# Symbiotic Promiscuity among Rhizobia Isolated from Tree Legumes

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## ABSTRACT

Twenty nine strains of *Rhizobium* were isolated from *Acacia, Prosopis* and *Albizia* species. Two efficient strains each of *Rhizobium* nodulating *Acacia* (AC-2 and AC-11), *Prosopis* (P-6 and P-7) and *Albizia* (Alb-4 and Alb-9) were tested for their host range for nodulation and nitrogen fixing ability on different species of *Acacia, Prosopis* and *Albizia*. *Rhizobium* sp.(*Acacia*) strains AC-2 and AC-11 were able to nodulate, *A. nilotica, A. tortilis, A. auriculiformis, A. farnisiana, A. arabica, A. molisma* and *A. senegal. Rhizobium* sp. (*Prosopis*) strains P-6 and P-7 nodulated *P. juliflora* and *P. Specigara* while *Rhizobium* sp (*Albizia*) strains Alb-4 and Alb-9 nodulated. *A. lebbek, A molucana, A. richardiana* and *A. procera*. *Rhizobium* strains isolated from *Acacia*, *Prosopis* and *Albizia* had wide host range and were able to nodulate heterologous hosts. Variation in effectiveness with homologous and heterologous host nodulated by different rhizobia were also observed.

**Key word :** *Acacia, Albizia, Rhizobium, host range, Prosopis*

## 1. INTRODUCTION

Leguminous trees like cultivated legumes form nitrogen fixing root nodules in symbiotic association with *Rhizobium* and *Bradyrhizobium*. Nitrogen fixing trees (NFTs) with high potential for nitrogen fixation have been identified for agroforestry systems, alley cropping and alley farming (Kang and Mulongoy, 1992; Franco and Faria, 1997). Among the family Leguminoseae, nodulation capacity has been surveyed in 3395 species expressing 20% of leguminous species and including about 57% of legume genera (Faria et al., 1989). Globally *Acacia, Callindra, Faidlerbia, Flemingia, Gliricidia, Leucaena, Prosopis* and *Sesbania* are few nodulating species which have been recommended for agroforestry (Sprent and Paarson, 2002).

Nodulation and nitrogen fixation potential of tree legumes is affected by host species, *Rhizobium* strain and abiotic and biotic factors (Dommergues, 1995; Toky et al., 1995). Inoculation with *Rhizobium* helps in producing healthy seedlings of tree legume (Beniwal et al., 1991). However, unlike cultivated legumes, tree legumes are rarely inoculated because *Rhizobium* inoculants for tree legumes have not been developed so far. This is perhaps due to the lack of knowledge on host range of nodulation and nitrogen fixing potential in combination with different hosts (Danso, 1992). The present investigation was initiated to test the host range of *Rhizobium* on different species of *Acacia, Prosopis* and *Albizia*.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Nodule Samples and Isolation of *Rhizobium*

Nodules of *Acacia nilotica, Prosopis juliflora* and *Albizia lebbek* were collected from 3-6 months old seedlings growing in and around Haryana Agricultural University farm after monsoon. Isolation of rhizobia were made from surface sterilized nodules as described earlier (Bala et al., 1990). Nodules were sterilized with 0.1% mercuric chloride solution for 3 min. and repeatedly washed 6-7 times with sterilized distilled water. Nodules were crushed with the help of a sterilized glass rod and nodule sap was streaked on yeast extract mannitol agar (YEMA) plates with congo red (10 ml/l. of 0.25% aqueous solution). *Rhizobium* isolates were preserved on YEMA slopes and authenticated using the nodulation using homologous host (Beniwal et al., 1991).

### 2.2 Host Species

Seeds of *Acacia nilotica, A. tortilis, A. molisima, A. senegal, A. auriculiformis, A. farnisiana, A. arabica, Prosopis juliflora, P. specigera, Albizia lebbek, A molucana, A. richardiana, A. procera,* were procured from Department of Agroforestry, HAU, Hisar and Forestry Research Institute, Dehradun, India.

