Cross inoculation studies: Response of *Vigna mungo* to inoculation with rhizobia from tree legumes growing under arid Environment

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**ABSTRACT:** Cross-inoculation experiments were conducted in the greenhouse to test the rhizobia isolated from nodules of seven tree legumes for their effectiveness in *Vigna mungo* plants. The tree legumes included *Albizia lebbeck*, *Dalbergia sissoo*, *Leucaena leucocephala*, *Pithecellobium dulce*, *Prosopis cineraria*, *Prosopis glandulosa* and *Prosopis juliflora*, all growing under arid environment. Rhizobia from these legumes formed nodules on the roots of *Vigna mungo* except isolates from *Albizia lebbeck*. Dry weight and nitrogen contents of *Vigna mungo* plants increased significantly (P<0.05) in response to cross inoculation as compared to uninoculated control. Rhizobia from *Leucaena leucocephala* and *Prosopis glandulosa* showed significant increase in dry weight (P<0.05) and nitrogen contents (P<0.05) than other inoculated treatments. The natural rhizobia of wild tree legumes growing under arid environment show higher tolerance to prevailing adverse conditions like salt stress, elevated temperatures and drought. These rhizobia may be used to inoculate wild as well as crop legumes cultivated in reclaimed desert lands. These rhizobia may have specific traits that can be transferred to other rhizobia through genetic engineering tools. The cross infection of agriculturally important legumes with isolates from wild legumes may prove a useful means of increasing nitrogen contents within these plants.

**Key words:** Tree legumes, rhizobia, cross inoculation, *Vigna radiat*, nodulation, nitrogen fixation

**INTRODUCTION**

Awareness of the benefits of cross inoculation as a means of comparing symbiotic effectiveness of wild rhizobial strains with cultivated strains has increased in the past (Amarger, 2001; Vessey, et al., 2004; Zhang, et al., 1991). The cross-inoculation of legumes of agricultural importance with rhizobial isolates from wild legumes resulted in an increase in dry matter and total nitrogen contents of cross infected plants (Iqbal and Mahmood, 1992; Lalani Wijesundra, et al., 2000; Zahran, et al., 1999). *Vigna mungo* is a grain legume widely cultivated in Pakistan, India and other Asian countries.

It is a cheap source of protein and part of diet for millions of people in these countries. Seeds are commonly consumed and contain 17-34% of protein (Gour, 1993). The experiments were conducted in a greenhouse to test the rhizobia isolated from nodules of tree legumes for their effectiveness in *Vigna mungo*. The impact of cross inoculation on nodulation, dry matter production and total nitrogen contents of host species was studied.

**MATERIALS AND METHODS**

Nodules were collected from seven tree legumes growing in and around Karachi under arid environment. Tree species were selected because of their importance in agroforestry and reforestation programs. The tree legumes studied including *Albizia lebbeck* (L) Benth., *Dalbergia sissoo* Roxb., *Leucaena leucocephala* (Lam.) de Wit., *Pithecellobium dulce* (Rox.) Benth., *Prosopis cineraria* (L.) Druce, *Prosopis glandulosa* Torr. and *Prosopis juliflora* (Swartz.) DC. Rhizobia were isolated from the nodules as described by Somasegaran and Hoben, (1994). Rhizobial isolates from nodules of *Vigna mungo* (L.) Hepper were also included for comparison. Cultures were maintained on yeast extract mannitol agra medium (YMA) slants as described by Somasegaran and Hoben, (1994). The bacterial isolates were examined for Gram reaction, motility, colony characteristics on YMA medium, YMA with Congo red and YMA with bromothymol blue. They were characterized as rhizobia (Somasegaran and Hoben, 1994). The motility of isolates was determined by hanging drop method (Somasegaran and Hoben, 1994). Cross-inoculation experiments were performed in chillum jar assemblies...
developed by Dahiya and Khurana, (1981) which is a modified form of Leonard bottle jar assembly (Somasegaran and Hoben, 1994). The chillum jar unit consisted of a top earthen vessel round at one end and tapering at the other. The lower half (the reservoir) consisted of a glass jar of such dimensions that the chillum vessel sat snugly on its rim and the tapering end of the chillum came to within 2-4 cm of the bottom of the jar. A wick was provided in the tapering end of chillum to help the capillary rise of the liquid from the reservoir to the top of the growth vessel. Coarse river sand, previously acid washed thoroughly, was added to chillum units within 5 cm of the top. Hoagland nitrogen-free nutrient solution (200 mL) was added to each reservoir unit. Top of the growth vessel was covered with glass petri dish and the whole unit was wrapped with thick paper and secured with rubber bands. The units were autoclaved at 121 °C for 2 h. for two alternate days and kept intact until they were brought into use. Undamaged Vigna mungo seeds (courtesy of Dr. Zahoor Aslam, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan) of uniform size were selected by hand sorting. Seeds were surface sterilized through treating with 0.2% HgCl₂ (acidified with 5 mL/L conc. HCl) for 3 to 5 min. were surface sterilized through treating with 0.2% HgCl₂ (acidified with 5 mL/L conc. HCl) for 3 to 5 min. followed by repeated washing with sterile water. The inoculum was applied direct to seed coat by soaking the seeds for half an hour in rhizobial suspension with 10% sucrose solution (4 days old YMA culture). Four inoculated seeds were sown in each assembly immediately after inoculation. In some cases, pre-germinated seedlings were inoculated by adding L/mL of suspension of the culture to their bases.

