PRELIMINARY STUDIES FOR SELECTION OF EFFICIENT RHIZOBIUM STRAINS FROM DALBERGIA SISOO

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ABSTRACT

The present investigation was carried out to isolate and evaluate the effective strains of *Rhizobium* from *Dalbergia sisoo* seedlings. Studies were conducted under greenhouse condition to assess the compatibility and growth-promoting ability of isolated *Rhizobium* strains. From isolated strains thirteen strains were screened as *Rhizobium* out of which strain nos. 382, 386, 388, 392 and 393 performed significantly better in nodulation (54.65 to 51.12/plant), shoot length (34.4 to 31.4 cm/plant) and root length 27.8 to 23.4 cm/plant) over untreated control. Likewise, dry wt and N content of root, shoot and nodule also increased significantly. The overall results indicated that strain no. 388, 393, 392 and 382 were most efficient among the isolates.

INTRODUCTION

High quality tree seedlings are prerequisite for the successful establishment of hardwood plantations particularly on substandard soil where fertility problems are common (Jha et al., 2000). Nitrogen fixing species assume considerable importance in this context for their soil ameliorating characteristics. Among multipurpose leguminous tree species, *Dalbergia sisoo* is known for its timber quality, fuel, fodder and also recorded to enhance soil fertility through its association with effective rhizobia and proficient litterfall (Dart et al., 1991). Rapid early growth of these trees in nurseries unable carry field establishment of seedlings and also improves the efficiency of nursery operations, but this species usually takes more time for growth at initial nursery conditions (Bary et al., 1985). Hence, consistent nursery production of such seedlings is often a major constraint to implement plantation programme mostly in social forestry and in degraded soil. To overcome this problem rhizobial inoculation is often proved to be an alternative solution (Ferrera-Cerrato et al., 1985; Nautiyal, 1994) which could result in production of high quality nursery stock. However, the differential interaction between host ‘ and *Rhizobium* strain for nitrogen fixing potential indicates the need for identification of efficient strain for the particular locality. Effective symbiotic nitrogen fixation depends upon the proper establishment of interrelationship between a particular legume and specific strain of *Rhizobium* (Dart et al., 1976; Singh et al., 2000). In many cases the introduced strain fails to compete with the native *Rhizobium*, indicating the need for identifying the efficient strains for the native inoculation. Keeping this point in view the present study was undertaken to screen the efficient *Rhizobium* isolates from *Dalbergia sisoo* plantlets collected from the nurseries of three districts of Assam and investigate their influence on growth, nodulation and nitrogen status of seedlings.

MATERIAL AND METHODS

Isolation: The root nodules from six months old healthy seedlings of *Dalbergia sisoo* were collected from different forest nurseries of Sibsagar, Jorhat and Golaghat districts of Assam. Nodules were sterilized and rhizobia was isolated using Yeast Extract Mannitol Agar (YEMA) media (Vincent, 1970).

Identification: The identification of isolates was done by performing various morphological and biochemical tests like Gram strains, Congo red test, Keto lactose test, Peptone glucose agar test, Hofer's alkaline broth test, H₂S production, Methyl red test,
V.P. test, Indole production, Growth in litmus milk. Starch hydrolysis, Reduction of 2, 3, 5 TTC etc. according to Laboratory Manual of Microbiology. Strains were identified according to Buchanan and Gibbons (1974). Rhizobia were authenticated by plant infection test in laboratory adopting nitrogen free medium in aqua culture (Suba Rao, 1984) and in modified Leonard Jar assembly (Leonard's, 1944). All the cultures were maintained in yeast extract mannitol agar (YEMA) slants and stored at 4°C.

Evaluation of different strains for Nitrogen fixing ability: The study was conducted in greenhouse condition in the nursery of Deovan, RFRI, Jorhat in the year 2001. Soils were dried, sieved and filled with 6 x 8 cm sized polybags. The inoculum of various strains of Rhizobium were grown on yeast extract mannitol broth on a rotary shaker at 30°C for 48 hours. The culture was harvested by centrifugation at 7500 rpm, washed twice with sterilized water. Seeds of Dalbergia sissoo that were uniform in shape, size and weight were collected from local provances and surface sterilized using ethyl alcohol and mercuric chloride solution (Somasegaran and Hoben, 1994). Seeds were then placed into the polybags in the nursery for germination. After germination 1 ml of sterile suspension of bacterial culture was used as inoculants in soil. Control was also maintained without inoculation of the suspension. For each strain 10 replications were made.

The parameters analysed in the study could be broadly categorized into biometric parameters such as root length, shoot length, dry biomass and N content of seedlings etc. and symbiotic parameters like number of nodules per plant, nodule biomass etc. These parameters were recorded after two months of growth of seedlings. For this purpose all the surviving seedlings were uprooted gently and analysed individually. For biomass production samples were dried at 80°C for 72 hours. From dried samples nitrogen were analysed with the help of Nitrogen auto analyzer.