### 2.3 Nodulation and Cross Infectivity

Nodulation studies on homologous and heterologous hosts were carried out as described by Toky et al., (1995). Seeds of *Acacia, Prospis* and *Albizia* species (10g) were surface sterilized using 0.2% mercuric chloride solution for 5 min. Seeds were washed 5-7 times with sterilized distilled water and inoculated with 1 ml 48 h old *Rhizobium* culture (10 $^{7}$cells ml$^{-1}$). Inoculated seeds were sown in sterilized chillum jar (modified Leonard jars) (Dahiya and Khurana, 1983). Each jar had five plants and each treatment had three replications. After 60 days of growth, plants were uprooted and data on nodule number, nodule

Table 1. Symbiotic performance of *Rhizobium* sp. (*Acacia*) with different species of *Acacia*

| Species | Treatment | Nodule number plant$^{-1}$ | Nodule dry weight plant$^{-1}$(mg) | Plant dry weight plant$^{-1}$(mg) | ARA µ Mole pl$^{-1}$h$^{-1}$ |
|---|---|---|---|---|---|
| *A. nilotica* | Control | – | – | 195±9.6 | – |
| | AC–2 | 21+4.9 | 24±6.6 | 587±41.5 | 2.5±3.8 |
| | AC–11 | 13±2.4 | 10±1.9 | 254±7.6 | 2.6±1.5 |
| *A. tortilis* | Control | – | – | 68±13 | – |
| | AC–2 | 8±0.5 | | 97±3.1 | 0.3±0.09 |
| | AC–11 | 7±1.6 | | 103±2.8 | 1.0±0.2 |
| *A.auricuriformis* | Control | – | – | 40±2.5 | |
| | AC–2 | 4±0.4 | 6±1.8 | 77±3.5 | 0.27±0.5 |
| | AC–11 | 8±3.7 | 11±3.0 | 133±7.5 | 0.3±0.1 |
| *A. farnesiana* | Control | – | – | 122±5.9 | – |
| | AC–2 | 10±0.8 | 15±37 | 168±3.2 | 2.61±1,5 |
| | AC–11 | 14±1.0 | 16±6.8 | 291±28.0 | 2.5±0.2 |
| *A. arabica* | Control | – | – | 432±62 | – |
| | AC–2 | 26±5.8 | 19±1.3 | 499±16.0 | 1.91±0.6 |
| | AC–11 | 24±4.7 | 17±4.0 | 568±18.9 | 2.9±1.1 |
| *A. molisima* | Control | – | – | 105±8.1 | – |
| | AC–2 | 23±3.7 | 13±4.4 | 154±6.1 | 1.2±0.5 |
| | AC–11 | 22±5.3 | 16±2.2 | 180±4.1 | 0.9±0.14 |
| *A. senegal* | Control | – | –119±8.3 | – | |
| | AC–2 | 15±5.0 | 13±1.4 | 453±14.0 | 4.1±1.0 |
| | AC–11 | 15±1.6 | 15±0.8 | 367±4.7 | 7.3±1.0 |
| LSD at 1% | 2. | 2.6 | 3.1 | 20.8 | 1.4 |

Values are average of three replicates, ± standard deviation.

dry weight and plant dry weight was recorded. Nitrogen activity in nodule was estimated by acetylene reduction assay as described by Hardy et al. (1968).

## 3. RESULTS AND DISCUSSION

### 3.1 Isolation and Characterization of *Rhizobium* spp.

Twenty nine strains of *Rhizobium* spp. were isolated from nodules collected from *Acacia* (15), *Prosopis* (6) and *Albizia* (8). Majority of the strains (86.2%) were fast growers and all the strains were able to nodulate their respective host under chillum jar conditions. On the basis of their efficiency strains AC-2 and AC-11 of *Rhizobium* sp. (*Acacia*), P-6 and P-7 of *Rhizobium* sp. (*Prosopis*) and Alb-4 and Alb7 of *Rhizobium* sp. (*Albizia*) were selected for further studies.

### 3.2 Host Range of Nodulation

*Rhizobium* sp. (*Acacia*) strain AC-2 and AC-11 were able to nodulate seven species of *Acacia* i.e. *A. nilotica, A. tortilis, A. auriculiformis, A. farnisiana., A. arabica, A. molisima* and *A. senegal* under sterilized conditions (Table 1). Inoculated plants showed better growth than the uninoculated controls and accumulated significantly higher plant dry weight. The maximum number of nodules was formed on *A.arabica* by strains AC-2 and AC-11, while minimum number of nodules was formed on *A. auriculiformis*. There was no host specificity for nodulation, however, efficacy of strains AC-2 and AC-11 with different hosts was different with different species of *Acacia*. *A. nilotica, A.farnisiana, A.arabica* and *A.senegal* showed significantly higher nitrogenase activity when inoculated with AC-2 and AC-11 than the other three species. Earlier Barnett and Catt (1991) isolated fast growing *Rhizobium* from *Acacia* and most of these strains tested had wide host range of nodulation which was extended to family Mimosoideae and Fabaceae. On the contrary Dreyfus and Dommergues (1981) reported host specificity among *Rhizobium* isolated from *Acacias*. Some species of *Acacia* like *A. albida, A. holoseracia, A. mearensii, A. nilotica, A. longiflolia, A. nilotica, A. radians* and *A. senegal* were nodulated only by fast growing *Rhizobium* while *A. seyal* and *A. salinga* were nodulated by both *Rhizobium* and *Bradyrhizobium*.