The covering on the top of the assemblies was removed after germination of seeds and sterilized dry gravels were spread over the surface of the sand to check contamination. The assemblies were arranged in randomized blocks using three replicates for each treatment. Uninoculated treatment served as control. Nitrogen free sterile Hoagland solution was supplied to each unit after an interval of 24 h. The assemblies were kept in controlled environment chamber operating at 27 °C with 16 h. illumination. The plants were harvested after six weeks. Nodules were counted on each plant and plants were dried in an oven at 72 °C for 48 h. and weighed. Dry plants were ground and total nitrogen content was determined by Kjeldahl method (Nelson and Sommers, 1980). The data were analyzed statistically and the least significant differences among treatments were evaluated at the 0.05 level of probability.

RESULTS AND DISCUSSION
Rhizobial isolates from tree legumes produced translucent, round and gummy colonies which varied in size between 1.5 and 2.0 mm. The isolates were motile and did not take Congo red stain. They showed varied reaction with bromothymol blue. Rhizobial isolate from Prosopis cineraria and Vigna mungo gave acidic reaction, while Dalbergia sissoo, Leucaena leucocephala, Pithocellobium dulce, Prosopis glandulosa and Prosopis juliflora gave alkaline reactions (Table 1). All the rhizobial isolates from tree legumes except Albizia lebeck formed nodules in Vigna mungo plants. The nodules were globose to be elongated in shape; they were formed on primary as well as secondary roots (Table 2). All the isolates produced moderate nodulation except Vigna mungo which showed abundant nodulation (Table 2).

No nodules were observed in uninoculated control plants. Vigna mungo was successfully cross inoculated with the rhizobial isolates from tree legumes. The number of the formed nodules, dry matter contents and total nitrogen contents of cross infected plants is presented in Table 3. The highest numbers of nodules were produced by isolates from Prosopis glandulosa followed by Dalbergia sissoo which were significantly different (P<0.05) from Vigna mungo and other inoculated treatments. The highest dry weight and nitrogen content were observed in treatment inoculated by Dalbergia sissoo followed by Leucaena leucocephala and Prosopis glandulosa which were comparable with Vigna mungo and were significantly different (P<0.05) from other inoculated treatments and uninoculated control treatment.

Early reports of rhizobia associated with tree legumes described them as either of the slow growing type or of the cowpea miscellany type (Basak and Goyal, 1980). There have been reports of alkali producing Rhizobium strains (Hernandez and Focht, 1984) and acid producing Bradyrhizobium strains (Moerira, et al., 1993; Padmanabhan, et al., 1990). Legume trees are infected as much by fast growing rhizobia as by slow growing rhizobia (Moerira, et al., 1998; Trinick, 1980; Turk and Keyser, 1992; Zhang, et al., 1991). Some tree rhizobial strains are host specific, whereas others have a wide host range (Dommergues,
Table 1: Colony morphology and staining reactions of rhizobia isolated from nodules of tree legumes and Vigna mungo

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Congo red medium</th>
<th>Grams staining</th>
<th>Motility</th>
<th>Reaction with bromothymol blue medium</th>
<th>Colony morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albizia lebbeck</td>
<td>-</td>
<td>Negative</td>
<td>+</td>
<td>-</td>
<td>2 mm, translucent, round, gummy</td>
</tr>
<tr>
<td>Dalbergia sissoo</td>
<td>-</td>
<td>Negative</td>
<td>+</td>
<td>Alkaline</td>
<td>2 mm, translucent, round, gummy</td>
</tr>
<tr>
<td>Leucaena leucocephala</td>
<td>-</td>
<td>Negative</td>
<td>+</td>
<td>Alkaline</td>
<td>2 mm, translucent, round, gummy</td>
</tr>
<tr>
<td>Pithecellobium dulce</td>
<td>-</td>
<td>Negative</td>
<td>+</td>
<td>Alkaline</td>
<td>1.5 mm, translucent, round, gummy</td>
</tr>
<tr>
<td>Prosopis cineraria</td>
<td>-</td>
<td>Negative</td>
<td>+</td>
<td>Acidic</td>
<td>2 mm, translucent, round, gummy</td>
</tr>
<tr>
<td>Prosopis glandulosa</td>
<td>-</td>
<td>Negative</td>
<td>+</td>
<td>Alkaline</td>
<td>2 mm, translucent, round, gummy</td>
</tr>
<tr>
<td>Prosopis juliflora</td>
<td>-</td>
<td>Negative</td>
<td>+</td>
<td>Alkaline</td>
<td>2 mm, translucent, round, gummy</td>
</tr>
<tr>
<td>Vigna mungo</td>
<td>-</td>
<td>Negative</td>
<td>+</td>
<td>Acidic</td>
<td>2 mm, translucent, round, gummy</td>
</tr>
</tbody>
</table>

