Role of arbuscular mycorrhiza fungi on tolerance to salinity of the tree legume *Albizia lebbeck* (L.) inoculated by *Rhizobium*

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1. Introduction

Current development in sustainability involve a rational exploitation of soil microbial activities [1] and the use of less expensive, though less bioavailability sources of plant nutrients like Rock phosphates (RP), which may be made available by microbiologically mediated processes [2]. It is known that microorganisms are activated in the soil-plant interface where a microcosm system, the rhizosphere, develops [3-5]. The role of other microorganisms e.g., the so called plant growth promoting rhizobacteria (PGPR), as modifiers of soil fertility and facilitators of plant establishment is being considered [4, 6-10]. Mycorrhizal symbiosis plays an important role in nutrient cycling in agricultural and natural ecosystems [11]. AM fungi colonize the root cortex of plants and develop an extrametrical hyphal network that can absorb nutrients from the soil. The structure and functionalities of these AM associated microbial communities differ from those of the rhizosphere [12] and this microbial compartment has been named “mycorrhizosphere” [13]. Conversely, soil microorganisms can affect AM formation and function [1-3]. The mycorrhiza helper bacteria are known to stimulate mycelial growth of mycorrhizal fungi or to enhance mycorrhizal formation [1, 14, 15]. The microbiologically solubilized phosphate could, however, be taken up by a mycorrhizal mycelium, thereby developing a synergistic microbial interaction [1]. Phosphorus (P) is added in the form of phosphatic fertilizers, part of which is utilized by plants and the remainder converted into insoluble fixed forms [16].
The contribution of their process to plant nutrition is unclear and because of the possible refixation of solubilized phosphate ions on their way to the root zone [8, 17]. According to Moradi et al. Arbuscular Mycorrhizal Fungi (AMF) and Rhizobium significantly increased fresh and dry weights of shoot and root inoculation with AMF and/or Mesorhizobium can increase plant growth, significantly in the soil with low nutrient content.

The requirement of P is high in legumes [18] and therefore leguminous plant respond more to mycorrhizal infection than cereals, which indirectly enhance the biological nitrogen fixation through increased P availability specially in soil with low P content [19, 20]. Rosendahl and Rosendahl reported that the vesicular arbuscular mycorrhizal fungi have the ability to protect plants from salt stress [21]. Mycorrhizal association helps the plants to withstand stress maximum NaCl regime [22]. No reports are available on the levels of mycorrhirization on growth and nutrient uptake of tree legume grown in Rajasthan under salinity stress condition. In the present study, the role of AMF (the most preferred AM Acaulospora laevis) and the percentage of AM colonization on growth and nutrient uptake of tree legume Albizia lebbeck (L.) under salinity are discussed.

2. Materials and methods

Role of most preferred AMF on tolerances to different salinity (NaCl) levels of test plants inoculated with most efficient Rhizobium was studied. A pot culture experiment was conducted for six months with sterilized soil using plastic pots to prevent leaching of NaCl from the bottom. The surface sterilized seeds pelleted with rhizobial isolates were sown in pots on AMF inoculums pad containing 250 spores/50 gm soils. After 15 days of seedling establishment the pots were saturated with different concentrations of NaCl viz., 0.5%, 1.0%, 2.0%, 3.0% and 4.0%. Pots with no addition of salt (NaCl) served as control. The surface sterilized seeds pelleted with rhizobial isolates were sown in pots on AMF inoculums pad containing 250 spores/50 gm soils. After 15 days of seedling establishment the pots were saturated with different concentrations of NaCl viz., 0.5%, 1.0%, 2.0%, 3.0% and 4.0%. Pots with no addition of salt (NaCl) served as control. Three replicates of pots, each containing five seedlings were maintained for each treatment and were periodically irrigated with the sterilized water. The various treatments included were T0-0%, T1-0.5%, T2-1.0%, T3-2.0%, T4-3.0% and T5-4.0% NaCl. Each of these treatments consisted of uninoculated control, Rhizobium alone and Rhizobium + AMF inoculated sets. The parameters selected for the study were shoot-root length, shoot and root dry weight, total plant protein [21], total chlorophyll [24], total nitrogen [25] and total phosphorus content [26], nodule number, nodule dry weight and maximum nodule size [27], nitrogenase activity [28] of root nodules and AMF colonization by roots (%).

