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### *Original Research Article*

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

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#### **ABSTRACT**

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 $N_2$  fixation and salinity.

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effect of different level of salinity on growth, nodulation and Nitrogen fixation of single and dual inoculated tree legumes which help arbascular mycorrhizal fungi (AMF) *Acaulospora laevis* Gerd. and Trappe with *Rhizobium* bacteria in the presence of different levels of salinity (concentration of NaCl) conducted in earthen pots in a completely randomized block design with three replications resulted in effective plant growth, shoot and root biomasses, nodulation and  $N_2$  fixation. The effect of different levels of salinity (concentration of NaCl) on growth, nodulation and  $N<sub>2</sub>$  fixation of single (*Rhizobium* or AMF alone) and dual inoculated (*Rhizobium* + AMF ) tree legume has a pot culture experiment using The parameter growth , nodulation, nitrogen fixation and % AMF colonization of roots were considerable influenced with the increase in salt concentration from 0.5 % to 4.0 % NaCl. It was observed that the mycorrhizal tree legume (inoculated with the most preferred AMF *Acaulospora laevis*) performed better in the increasing levels of salinity in comparison to non-mycorrhozal ones. These investigations suggested a protective role play by AMF in providing resistance to the tree legume against injurious effects of salinity. Inoculation of efficient strain of AMF (*Acaulospora laevis*) during the course of study, prevented the injurious effects of salinity in the test plants due to enhanced water and sustainable nutrient uptake thereby promoting growth, nodulation and biogeochemical  $N_2$  cycle (fixation of nitrogen) of the tree legume under investigation.

*Myrica esculenta* (Myricaceae) and *Syzygium cumini*(Myrtaceae), the Indian traditional fruits The

#### **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].

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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 exported that the vesicular arbuscular mychorrhizal fungi have the ability to protect plants from salt stress [21]. Mychorrhizal association helps the plants to withstand stress maximum NaCl regime [22]. No reports are available on the levels of mychorrhization 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. 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  $T_0$ -0%,  $T_1$ -0.5%, T<sub>2</sub>-1.0%, T<sub>3</sub>-2.0%, T<sub>4</sub>-3.0% and T<sub>5</sub>-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 [23], 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 arbascular 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  $(0.103 \text{ g})$ , root length  $(68.1 \text{ cm})$  and root dry weight  $(0.105 \text{ g})$ was observed in *Rhizobium* isolated AL *Rhz* + *Acaulospora laevis* inoculated plants at the first level of salinity  $(T_0-0)/NaCl$ which also served as control. The same parameters in other dual inoculated plants decreased at the subsequent levels  $(T_0-0\% , T_1-$ 0.5%,  $T_2$ -1.0%,  $T_3$ -2.0%,  $T_4$ -3.0% and  $T_5$ -4.0% NaCl) of salinity. Among *Rhizobium* (AL *Rhz*) or AMF( *Acaulospora laevis*) alon 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  $T_0$  (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  $(T_0)$  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 seeding 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 1.7 $\mu$  mol C<sub>2</sub>H<sub>4</sub> g<sup>-1</sup> fresh nodule h<sup>-1</sup> in seedling inoculated with dual combination of *Rhizobium* (AL *Rhz*) + *Acaulospora laevis* in comparison to the nitrogenase activity of 1.3  $\mu$  mol C<sub>2</sub>H<sub>4</sub> g<sup>-1</sup> fresh nodule h<sup>-1</sup> 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)** 





**Original Research Article** 47



**(2.0% NaCl)-13**. uninoculated control, **14.** *Acaulospora laevis,* 15. AL *Rhz*, 16. AL *Rhz* + *Acaulospora laevis,* **T<sup>4</sup> (3.0% NaCl)-17** uninoculated control, **18.** *Acaulospora laevis,* **19.** AL *Rhz*, **20.** AL *Rhz* + *Acaulospora laevis*

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  $(T_0)$  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  $(T_0)$ . 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 the level 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 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 nitrogenase activity under salinity stress condition. The above

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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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