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CROSS INOCULATION STUDIES 11: RESPONSE OF *VIGNA RADIATA* (L.) WILCZEK. TO INOCULATION WITH RHIZOBIA FROM TREE LEGUMES

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ABSTRACT

Rhizobia from nodules of *Acacai nilotica* (L.) Delile., *Albizia lebbeck* (L) Benth., *Dalbergia sissoo* Roxb. *Leucaena leucocephala* (Lam.) deWet., *Pithecellobium dulce* (Roxb.) Benth., *Prosopis cineraria* (L.) Druce., *P. glandulosa* Torr. and *P. juliflora* (Swartz.) DC. were tested for their ability to produce roots nodules on *Vigna radiata* host. Isolates from all the leguminous plants produced roots nodules on *V. radiata*. Isolates from *L. leucocephala* were found most effective in nitrogen fixation and induced substantial increase in dry weight, followed by isolates from *P. juliflora* and *V. radiata.*

Key-words: *Vigna radiate, Rhizobium* spp. Cross inoculation studies, legume trees

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 (Basak and Goyal, 1980; Sirivastiva snd Tewari, 1982; Becking, 1983; Herrera *et al*., 1985; Zhang *et.al*, 1991; Rubina and Mahmood, 1992; Aryal *et al*., 1999; Bala and Giller, 2001; Mahmoodand Javed, 2005). Cross inoculation of legumes of agriculture importance with rhizobial isolates from tree legumes resulted in an increase in dry matter and total nitrogen contents of cross- inoculated plants (Sirivastiva and Tewari, 1982; Rubina and Mahmood, 1992). In the present study rhizobial isolates obtained from 8 tree legumes were tested for symbiosis with *V. radiata* and impact of symbiosis on nodulation, dry matter production and total nitrogen contents of host species was recorded. The aim of the present study was to explore the possibility whether cross infection of agriculturally important legumes with isolates from tree legumes may prove a useful means of increasing dry matter and nitrogen contents within these plants.

MATERIALS AND METHODS

Isolation of rhizobi from the nodules

Rhizobia were isolated from the nodules of tree legumes growing in and around Karachi. The legumes included *A. nilotica, A. lebbeck, D. sissoo, L. leucocephala, P. dulce, P. cineraria, P. glandulosa* and *P. juliflora*. Rhizobial isolates were also obtained from the nodules of *V. radiata* that were grown from seeds, and inoculated with strain specific in order to compare the effectiveness in nodule formation, dry matter production and total nitrogen content with tree rhizobia. In addition non inoculated *V. radiata* and un inoculated *V. radiata* supplied with 0.05 KNO3 (Nitrogen control) were used as control. *V. radiata* is a grain legume widely cultivated in Pakistan and other Asian countries. It is a cheep source of protein for million of people in these countries (Allen and Allen, 1981). Seeds of commonly consumed legumes contain 17-34% protein (Gaur *et al*, 1993). 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 roots inhabiting pathogenic micro-organisms. Nodules were placed in screw capped vials containing an-hydrous calcium chloride for storage (Somasegaran and Hoben, 1985). The isolation of rhizobia from the nodules and smear preparations were made following Vincent (1970).

Characterization of the isolates:

The rhizobial isolates obtained from nodules of tree legumes and from the nodules of *V. radiata* were examined for Gram's staining , motility and verified by their colonial characteristics on YMA medium, YMA with cango red and YMA with Bromothymol blue. Cultures were maintained on YMA slants as described by (Vincent, 1970). Motility of the isolates was determined by hanging drop method (Vincent, 1970).

Cross Inoculation Experiments:

Cross inoculation experiments were performed in Chillum jar assemblies developed by Dahiya and Khurana (1981) which is a modified form of bottle jar assembly (Leonard, 1944). Undamaged seeds of *V. radiata* were selected. Seeds were surface sterilized by treating them with 0.2% $HgCl_2$ for 3-5 minutes, followed by repeated

washing with sterile water. 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). The assemblies were arranged in randomized blocks using 3 replicates of each treatment along with a set of nitrogen control 0.05KNO3 and uninoculated *V. radiate* plants were kept as control (Trinck, 1968). Plants were harvested after six weeks. Plants were kept in a growth chamber at 25º-28º C with 16h illumination and watered with nitrogen free nutrient solution (Hoagland and Arnon, 1950). Nodule number, Plant dry weight and total nitrogen contents were determined by Microkjeldahl method (Bergersen, 1980). Statistical analysis was performed following Zar (1995)