Table 2: Morphology and nodule frequency of Vigna mungo cross-inoculated with rhizobia isolated from tree legumes

<table>
<thead>
<tr>
<th>Host of isolation</th>
<th>Nodule shape</th>
<th>Nodule frequency</th>
<th>Nodule distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albizia lebbeck</td>
<td>Globose</td>
<td>*</td>
<td>Secondary roots</td>
</tr>
<tr>
<td>Dalbergia sissoo</td>
<td>Globose</td>
<td>*</td>
<td>Secondary roots</td>
</tr>
<tr>
<td>Leucaena leucocephala</td>
<td>Globose</td>
<td>*</td>
<td>Secondary roots</td>
</tr>
<tr>
<td>Pithecellobium dulce</td>
<td>Globose</td>
<td>*</td>
<td>Secondary roots</td>
</tr>
<tr>
<td>Prosopis cineraria</td>
<td>Globose</td>
<td>*</td>
<td>Primary and Secondary roots</td>
</tr>
<tr>
<td>Prosopis glandulosa</td>
<td>Globose</td>
<td>*</td>
<td>Primary and Secondary roots</td>
</tr>
<tr>
<td>Prosopis juliflora</td>
<td>Globose</td>
<td>*</td>
<td>Primary and Secondary roots</td>
</tr>
<tr>
<td>Vigna mungo</td>
<td>Semi globose</td>
<td>**</td>
<td>Primary and Secondary roots</td>
</tr>
</tbody>
</table>

et al., 1984). In the present studies, the isolates from root nodules of Albizia lebbeck did not form nodules on Vigna mungo host. Albizia lebbeck rhizobia also failed to nodulate Vigna radiata host. Duhoux and Dommergues, (1985) have stated that Albizia lebbeck is specific in its rhizobial requirements. It is nodulated only by Bradyrhizobium. Failure of Albizia lebbeck rhizobia to infect Vigna mungo and Vigna radiata hosts would need further investigations. One interesting application of rhizobia of wild tree legumes is their use as inoculum for crop legumes. Many tree strains effectively nodulate herbaceous legumes (Herrera, et al., 1985). Several reports describe successful experiments where wild rhizobia are more effective in nitrogen fixation than their compatible hosts (Zahran, 2001). Wange, (1989) obtained effective symbiosis between Acacia rhizobia and each of peanut and cowpea. Zhang, et al., (1991) performed cross inoculation experiments between rhizobia isolated from Acacia and Prosopis and found effective symbiosis with Medicago sativa, Phaeolus vulgaris and Vicia faba. Iqbal and Mahmood, (1992) tested
isolates from seven legumes for their ability to produce root nodules on *Leucaena leucocephala* host. Isolates from *Albizia lebbeck*, *Pithecellobium dulce* and *Vigna unguiculata* were most effective in nitrogen fixation and induced substantial increase in dry weight and nitrogen contents of the host plant. Zahran, *et al.*, (1999) studied the symbiotic performance of rhizobia isolated from wild tree legumes (*Acacia nilotica* and *Sesbania sesban*) and the herbaceous legumes (*Alhagi murarum*, *Melilotus indicus* and *Trifolium resupinatum*) with some grain legumes. About 30% of rhizobial isolates formed effective symbiosis with four legumes (*Medicago sativa*, *Pisum sativum*, *Vicia faba*, *Vigna sinensis*). Similarly, isolated rhizobia from the tree legume, *i.e.* *Pericopsis moonians*, formed nitrogen-fixing nodules in a broad spectrum host, *Macroptillium atropurpureum* (Lalani Wijesundara, *et al.*, 2000). The high nitrogen-fixing ability of rhizobia associated with *Dalbergia sissoo* and *Leucaena leucocephala* in Pakistani soils has been reported by Javid and Fisher, (1989) and Iqbal and Mahmood, (1992), respectively. Higher nitrogen activity in *Leucaena leucocephala* has been reported by Aryal, *et al.*, (1999) from Indian soils. The present results on the cross-inoculation of *Vigna mungo* with rhizobia from tree legumes are very encouraging and in conformity with previous studies (Iqbal and Mahmood, 1992; Lalani Wijesundara, *et al.*, 2000; Wange, 1989; Zahran, *et al.*, 1999). The natural rhizobia of wild tree legumes growing in arid zones exhibit higher tolerance to prevailing adverse conditions like salt stress, elevated temperatures and drought (Zahran, 2001). These rhizobia may be used to inoculate wild as well as crop legumes cultivated in reclaimed desert lands. These rhizobia may have specific traits that can be transferred to other rhizobia through genetic engineering tools. However, field trials are needed to prove the effectiveness of these isolates from wild legumes in increasing nitrogen contents of cultivated plants. The cross-infection of agriculturally important legumes with isolates from wild legumes may prove a useful means of increasing nitrogen contents within these plants.

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