Mycorrhizal dependency and growth response of *Gliricidia sepium* (Jacq.) Kunth ex Walp. under saline condition

Bhoopander Giri

Department of Botany, Swami Shraddhanand College, University of Delhi, Delhi 110036, India

**Abstract**

In pursuit of salinity-mycorrhiza interaction, a pot experiment was conducted to determine the dependence of *Gliricidia sepium* on arbuscular mycorrhizal association under salinity stress, which was imposed using different concentrations of sodium chloride solutions. The present study revealed that arbuscular mycorrhizal fungus; *Rhizophagus fasciculatus* significantly increased growth and biomass of *G. sepium* plants under saline condition. *G. sepium* showed a high degree of dependence on mycorrhizal symbiosis under saline as compared to non-saline condition. Under non-saline condition (SS0), *G. sepium* plants exhibited 23.9% dependence on *R. fasciculatus*, which increased with increase in the levels of salinity. At SS3 level, *G. sepium* plants showed 46.6% mycorrhizal dependency followed by SS2 and SS1 levels of salinity. However, there was no significant difference between mycorrhizal dependency of *G. sepium* at SS1 and SS2 levels of salinity. Improved growth of *G. sepium* under salinity stress revealed *R. fasciculatus* a promising inoculant for the reclamation of degraded saline soils.

**Keywords**

soil salinity; arbuscular mycorrhiza; nutrition uptake; biomass production; waste land reclamation

**Introduction**

Soil salinity has emerged as a significant threat to sustainable agriculture globally, as soluble salts under saline conditions adversely affect growth and development of plant (1). Salinization of soils has grown rapidly due to changing environmental conditions, which is covering about 800 million ha of the area (2). According to the Land and Plant Nutrition Management Services (FAO), more than 6% of the world’s land is affected due to excessive accumulation of soluble salts (3). Indeed, soil microbes like arbuscular mycorrhizal fungi (AMF) are of great ecological significance as they develop symbiotic associations with the roots of more than 90% of terrestrial plant species (4,5) and are considered promising candidates for sustainable agriculture (6). The extensive extraradical hyphae of AMF increase effective absorptive area of the roots and explore more soil volume (7); therefore, AMF enhance nutrient absorption and development of their partner plants. However, plants growing in saline soils could deprive of important nutrients...
like P as the phosphate ions get precipitated with the ions of other elements (8). Further, excessive salts in soil could induce osmotic, ionic, and oxidative stresses that severely affect plant growth. Several researchers have demonstrated that mycorrhizal fungi mitigate negative effects of salinity stress and promote plant growth (9-11).

Gliricidia sepium, a fast growing multipurpose leguminous plant, grow in many part of the world including South Asia, Central and Tropical Americas, West Indies, Mexico and West Africa and has been widely used in alley cropping systems, soil stabilization and as a green manure to improve soil fertility (12). Gliricidia sepium well adapts to the wide range of soil conditions viz., degraded wasteland, dry and infertile, acidic, calcareous, heavy clay and alkaline soils. Thus, determining growth response and mycorrhizal dependence of Gliricidia sepium under saline conditions could provide a practical solution to exploit mycorrhizal fungi for the reclamation of degraded salt-affected soils predominantly occurring throughout the world. Mycorrhizal dependency is a measurement of the degree of dependence of a plant on mycorrhizal association to produce its maximum growth or yield at the given level of soil fertility (13). To the best of our knowledge, there is no evidence available regarding dependence of Gliricidia sepium on AM symbiosis. Chasing salinity-mycorrhiza interaction and significance of AMF under saline condition, in this work, we report dependence and growth response of Gliricidia sepium on AMF, Rhizophagus fasciculatus (Thaxter.) C. Walker & A. Schüßler.

Materials and methods
Experimental design and biological materials
A completely randomized design experiment was conducted with four levels of salinity viz., (0, 50, 100, 200 mM NaCl) and two levels of mycorrhiza inoculation (un inoculated and inoculated with AMF). A total of eight treatments were carried out viz., Control (SS0), Mycorrhizal (SS0+M), 50 mM NaCl (SS1), Mycorrhiza with 50 mM NaCl (SS1+M), 100 mM NaCl (SS2), Mycorrhiza with 100 mM NaCl (SS2+M), 200 mM NaCl (SS3), and Mycorrhiza with 200 mM NaCl (SS3+M). The salinity was imposed by irrigating with 50 ml of NaCl solutions of respective concentrations once in a week. Plants were treated until the end of experiment. Control plants were irrigated with autoclaved distilled water. The soil-based inoculum of arbucular mycorrhizal fungus, Rhizophagus fasciculatus was produced in the open pot soil culture using two (Trigonella foenum-graecum and Sorgham helenpe) alternative trap plants (14). The roots of trap plants were chopped into small pieces and mixed with the rhizosphere soil to obtain a homogenous inoculum. Soil-based AMF inoculum containing about 100 fungal spore/10 g of soil along with chopped mycorrhiza-colonized roots (colonization 80%) was applied as a layer below each seedling at the time of transplantation. The control plants were inoculated with non-mycorrhizal soil inoculum.