3. Results and discussion

Influence of arbucular mycorrhizal inoculation on plant growth (shoot and root length), plant dry matter production (shoot and root dry weight) and nutrient uptake level under different levels of salinity stress condition are given in Table1. The highest value of growth in terms of shoot length (49.1 cm), shoot dry weight (0.103 g), root length (68.1 cm) and root dry weight (0.105 g) was observed in Rhizobium isolated AL Rhz + Acaulospora laevis inoculated plants at the first level of salinity (T0-0%NaCl) which also served as control. The same parameters in other dual inoculated plants decreased at the subsequent levels (T1-0.5%, T2-1.0%, T3-2.0%, T4-3.0% and T5-4.0% NaCl) of salinity. Among Rhizobium (AL Rhz) or AMF(Acaulospora laevis) alone inoculated seedling, highest values of shoot length (36.8 cm), shoot dry weight (0.88 g), root length (60.5 cm) and root dry weight (0.94 g) was recorded in AL Rhz -9 inoculated plants at T0 (0% NaCl). All the above parameters decreased with the increasing salinity levels. The uninoculated sets recorded the least values of all the above parameters.

The dual inoculated seedling recorded maximum values of total plant protein, total chlorophyll, total N and Total P at the level (T0) of salinity. The values decreased at the subsequent levels. Among single inoculation plants, the values in Rhizobium (AL Rhz) inoculated plants were highest at first level as compared to other levels of salinity. The uninoculated seedling recorded the lowest values (Table 1).

The nodulation (nodule number, nodule dry weight, maximum nodule size) and nitrogen fixation in term of nitrogenase activity by nodules were recorded highest in seedling inoculated with dual combination of Rhizobium (AL Rhz) + AMF(Acaulospora laevis) (Fig.1) the maximum nitrogenase activity of nodulated roots was recorded 1.7µ mol C2H4 g-1 fresh nodule h-1 in seedling inoculated with dual combination of Rhizobium (AL Rhz) + Acaulospora laevis in comparison to the nitrogenase activity of 1.3 µ mol C2H4 g-1 fresh nodule h-1 in the seedling inoculated with Rhizobium (AL Rhz) alone, at the first level of salinity. The phenomena of nodulation and nitrogen fixation very poor and decrease drastically at the subsequent level of salinity (Fig.1).

Table1. Effect of salinity (Salt concentration ) on growth, nodulation, N2-fixation and root colonization of Albizia lebbeck inoculated with Rhizobium (AL Rhz) and AM (Acaulospora laevis). (Values are Mean ± Standard deviation of 15 replication)
| Salinity levels | Treatments              | Shoot length (cm) | Shoot dry weight (g) | Root length (cm) | Root dry weight (g) | Total plant dry protein (mg/g) | Total Plant chlorophyll (mg/l) | N-conten t (dry weight) (%) | P-conten t (dry weight) (%) | AM colonization (%) |
|-----------------|-------------------------|-------------------|----------------------|------------------|---------------------|-------------------------------|-------------------------------|---------------------------|--------------------------|-------------------|
| T₀ (0% NaCl)    | Uninoculated Control    | 3.0 ± 0.75        | 62.4 ± 0.79          | 1.2 ± 0.45       | 2.0 ± 0.24          | 1.0 ± 0.25                    | 0.0 ± 0.08                    | 1.0 ± 0.36                | 0.0 ± 0.02              | Zero               |
|                 | Acaulospora laevis      | 3.6 ± 0.88        | 66.3 ± 0.84          | 2.7 ± 0.12       | 2.72                | 1.18 ± 0.38                   | 0.21 ± 0.02                   | 1.45 ± 0.17              | 0.01 ± 0.17             | 30.5 ± 4.1          |
|                 | AL Rhz                  | 2.8 ± 0.08        | 60.5 ± 0.94          |                 |                     |                              |                              |                          |                          | Zero               |

| T₁ (0.5% NaCl)  | Uninoculated Control    | 4.1 ± 0.01        | 4.5 ± 0.03           | 1.0 ± 0.5        | 2.72                | 1.18 ± 0.38                   | 0.21 ± 0.02                   | 1.45 ± 0.17              | 0.01 ± 0.17             | Zero               |
|                 | Acaulospora laevis      | 3.0 ± 0.85        | 51.2 ± 0.02          | 2.72             | 1.18 ± 0.38         | 0.21 ± 0.02                   | 1.45 ± 0.17                   | 0.01 ± 0.17              | 30.5 ± 4.1             | Zero               |
|                 | AL Rhz                  | 2.8 ± 0.08        | 83.0 ± 0.05          | 4.1              | 1.49 ± 0.42         | 0.01 ± 0.02                   | 1.45 ± 0.17                   | 0.01 ± 0.17              | 30.5 ± 4.1             | Zero               |

| T₂ (1.0% NaCl)  | Uninoculated Control    | 2.8 ± 0.07        | 5.0 ± 0.05           | 0.9 ± 0.5        | 2.72                | 1.18 ± 0.38                   | 0.21 ± 0.02                   | 1.45 ± 0.17              | 0.01 ± 0.17             | Zero               |
|                 | Acaulospora laevis      | 2.4 ± 0.09        | 7.1 ± 0.12           | 2.19             | 1.18 ± 0.22         | 0.13 ± 0.00                   | 30.5 ± 4.1                    | Zero                      |
|                 | AL Rhz                  | 4.7 ± 0.03        | 4.2 ± 0.03           | 0.51             | 1.35 ± 0.02         | 0.05 ± 0.01                   | 30.5 ± 4.1                    | Zero                      |

