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CROSS INOCULATION STUDIES I: RESPONSE OF COWPEA (*VIGNA UNGUICULATA*) TO INOCULATION WITH RHIZOBIA FROM TREE LEGUMES

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ABSTRACT

Rhizobia from root nodules of *Albizia lebbeck, Dalbergia sissoo, Leucaena leucocephala, Pithecellobium dulce, Prosopis glandulosa, P. juliflora*, and *Vigna unguiculata* were tested for their ability to produce root nodules on *V. unguiculata*. Isolates from all the leguminous plants produced nodules on *V. unguiculata*. Isolates from *D. sissoo, L. lencocephala, P. dulce* and *P. glandulosa* were most effective in nitrogen fixation and produced substantial increase in dry weight and nitrogen contents of the host plant.

Key-words: Cow pea, *Vigna unguiculata*, rhizobia, tree legumes

INTRODUCTION

Awareness of the benefits of cross inoculation as a means of comparing symbiotic effectiveness of wild strains with cultivated strains has increased in the past (Basaak and Goyal,1980; Srivastava and Tewari, 1982 and Iqbal and Mahmood, 1992). The cross infection of legumes of agriculture importance with rhizobial isolates from wild legumes resulted in an increase in dry matter and total nitrogen contents of cross inoculated plants (Srivastava and Tewari, 1982; Iqbal and Mahmood, 1992). In the present study ability of rhizobial isolates obtained from tree legumes to symbiose effectively with *V. unguiculata* (L) Walp was tested and impact of symbiosis on nodulation, dry matter production and total nitrogen contents of host species was recorded.

MATERIALS AND METHODS

Collection of nodules from trees and isolation of rhizobia from the nodules

The nodules were collected from the trees following somasegaran and Hoben (1985), special care was taken to distinguish root nodules from the kinds of malformations such as caused by nematodes, insects or other root inhabiting pathogenic micro-organisms. Nodules were placed in screw capped vials containing anhydrous calcium chloride for storage (Somasegaran and Hoben, 1985). The isolation of Rhizobia from root nodules and smear preparations were made following Vincent (1970).

Rhizobia were isolated from the nodules of 6 tree legumes growing in and around Karachi. The legumes included: *Albizia lebbeck* (L.) Benth; *Dalebergia sissoo* Roxb., *Leucaena leucocephala* (Lam.) de Wit., *Pithecellobium dulce* (Roxb.) Benth., *Prospis glandulosa* Torr. and *Prosopis juliflora* (Swartz). DC. Rhizobial isolates were also obtained from *Vigna unguiculata* (L.) Walp grown from seeds, and inoculated with strain specific in order to compare the effectivness in nodule formation, dry matter production and total nitrogen contents with tree rhizobia. In addition non inoculated *V. unguiculata* was also used as control.

Characterization of the isolates

Yeast extract mannitol medium (YEMA), YMA with congo red and YMA containing. Bromothymol blue were prepared following Vincent (1970). The motility of isolates was determined by hanging drop method (Vincent, 1970). The isolated bacterial cultures were examined for Gram reaction, motility, colonial characteristics on YEMA medium, YMA with Congo red and YMA with Bromothymol blue and were characterized (Vincent, 1970). Cultures were maintained on YMA slants as described by Vincent (1970).

Cross inoculation experiments

Cross Inoculation experiments were preformed in chillum jar assemblies developed by Dahiya and Khurrana (1981) which is a modified form of the bottle jar assembly (Leonard, 1944). 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 2-4cm 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. Thoroughly acid washed coarse river sand was added to "Chillum" units to within 5cm of the top. To each reservoir unit 200ml of Hoagland's nitrogen free nutrient solution

was added. Top of the growth vessel was covered with glass petridish half 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.

Pretreatment of seeds

Undamaged seeds of equal size of *V. unguiculata* were selected. Seeds were surface sterilized by treating them with 0.2% $HgCl_2$ (acidified with 5ml/Litr. Conc, HCl) for 3 to 5 minutes, followed by repeated washing with sterile water.

Inoculation of seeds

The inoculum was applied direct to seed coat by soaking the seeds for half an hour in bacterial suspension with 10% sucrose solution. (4 days old YMA culture). Inoculated seeds were sown in the assemblies immediately after inoculation (Burton, 1976). In some cases pregerminated seedlings were inoculated by adding 1ml of suspension of the culture to their bases (Trinick, 1968). The covering on the top of the assembler 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 along with a set of nitrogen control (0.05% KNO_3) and untreated seeds as control (Trinick, 1968). 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. Nodule number and plant dry weight were determined. Total nitrogen contents were determined by microkjeldahl method (Bergerson, 1980).

The statistical analysis was performed following Zar (1995).

RESULTS AND DISCUSSION

All the isolates from tree legumes induced nodulation on *V. unguiculata* host. The nodules formed were globose to semiglobose in shape (Table 1). The color of the nodules varied between white, brown and pink and were formed on primary as well as on secondary root (Table 1). All the isolates produced moderate nodulation except *P. dulce*, *P. juliflora* and *V. unguiculata* that produced abundant nodulation (Table 1).