Before sowing, seeds of Gliricidia sepium were soaked in water overnight then disinfected using 5% NaOCl solution for 15 min and germinated in the polythene bags filled with the mixture of sand and farmyard manure (ratio 1:1). After a week, five seedlings were transferred to each pot (20-cm height × 22-cm diameter) containing 2 kg of sandy loam soil (Table 1). Seedlings were later reduced to three per pot by manual thinning and allowed to grow under natural conditions. Soil extract was prepared according to Singh et al (15). Soil pH was determined with the help of pH meter and EC with electrical conductivity meter (Model LF 539, Germany). The determination of soil organic carbon was based on the Walkley and Black method (16), N on the Jackson’s method (17), P on the Olsen’s method (18) and K on an ammonium acetate method (19).

Table 1: Experimental soil physico-chemical properties

<table>
<thead>
<tr>
<th>Soil texture</th>
<th>Soil- pH</th>
<th>EC-S/m</th>
<th>Organi c- C (%)</th>
<th>N (%)</th>
<th>Available e-K</th>
<th>P - (mg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandy loam</td>
<td>7.6</td>
<td>0.12</td>
<td>1.1</td>
<td>0.58</td>
<td>65</td>
<td>49</td>
</tr>
</tbody>
</table>

Assessment of mycorrhizal root colonization
The assessment of roots for AMF colonization was made at the end of the experiment by random sampling of plants, which were separated in to root and shoot. The roots were clarified with KOH and stained in trypan blue (20). Small root segments were observed under the light microscope to determine AMF structures. The extent of the root colonization and frequency of AMF structures (arbuscules, vesicles and intraradical hyphae) was determined (21).

Analysis of plant growth, mycorrhizal dependency (MD) and phosphorus
Plant growth performance was determined 45 and 90 days after germination (DAG) of Gliricidia sepium seedlings. Root and shoot length was measured with the help of measuring scale and dry biomass was determined after drying the plant tissues in an oven at 75°C for 72 h or till a constant weight was recorded. Oven-dried root and shoot tissues (90 DAG) were ground and sieved through a 0.5 mm sieve. Ground material (0.2 g) of dried shoot samples was analyzed for the P concentration by digesting in an acid mixture of HNO3 and HClO4 (4:1, v/v). The resultant solution was diluted to a volume of 50 ml with de-ionized water. The concentration of P was determined...
according to the molybdenum blue method (22) using UV spectrophotometer (Shimadzu UV-1601, Japan). Mycorrhizal dependency is a measurement of the degree to which a plant species is dependent on mycorrhizal condition to produce the highest growth at the given level of soil fertility (13), which was determined as per the formula of Plenchette et al (23).

**Statistical analysis**

Treatment effects were determined by one-way analysis of variance (ANOVA). The differences between treatments were confirmed by Duncan’s Multiple Range Test (DMRT) (Cohort Berkeley, California). A significance level of 95% was applied.

**Results**

**Estimation of Rhizophagus fasciculatus colonization in Gliricidia sepium roots**

The present study showed that *Rhizophagus fasciculatus* successfully colonized *Gliricidia sepium* roots under saline condition (Fig 1a). Mycorrhizal roots showed presence of characteristic AM structure i.e. the inter-and intracellular hyphae, arbuscules, terminal and intercalary vesicles. Due to toxic effects of soluble salts, the degree of mycorrhizal colonization of *Gliricidia sepium* roots decreased as the levels of salinity increased. A maximum of 82% root colonization was recorded in case of mycorrhiza-inoculated plants, which decreased with increase in the levels of salinity. At SS1+M level of salinity, the mycorrhizal colonization of *Gliricidia sepium* roots decreased markedly. However, no traces of AMF were observed in case of nonmycorrhizal plants at any level of salinity. The mycorrhizal colonization of *Gliricidia sepium* roots decreased up to 50% at SS3+M (200 mM NaCl) and 43% at SS2+M (100 mM NaCl) as compared to the mycorrhizal colonization of control plants (0 mM NaCl condition).