RESULTS AND DISCUSSION

The nurseries from where the seedlings were collected and isolation was made was presented in Table 1. After 48 hours of incubation the growth the colonies were recorded and which diameter of ranged between 3.5 to 5mm. Most of the colonies were transparent while some were creamy white to opaque. All the isolates were found to be gram -ve, short rods, non spore forming, do not absorb Congo red dye, not grown in peptone glucose agar and Hofer's alkaline broth. Based on these characteristics the

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>Host plant</th>
<th>Name of the nursery</th>
<th>District</th>
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<tbody>
<tr>
<td>381</td>
<td>Dalbergia sissoo</td>
<td>Phulbari: Bokakhat</td>
<td>Golaghat</td>
</tr>
<tr>
<td>382</td>
<td></td>
<td>Phulbari: Bokakhat</td>
<td>Golaghat</td>
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<td>383</td>
<td></td>
<td>Bokakhat H.Q.</td>
<td>Golaghat</td>
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<td>386</td>
<td></td>
<td>Nawjan: Morajan</td>
<td>Golaghat</td>
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<tr>
<td>387</td>
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<td>Nawjan: Morajan</td>
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<td>388</td>
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<td>Jonaki Nagar</td>
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<td>384</td>
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<td>Holongapara: Mariani</td>
<td>Jorhat</td>
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<td>385</td>
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<td>393</td>
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<td>391</td>
<td></td>
<td>Bahgarh: Nazira</td>
<td>Sibsagar</td>
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<td>392</td>
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<td>Bahgarh: Nazira</td>
<td>Sibsagar</td>
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Fig. 1. Effect of *Rhizobium* inoculation on biometric parameters of *Dalbergia sissoo*
Fig. 2. Effect of *Rhizobium* inoculation on symbiotic parameters of *Dalbergia sissoo*
isolates were identified as *Rhizobium* spp. After screening the isolates for nodulation in aqua culture and modified Leonard Jar using nitrogen free media, 13 strains were observed to produce nodules in the host plant. These strains were preserved as mother culture for further study. The study revealed significant variation in growth, nodulation, biomass production and nitrogen content in plants due to inoculation with various isolates of *Rhizobium*. Fig. 1 revealed that root length and plant height were significantly high in strain nos. 382, 386, 388, 392 and 393 inoculated seedlings which ranges from 50.2% to 26.4% and 40.9% to 28.6% more in comparison to uninoculated control. The value of root and shoot dry weight also follows the same trend with growth (Fig. 2). The maximum value of root and shoot dry weight was recorded 50% and 41% more in case of strain nos. 382 inoculated seedlings over control followed by strain nos. 386, 388, 393 and 392. Verma et al. (1996) also reported that the application of *Rhizobium* broth (5ml/pl) significantly influenced the plant height, collar diameter, root length and biomass production of *D. sisoo* seedlings. Tremendous increase of nodulation was recorded in inoculated plants compared to control.

The number of nodules in inoculated plants was almost double in compared to uninoculated plant except the strain nos. 381, 384 and 390 inoculated seedlings (Fig. 2). On the other hand the maximum dry weight of nodule biomass was found in strain no. 388 which was recorded as 0.056 g/plant (Fig. 2). From the results it was observed that although the nodule number was equal, the size of the nodules was relatively big. Variation of nodulation and dry weight of nodules due to inoculation with different strains of *Rhizobium* were also reported by Nambiar (1985). Maximum percentage of nitrogen content in root, shoot and nodule were observed in strain no. 393, which was recorded as 69.4%, 78.3% and 59.44% respectively followed by strain nos. 392, 386, 388 and 382 (Fig. 1 and 2). The strains in which dry biomass were observed more the percentage of nitrogen content were also recorded high. From the result it may be said that relatively big and healthy nodules can fix more nitrogen than the smaller nodules. The result was also supported by Chaukiyal and Pokhriyal (2001).

From this study it was observed that although the biometric parameters- shoot length, root length and dry weight were strongly interrelated, it was poorly related with symbiotic associated parameters. Of the symbiotic associated parameters the number and size of the nodules has significant influence in nitrogen content of plants. Therefore, symbiotic parameters may be considered more important in nitrogen fixation of plants.

The above observation reported significant improvement of growth parameters as a result of inoculation of efficient *Rhizobium*. Considering the overall effects of individual isolates it can be concluded that the strain nos. 382, 386, 388, 392 and 393 were suggested for further field performance evaluation of *Dalbergia sisoo*.

ACKNOWLEDGEMENT

The authors are grateful to the Director, Rain Forest Research Institute, Jorhat, for providing laboratory facilities and constant encouragement during the study.
REFERENCES