The isolates from tree legumes produced translucent, rounded and gummy colonies which varied in size between 1.5-2.0mm. The isolates were motile and did not take congo red stain. They showed varied reaction with Bromothymol blue. *V. unguiculata* gave acidic reaction while *A. lebbeck*, *L. leucocephala*, *P. dulce*, *P glandulosa*, *P. juliflora* and *D. sissoo* gave alkaline reactions (Table 2). Early reports of rhizobia associated with woody legumes described them as either of the slow growing type or the cowpea miscellany (Basak and Goyal 1980; Habish and Khairi, 1970). But there have been reports of alkali producing *Rhizobium* strains (Hernan-dez and Focht, 1984) and acid producing *Bradyrhizobium* strains (Moerira *et al.*, 1993; Padmanabhan *et al.*, 1990). Leguminous trees are infected as much by fast growing rhizobia as by slow growing rhizobia. Some tree rhizobial strains are host specific where as others have a wide host range (Dommergues, 1984). Many tree strains even effectively nodulate herbaceous legumes (Herrera *et al.*, 1985). Odee *et al.* (1997) have studied the phenotypic characteristics and composition of rhizobia associated with woody legumes growing in diverse, Kenyan conditions. Their data demonstrated a high diversity of tropical rhizobia associated with trees.

Table 1. Morphology of nodules of *Vigna unguiculata* cross inoculated with isolates from tree legumes.

HOST OF ISOLATION	SHAPE OF NODULES	COLOUR	FREQUENCY	DISTRIBUTION
Albizia lebbeck	globose	white	++	secondary roots
Dalbergia sissoo	globose	brown	++	secondary roots
Leucaena leucocephala	globose	brown	++	secondary roots
Pithecellobium dulce	"	brown	++	pri. & sec. roots
Prosopis glanduloza	"	brown	++	secondary roots
Prosopis juliflora	semi globose	pink	+++	pri. & sec. roots
Vigna unguiculata	semi globose	pink	+++	pri.& sec. roots

++, indicates moderate nodulation, +++ indicates abundant nodulation

Table 2. Colonial morphology and staining reactions of rhizobia isolated from nodules of tree legumes.

Plant Species	Congo red medium	Grams staining	Motility	Reaction with Bromothymol blue medium	Colononial Size and Morphology
Albizia lebbeck (L.) Benth.	-ve	-ve	+	alkaline	1.5mm, translucent, rounded, gummy
Dalbergia sissoo Roxb	-	-	+	alkaline	2mm, as above
Leucaena Leucocephala (Lam.) de Wit.	-	-	+	alkaline	1mm, as above
Pithecellobium dulce (Roxb.) Benth	-	-	+	alkaline	2mm, as above
Prosopis glanduloza Torr.	-	-	+	alkaline	2mm, as above
Prosopis juliflora (Swartz.) DC.	-	-	+	alkaline	2 mm, as above
Vigna unguiculata (L.) Walp.	-	-	+	acidic	2mm, as above

Table 3. Effectiveness of rhizobial strains isolated from tree legumes on nodulation, dry weight and nitrogen fixation in *Vigna unguiculata* (L.)Walp.

Host of Isolation	Nodule No/Plant	Total dry weight/plant (mg)	N. content/plant(mg)
Control	-	105.3	0.79
Albizia lebbeck (L.) Benth	5.0	108.4	0.83
Dalebergia sissoo Roxb.	10.4	316.6	4.11
Leucaena Leucocephala (lam.)de Wit	16.2	277.5	3.80
Pithecellobium dulce (Roxb.) Benth	8.0	288.3	2.90
Prosopis glanduloza Torr.	6.0	279.0	2.95
Prosopis juliflora (Swartz).DC.	13.6	136.0	1.53
Vigna unguiculata (L.) Walp.	13.4	182.4	1.93
Mean	10.37	215.87	2.41
SE	± 1.59	± 35.63	± 0.517
Difference	7.78	174.39	2.53

Vigna unguiculata was cross inoculated with the rhizobial isolates obtained from tree legumes. Number of nodules, dry matter contents and total nitrogen contents of cross infected plants were determined (Table 3). The highest number of nodules were produced by *L. leucocephala* followed by *P. juliflora*. The highest nitrogen content values were scored for *D. sissoo*, followed by *L. leucocephala*, *P. glandulosa* and *P. dulce* in that order (Table 3). The high nitrogen fixing ability of rhizobia associated with *D. sissoo* and *L. Leucocephala* in Pakistani soils has also been reported by Javid and Fisher (1989) and Iqbal and Mahmood (1992).

Srivastava and Tewari (1982) used *Rhizobium* cultures obtained from 14 legumes growing wild in the Varanase area of India in cross inoculation experiments with *V. radiata* and *V. unguiculata* in sand and solution cultures. Seven of the cultures were effective in nodule formation on *Vigna unguiculata* host and increased the total N_2 contents of inoculated plants by 12-48%. The most effective strains were from *Uraria picta* and *Zornia diphylla* with *V. radiata* in sand culture. Only the *Rhizobium* isolated from a plant classified locally as *Phaseolous psoraleoides* were effective in promoting plant development. In tube culture, however, isolates from *Cassia absus* and *Z. diphylla* were also highly effective. Known strains of rhizobium for *V. unguiculata* and *V. radiata* included as controls, were generally poor in N_2 fixation with these hosts.

Iqbal and Mahmood (1992) tested isolates from 7 legumes for their ability to produce root nodules on *L. leucocephala* host. Isolates from *V. unguiculata*, *A. lebbeck* and *P. dulce* were most effective in nitrogen fixation and induced substantial increase in dry weight and nitrogen contents of the host plant.

Results of Srivastava and Tewari (1982) and Iqbal and Mahmood (1992) and our present results are very encouraging. More experiments including field trials are needed to prove the effectiveness of isolates from wild legumes in increasing N_2 contents of cultivated plants. 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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