**Growth response**

High concentration of NaCl decreased growth of *Gliricidia sepium* seedlings and showed a negative correlation between plant growth and increasing levels of salinity. Indeed, *Rhizophagus fasciculatus* exhibited profound effect as its inoculation significantly (P <0.05) increased root and shoot length and dry biomass under saline condition (Fig 2). The maximum increase in root and shoot length was recorded in case of AMF inoculation under non saline condition, which decreased as the levels of salinity increased in both mycorrhizal and nonmycorrhizal plants. Root and shoot length and biomass accumulation drastically decreased at SS3 (200 mM NaCl) level of salinity in the case of nonmycorrhizal than mycorrhizal plants (Fig 2). Root and shoot length decreased about 40% and 42%, and root and shoot biomass reduced up to 53% and 43% respectively in uninoculated as compared to AMF-inoculated plants at SS3 level of salinity. Although highest inhibitory effect of salinity was recorded at SS3 level followed by SS2 level of salinity, salt-induced inhibition and its intensity significantly (P<0.05) reduced on mycorrhizal inoculation at each level of salinity. It was noteworthy that in mycorrhizal plants, root biomass accumulation at SS1 level of salinity (100 mM NaCl) supersedes even those which grow at SS0 level of salinity (non-saline).

**Nutrient acquisition**

In present study, the effect of *R. fasciculatus* was determined on P in both root and shoot tissues of *Gliricidia sepium* seedlings (Fig 3). The accumulation of P was greater in root than shoot tissues. Though excessive accumulation of soluble salts lessens availability of P, mycorrhizal inoculation of *Gliricidia sepium* significantly (P<0.05) improved nutrient acquisition capacity of its partner plant as evidenced by the increased accumulation of P in both root and shoot tissues (Fig 3). At lower concentration, NaCl did not considerably affect uptake of P; however, as the concentration of NaCl increased in the soil, the accumulation of P decreased in both mycorrhizal and nonmycorrhizal plants. Instead, mycorrhizal plants had significantly higher concentrations of P than nonmycorrhizal plants.

**Relative mycorrhizal dependency**

The relative mycorrhizal dependency of *Gliricidia sepium* was determined by expressing the dry weights of mycorrhizal plants as a percentage of the dry weights of non-mycorrhizal plants (Fig 1b). The relative dependence of *Gliricidia sepium* on mycorrhizal fungus, *R. fasciculatus* changed with increasing levels of soil salinity. *Gliricidia sepium* plants showed significantly higher mycorrhizal dependency at SS3 level followed by the SS2 and SS1 levels of salinity. Inversely, mycorrhizal dependence of *Gliricidia sepium* plants on *R. fasciculatus* decreased under non-saline condition. However, there was no significant difference between mycorrhizal dependence of *Gliricidia sepium* between SS1 and SS2 levels of salinity.

**Discussion**

Salinization of soil is one of the major factors that restrict plant growth and makes them remain unproductive (24,25). Though salinity exhibited inhibitory effects, inoculation of *Gliricidia sepium* seedlings with *R. fasciculatus* noticeably reduced injurious effects of salinity stress. In this study, we observed that the high concentration of NaCl was detrimental to both mycorrhiza and its host plant, affecting both fungal attributes and plant growth. Salinity had a negative impact on the mycorrhizal colonization of *Gliricidia sepium*, which is evidenced by the decreased colonization of Gliricidia sepium.
Gliricidia sepium roots at increasing levels of salinity (Fig 1a). Our results corroborate previous findings that excessive soluble salts in the soil could restrict fungal hyphae growth and thereby reduce the extent of spreading mycorrhizal colonization (26). Improved plant growth on inoculation with R. fasciculatus indeed substantiate the fact that salinity merely impacts the effectiveness of mycorrhizal fungi, and the beneficial effects of mycorrhizal symbiosis on plant growth are likely to be independent of percent root colonization (27,28).

Salinity-driven reduction in plant growth has been reported in several plant species such as Acacia auriculiformis (29), Sesbania aegyptiaca and Sesbania grandiflora (30), Acacia nilotica (31), Lotus glaber (32), Trifolium alexandrinum (33); Linum usitatissimum (34), Trigonella foenum-graecum (28) and Artemisia annua (10); however in all these cases mycorhiza-inoculated plants performed better than uninoculated plants. The beneficial effect of mycorrhizal colonization on plant growth under saline condition has been documented as an impact of improved uptake of soil nutrients by mycorrhizal plants. Nonetheless, improved nutrient, particularly P and water status has often thought to be the major factors contributing to the increased growth of plants under salt stressed condition (9,35). Increased uptake of P on inoculation of Gliricidia sepium seedlings with mycorrhizal fungus, R. fasciculatus is in agreement that the ability of mycorrhizal plant to absorb nutrients from the stressed soils is often greater than that of the non-mycorrhizal or control plants (Fig 3). In the present study, plant growth decreased with increase in the levels of salinity in both nonmycorrhizal and mycorrhizal plants, however the inoculation with R. fasciculatus attributed to significantly higher plant growth as compared to nonmycorrhizal plants at the equivalent levels of salinity. Increased plant growth in mycorrhizal plants suggested that mycorrhizal inoculant indeed supports its partner plant to combat environmental stresses like salinity and had restoration effect on salinity-induced damages to plants.