| T₃ (2.0% NaCl)  | Uninoculated Control    | 3.0 ± 0.68        | 49.5 ± 0.78          | 88.29            | 0.85 ± 0.10         | 30.5 ± 4.1                    |                          |                          |                          | Zero               |
|                 | Acaulospora laevis      | 3.5 ± 0.81        | 54.5 ± 0.82          | 121.01           | 1.03 ± 0.11         | 30.5 ± 4.1                    |                          |                          |                          | Zero               |
|                 | AL Rhz                  | 1.6 ± 0.08        | 6.3 ± 0.03           | 0.51             | 1.15 ± 0.72         | 0.01 ± 0.01                   | 30.5 ± 4.1                    | Zero                      |

| T₄ (3.0% NaCl)  | Uninoculated Control    | 3.0 ± 0.68        | 49.5 ± 0.78          | 88.29            | 0.85 ± 0.10         | 30.5 ± 4.1                    |                          |                          |                          | Zero               |
|                 | Acaulospora laevis      | 3.5 ± 0.81        | 54.5 ± 0.82          | 121.01           | 1.03 ± 0.11         | 30.5 ± 4.1                    |                          |                          |                          | Zero               |
|                 | AL Rhz                  | 4.3 ± 0.04        | 3.6 ± 0.02           | 0.51             | 1.26 ± 0.99         | 0.01 ± 0.01                   | 30.5 ± 4.1                    | Zero                      |

| T₅ (4.0% NaCl)  | Uninoculated Control    | 3.0 ± 0.68        | 49.5 ± 0.78          | 88.29            | 0.85 ± 0.10         | 30.5 ± 4.1                    |                          |                          |                          | Zero               |
|                 | Acaulospora laevis      | 3.5 ± 0.81        | 54.5 ± 0.82          | 121.01           | 1.03 ± 0.11         | 30.5 ± 4.1                    |                          |                          |                          | Zero               |
|                 | AL Rhz                  | 4.3 ± 0.04        | 3.6 ± 0.02           | 0.51             | 1.26 ± 0.99         | 0.01 ± 0.01                   | 30.5 ± 4.1                    | Zero                      |

Acaulospora laevis Plant survived for 10-15 days of the treatment

AL Rhz

AL Rhz + Acaulospora laevis

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Fig. 1 Effect of salinity (salt concentration) on growth, nodulation, N2-fixation and AM root colonization of *Albizia lebbeck* inoculated with *Rhizobium* (*Rhz*) and AM (*Acaulospora laevis*) (Value are Mean ± Standard deviation of 15 replicated)

Highest percentage of AMF root colonization (Table 1) was recorded to be 42.4% in seedling inoculated with dual combination of *Rhizobium* (AL *Rhz*) + *Acaulospora laevis* and in only AMF (*Acaulospora laevis*) inoculated plants, percentage of AM root colonization was recorded 39.5% at the level (T₀) of salinity. The value decreased with the increase in salinity levels. The salinity level did not support the growth of the test individuals. At 4% NaCl treatment individuals survived for 10-15 days only.

The result from the experiment clearly indicated that *Rhizobium* isolate AL *Rhz* and AMF *Acaulospora laevis* formed an effective dual combination resulting in highest values of all the parameter studies at the first level of salinity (T₀). The percentage AMF was associated with *Rhizobium*. These results were also supported by other workers [29-32].
The under salinity stress condition, it was found that the growth rate of plants decreased according to the increase of salinity. Even though the plants had low growth compared to the uninoculated control plants, the treated plants (treated with AM) showed more growth than their non-mycorrhizal counter parts.

The data presented in table suggested that inoculation of efficient strain of AMF (Acaulospora laevis) during the course of the study, prevented the injurious effects of salinity in the test plants strain of AMF (Acaulospora laevis) during the course of the study, prevented the injurious effects of salinity in the test plants. It was not that the percentage of mycorrhization due to enhanced water and nutrient uptake thereby promoting growth, nodulation and nitrogen fixation of the tree legume under investigation. It was not that the percentage of mycorrhization was directly proportional to the growth rate, nodule and investigation. It was not that the percentage of mycorrhization was directly proportional to the growth rate, nodule and nitrogenase activity under salinity stress condition. The above observations have been supported by Rosendahl and Rosendahl and Hirrel and Gerdemann [21, 22].

**Conflict of interest statement**

We declare that we have no conflict of interest.

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