RESULTS AND DISCUSSION

All the isolates from the nodules of tree legumes and isolate from *V. radiata* nodules induced nodulation on *V. radiata*. The nodules formed were globose and elongated in shape (Table-1). All the isolates produced moderate to abundant nodulation, and produced translucent, rounded and gummy colonies which varied in size between 1.0, 1.5 and 2.0mm. The isolates were motile and did not take congo red stain. They showed varied reaction with Bromothymol blue. *A. nilotica, P. cineraria* and *V. radiata* gave acidic reaction while *A. lebbeck, L. leucocephala, P. dulce*, *P. glandulosa, P. juliflora* and *D. sissoo* gave alkaline reactions. Early reports of rhizobia associated with woody legumes described them as either of 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 *Brabyrizobium* 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 other 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. Moreover both slow and fast growing rhizobia were sometime isolated from host plants belonging to the same genus, species or even nodules (Gao *et al*., 1984).

Table 1. Effectiveness of *Rhizobial* strains isolated from tree legumes on nodulation, dry matter production and total nitrogen content in *Vigna radiata*.

Host of isolation	**Nodule frequency**	**Average numbers of nodules per plant**	**Shape of nodules**	**Total dry weight per plant(mg)**	**Nitrogen contents per plant(mg)**
Control 1. uninoculated *Vigna radiata* (L) Wilczek	_	_	_	107.0 ± 9.8	0.84 ± 0.076
Nitrogen Control ***2.*** uninoculated *V.radiata* supplied with 0.05 KNO3 solution	_	_	_	131.0 ± 7.8	0.93 ± 0.10
Acacai nilotica (L.)Delile.,	+++	15.0± 0.5	Globose	180.0±11.13	1.89 ± 0.076
Albizia lebbeck (L) Benth.;	+++	27.3± 1.5	Globose	141.0 ± 2.5	1.49 ± 0.04
Dalbergia sissoo ***Roxb.***	+++	6.0± 1.2	Elongated	119.6 ± 19.2	1.14 ± 0.06
Leucaena leucocephala (Lam.) deWet	+++	16.2± 2.8	Elongated & Globose	373.6 ± 6.4	5.10 ± 2.1
Pithecellobium dulce (Roxb.) Benth.,	+++	11.0± 5.0	Elongated	138.4 ± 16.8	1.37 ± 16.4
Prosopis cineraria (L.)Druce.	++	12.3± 7.2	Globose	160.0 ± 07.1	1.63 ± 5.3
Prosopis glandulosa Torr.	+++	9.2± 2.7	Elongated	137.0 ± 8.6	1.76 ± 8.7
Prosopis juliflora (Swartz.)DC.	++	13.4± 1.2	Globose	176.8 ± 2.1	2.26 ± 6.4
Vigna radiata(L) Wilczek.	+++	14.2± 3.5	Elongated & Globose	197.7 ± 16.4	1.98 ± 2.1

++ = moderate nodulation, +++ = abundant nodulation.

Number of nodules, dry matter contents and total nitrogen concentration of cross inoculated *V. radiata*, plants with isolates from nodules of tree legumes were determined (Table 1).The highest number of nodules were produced

on the roots of *A. lebbeck* followed by *L. leucocephala* (Table 1). The highest dry matter contents were recorded for *L.leucocephala* followed by *V .radiata.* The highest nitrogen contents values were scored by *L. leucocephala* followed by *P. juliflora and V. radiata* (Table 1). The highest nitrogen fixing ability of rhizobia associated with *L. leucocephala* in Pakistani soils has also been reported by Javed and Fisher (1989) and Rubina and Mahmood (1992). Sirivastiva 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 i*n sand and agar cultures, 7 of the cultures were effective in nodule formation on *V. unguiculata* host and increased total nitrogen contents of inoculated plants by 12 - 48 %.The most effective strains were from *Uraria picta* and *Zornia diphylla* with *V. radiata i*n sand cultures, only the *Rhizobium* isolated from a plant classified locally as *Phaseoous psoraleoides* were effective in promoting plant development. In tube cultures however, isolates from *Cassia absus* and *Zornia diphylla* were also highly effective. Known strain of *Rhizobium* for *V. radiata* and *V. unguiculata* included in their trial as controls were generally poor in nitrogen fixation with these hosts.

Rubina 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 showed an increase in dry weight and nitrogen contents of the host plants. Results of Sirivastiva and Tewari (1982) and Rubina 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 nitrogen contents of cultivated plants. Cross inoculation 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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