus glaber is known to accumulate high levels of proline in response to salinity [29], but so far there is no information regarding the influence of AM fungal colonization on such accumulation.

The present work was aimed at evaluating the influence of the AM fungus *G. intraradices* on the polyamine and proline balances of two *L. glaber* genotypes differing in their tolerance to saline stress.

2. Materials and methods

2.1. Experimental design

The experiment consisted of a randomized block design with three factors: (1) mycorrhizal treatments (with or without AM fungus); (2) two salinity levels of 0 and 200 mM NaCl; and (3) *L. glaber* genotype. One salt shock of 200 mM NaCl showed to induced high titers of spermine and proline under long term salinization [29].

A total of 20 plants distributed in 5 pots (4 plants per pot) per treatment were used. Plants were harvested and processed at two different sampling times: just before salt addition (initial time, Ti) and 4 weeks after salt application (final time, Tf).

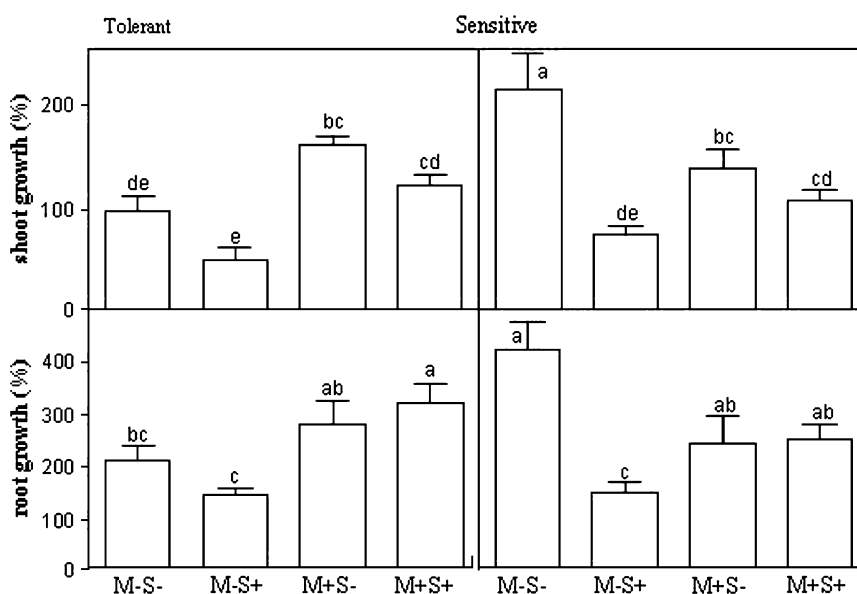


Fig. 1. Shoot and root growth (%) estimated as $(DW_f - DW_i)/DW_i \times 100$, where DW_f and DW_i represent dry weights at final and initial times, respectively. Two months old plants were watered with nutrient solution containing 0 or 200 mM NaCl during 4 weeks. M-: non-mycorrhizal, M+: mycorrhizal, S-: non-salinized plants, S+: salinized plants. Data are average of 5 replicates. Means ($\pm$ SD) with the same letter are not significantly different ($P < 0.05$).

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