Table 2. Symbiotic performance of *Rhizobium* sp (*Prosopis*) and different species of *Prosopis*

| Species | Treatment | Nodule number plant$^{-1}$ | Nodule dry weight plant$^{-1}$(mg) | Plant dry weight plant$^{-1}$(mg) | ARA µ Mole pl$^{-1}$h$^{-1}$ |
|---|---|---|---|---|---|
| *P.specigera* | Control | – | – | 62.±6.1 | – |
| | P-6 | 10±3.7 | 5±1.1 | 71±10.8 | 2.0±5 |
| | P-7 | 16±2.4 | 7±1.0 | 105±2.1 | 5.8±0.4 |
| *P.juliflora* | Control | – | – | 39±1.5 | – |
| | P-6 | 19±4.9 | 8±0.9 | 60±2.3 | 4.6±0.7 |
| | P-7 | 18±3.2 | 12±3.1 | 75±13.9 | 10.1±3.5 |
| LSD at 1% | | 2.3 | 1.5 | 7.6 | 1.3 |

Variabilty in host range of nodulation and nitrogen fixing potential in *Acacia* spp. has been observed by Aseefa and Kleiner. (1998), Beniwal et al. (1991), Toky et al. (1995) and Turk and Keyser (1992).

*Rhizobium* sp. (*Prosopis*) strains P-6 and P-7 isolated from *P. juliflora*, was able to nodulate *P.specigera* effectively. Inoculation was able to improve all the growth parameters of *Prosopis*. Nodule number, dry weight of nodule and acetylene reduction activity was significantly higher in case of *P. juliflora* than that of *P. specigera* (Table 2). Wide host range of nodulation of *Rhizobium* isolated from *Prospopis glandulosa* have been reported by Jenkins et al. ( 1987,1989), Thomas et al. (1994), Trinick et al. (1968).

*Rhizobium* strains Alb-4 and Alb-9 isolated from *A. procera* were able to nodulate three other species of *Albizia* i.e. *A.lebbek, A.molucana* and *A.richardiana*. (Table 3). *A. richardiana* and *A. procera* were fast growing species and accumulated maximum plant dry weight. However, acetylene reduction activity was highest in *A.procera* when inoculated with *Rhizobium* strain Alb-9 followed by *A. lebbek* inoculated with *Rhizobium* strain Alb-4. *Rhizobium* nodulating *Albizia* were not host specific and all the species of *Albizia* were effectively nodulated by *Rhizobium* strains isolated from *A. lebbek*. Puppeke and Broughton (1999) has reported that *Rhizobium fradii* isolated from soybean and *Rhizobium* NGR234 isolated from *Parasponia* were able to nodulate the species of *Albizia*.

### 3.3 Cross Infectivity of Rhizobium spp.

*Rhizobium* strains AC-2, P-6 and Alb-9 isolated from *Acacia nilotica, Prosopis juliflora* and *A. lebbek* respectively were tested for nodulation on heterologous host. Results indicated that *Rhizobium* strains were able to cross nodulate *Acacia, Prosopis* and *Albizia* but nodulation and nitrogen fixation efficiency was different with different hosts. (Table 4). In case of *A. nilotica* plants inoculated with *Rhizobium* sp. *Albizia* strain Alb - 9 formed significantly more nodule than that of plants inoculated with *Rhizobium* sp. (*Acacia*) strain AC-2 and