Janos (36) suggested mycorrhizal dependency as a measure of the benefit provided by mycorrhizal fungi that could depend on relative contribution of root and mycorrhiza-mediated nutrient supply to the plant. Examination of plant species in the natural ecosystems often shows a difference between intensity and consistency in the establishment of mycorrhizal colonization. Some plant species consistently have a high level of mycorrhizal colonization, some show moderate or intermediate, whilst a few species exhibit even no mycorrhizal formation in their roots (36,37). Present study suggested that Gliricidia sepium was less dependent on AM symbiosis under non-saline condition; however its dependence on mycorrhizal fungi increased as the levels of salinity increased (Fig 1b), which is in agreement with the results of Kumar et al (38) who revealed that mycorrhizal dependency of Jatropha increased 20.84% as the level of salinity increased from 0-0.4% and also with our previous findings that under degraded wasteland condition, mycorrhizal fungi increase uptake of mineral nutrients in Cassia siamea revealing that C. siamea has a high degree of dependence on mycorrhizal fungi for adequate supply of nutrients especially P, to accomplish optimal growth (39,40). In the present study, lower dependence of Gliricidia sepium plants under low or no saline condition may be owing to better availability and acquisition of soil nutrients. As the concentration of soluble salts in soil increased due to supply of NaCl solutions, the major nutrient like P becomes unavailable to the plant as the phosphate ions get precipitate with other ions (29,41). Therefore, under saline condition, to avail adequate amount of nutrients for achieving favorable growth, plants could seek assistance from its symbiotic partner to acquire nutrient from the soil through mycorrhizal hyphae (42). Mycorrhiza dependency has been found altered by a wide range of soil factors (42), stages of plant growth (30,40) and plant and fungal species (23,43). Giri et al. (44) demonstrated that under stress condition, mycorrhizal fungi first compete with the adverse effects of stress for their establishment and then they promote plant growth. Increased demand of mineral nutrients by plants is complemented by increase in mycorrhizal colonization of roots that gives rise to an increase in the mycorrhizal dependency at later stage of plant growth. Mycorrhizal dependency could decrease with increase in the level of available soil P, because under such situation plant uptakes enough phosphate from the soil at its own (42). At extremely high soil P, the growth of nonmycorrhizal and mycorrhizal plants become either similar or growth of nonmycorrhizal plants exceeds over the mycorrhizal ones revealing that beyond a certain limit of available soil nutrient particularly P, a parasitic relationship could exist between mycorrhizal fungi and their host plants (45). Present study corroborates previous findings revealing that the availability of certain nutrients could alter mycorrhizal dependency of plant species and also supports the fact that mycorrhizal dependency differs with the soil conditions (46,47). Present study demonstrates that salinity stress not only disturbs water potential but also makes the soil nutrient unavailable to plant; indeed mycorrhizal fungi attain rapid plant growth under saline condition; therefore, it may be of ecological importance inoculating seedlings with arbuscular mycorrhizal fungi under salt stress.

It is pertinent to conclude that Gliricidia sepium plants showed less mycorrhizal dependency under non-saline condition; however its dependence on AMF increased with increase in the levels of salinity. Salinity largely impacted plant growth and rendered plant stunted. However, improved growth of Gliricidia sepium
evidenced that deleterious effects of salinity can be impaired with *Rhizophagus fasciculatus* inoculation. This study substantiated the fact that AMF could be the potential candidate for efficient utilization of degraded wastelands and saline-and sodic soils largely occurring throughout the world.

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**Conflict of interest:** BG has no conflict of interest.
Fig. 3: Effect of salinity and Rhizophagus fasciculatus inoculation on P in the root and shoot tissues of mycorrhizal and nonmycorrhizal plants of Gliricidia sepium 90 DAG. Values are means of five replicates and different letters at the top of bars indicate significant differences at P< 0.05, ± SD. DAG-days after germination. Control (SS0), Mycorrhizal (SS0+M), 50 mM NaCl (SS1), Mycorrhiza with 50 mM NaCl (SS1+M), 100 mM NaCl (SS2), Mycorrhiza with 100 mM NaCl (SS2+M), 200 mM NaCl (SS3), and Mycorrhiza with 200 mM NaCl (SS3+M).

References