Table 3. Symbiotic performance of *Rhizobium* sp. (*Albizia*) on different species of *Albizia*

| Species | Treatment | Nodule number plant$^{-1}$ | Nodule dry weight plant$^{-1}$(mg) | Plant dry weight plant$^{-1}$(mg) | ARA µ Mole pl$^{-1}$h$^{-1}$ |
|---|---|---|---|---|---|
| *A. lebbek* | Control | – | – | 105±2.9 | – |
| | Alb–4 | 8±1.4 | 12±5.7 | 149±3.8 | 4.09± |
| | Alb–9 | 14±3.3 | 20±6.1 | 141±18.1 | 1.6± |
| *A. molucana* | Control | – | – | 105±2.9 | – |
| | Alb–4 | 8±1.6 | 9±3.8 | 37±8.3 | – |
| | Alb–9 | 5±1.8 | 3±1.3 | 30±8.4 | 1.96±0.11 |
| *A. richardiana* | Control | – | – | 780±140 | – |
| | Alb–4 | 16±2.0 | 45±16.1 | 1318±18.9 | 3.49±0.4 |
| | Alb–9 | 15±3.3 | 20±7.6 | 980±25.4 | 16.2±1.4 |
| *A. procera* | Control | – | – | 126 | |
| | Alb–4 | 19±4.3 | 24±6.7 | 203±4.7 | 2.05±1.6 |
| | Alb–9 | 16±2.6 | 12±4.1 | 111±4.7 | 5.3±1.1 |
| LSD at 1% | | 2.4 | 2.9 | 28.4 | 1.7 |

Table 4. Cross infectivity of *Rhizobium* sp. on *Acacia, Prosopis* and *Albizia*

| Host | *Rhizobium* | Nodule number plant$^{-1}$ | Nodule dry weight plant$^{-1}$(mg) | Plant dry weight plant$^{-1}$(mg) | ARA µ Mole $pl^{-1}h^{-1}$ |
|---|---|---|---|---|---|
| *A. nilotica* | AC–2 | 22±3 | 20±8.9 | 160±4.6 | 7.9±1.6 |
| | P–6 | 20±8.0 | 13.7±4.1 | 147±2.7 | 5.1±0.06 |
| | Alb–9 | 26±6.3 | 16±1.7 | 138±3.8 | 5.5±0.3 |
| | Control | – | – | 119±4.5 | – |
| *P. juliflora* | AC–2 | 6±1.1 | 2.7±1.6 | 36±13.7 | 14.7±2.6 |
| | P–6 | 15±2.4 | 13±4.2 | 68±15.5 | 0.72±0.2 |
| | Alb–9 | 13±2.5 | 24±7.6 | 25±44.1 | 5.54±1.5 |
| | Control | – | – | 20±6.8 | – |
| *A. lebbek* | AC–2 | 12 ±2.3 | 9± 1.4 | 145 ±17.8 | 24.1±3.3 |
| | P–6 | 13±2.8 | 8±3.9 | 115±2.9 | 9.3±1.8 |
| | Alb–9 | 12±3.2 | 7±3.7 | 145±17.1 | 30.6±3.3 |
| | Control | – | – | 98±9.5 | – |
| LSD at 1% | | 2.2 | 1.8 | 21.4 | 2.2 |

*Rhizobium* sp. (*Prosopis*) strain P-6. The acetylene reduction activity was highest in the plants inoculated with strain AC-2. In case of *P.juliflora* the nodule number in plant inoculated with strain P-6 and Alb-9 did not differ significantly however, the plants inoculated with strain P-6 accumulated significantly higher plant dry weight. In case of *A.lebbek*, the nodule number formed by the three strains did not differ significantly. However, the plants inoculated with strain AC-2 and Alb-9 accumulated significantly more plant dry weight.

Earlier studies have also shown that rhizobia isolated from *Prosopis, Leucaena., Acacia* and *Mimosa* were able to cross nodulated each other (Trinick,1980; Barnet and Catt, 1991; Frioni et al., 998; Thomas et al ,1994). *Acacia senegal, A. auriculiformus, A. salinga, A. albida* have been found to be nodulated by species of rhizobia like *Rhizobium* sp., *Rhizobium* strain NGR234, *Mesorhizobium plurifarum, Sinorhizobium kostiense, S. saheli, S. terranga* and *Bradyrhizobium* sp. (Dupuy et al., 1994; Khbhya et al., 1994; Nick ,1998; Nick et al., 1999). It appears that rhizobia which have been classified into different species on the basis of biochemical and genetical characters are not different on the basis of host specificity (Bala and Giller, 2001; Burdon et al. 1999; Dupuy et al. 1994; Khbhya et al. 1998; Zerhari et al. 2000).

## 4. CONCLUSIONS

The results indicated that rhizobia nodulating tree legume are promiscuous and show wide host range for nodulation. These rhizobia are able to nodulate different species of same genera as well as different genera of other legume trees from which they have been isolated.

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