Int. J. Environ. Sci. Tech., 5 (1), 135-139, Winter 2008
ISSN: 1735-1472

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

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Received 24 July 2007; revised 18 August 2007; accepted 19 October 2007; available online 26 December 2007

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 cheep 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.

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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 preformed 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% $HgCl_2$ (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. Dried 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 glandulasa* 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 glanduloga* 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 sisso* 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*

Plant Species	Congo red medium	Grams staining	Motility	Reaction with bromothymol blue medium	Colony morphology
Albizia lebbeck	-	Negative	+	-	-
Dalbergia sisso	-	Negative	+	Alkaline	2 mm, translucent, round, gummy
Leucaena leucocephala	-	Negative	+	Alkaline	2 mm, translucent, round, gummy
Pitchecellobium dulce	-	Negative	+	Alkaline	2 mm, translucent, round, gummy
Prosopis cineraria	-	Negative	+	Acidic	1.5 mm, translucent, round, gummy
Prosopis glandulosa	-	Negative	+	Alkaline	2 mm, translucent, round, gummy
Prosopis juliflora	-	Negative	+	Alkaline	2 mm, translucent, round, gummy
Vigna mungo	-	Negative	+	Acidic	2 mm, translucent, round, gummy

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

Host of isolation	Nodule shape	Nodule frequency	Nodule distribution
Albizia lebbeck	-	-	-
Dalbergia sissoo	Globose	*	Secondary roots
Leucaena leucocephala	Globose	*	Secondary roots
Pithecellobium dulce	Globose	*	Secondary roots
Prosopis cineraria	Globose	*	Primary and Secondary roots
Prosopis glandulosa	Globose	*	Primary and Secondary roots
Prosopis juliflora	Globose	*	Primary and Secondary roots
Vigna mungo	Semi globose	**	Primary and Secondary roots

- No nodule
* 5-10 nodules per plant
** >10 nodules per plant

Table 3: The effectiveness of rhizobial isolates from tree legumes on nodulation, dry weight and nitrogen content of *Vigna mungo*

Host of isolation	Nodule per plant	Total dry weight (mg)	N-Contents per plant (mg)
Un-inoculated control	-	102.3a	0.76a
Albizia lebbeck	-	107.6a	0.79a
Dalbergia sissoo	17.5b	298.6c	3.98c
Leucaena leucocephala	11.6a	288.3c	2.90b
Pithecellobium dulce	8.0a	197.1b	2.05b
Prosopis cineraria	9.5a	211.0b	2.10b
Prosopis glandulosa	23.0b	240.7	3.13c
Prosopis juliflora	4.3a	118.0b	0.85a
Vigna mungo	10.3a	235.8c	3.30c

The means sharing the same letters in each column are not significantly different at $P< 0.05$

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 Wijesundra, *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 nitrogenase 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 Wijesundra, *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.

ACKNOWLEDGEMENTS

The funding for this research was provided by NSRDB, Islamabad, Pakistan for the project No. B. Sc. (120)/KU/90. Thanks are due to Dr. Mohammad Qaiser and Dr. Surriya Khatoon from University of Karachi for their help in the identification of some of the plants. Sincere gratitude is expressed to Dr. Zahoor Aslam, Nuclear Institute for Agriculture and Biology, Faisalabad for an providing approved variety of *Vigna mungo* seeds.

REFERENCES

Amarger, N., (2001). Rhizobia in the field. Adv. Agron., 73, 109-168.

Aryal, U. K.; Hossain, M. K.; Mridha, M. A. U.; Xu, H. L.; Umemura, H., (1999). Effect of *Rhizobium* inoculation on growth, nodulation and nitrogenase activity of some tree species. J. Plant Nutr., 22, 1049-1059.

Basak, M. K.; Goyal, S. K., (1980). Studies on the biology of tree legumes-*Rhizobium* symbiosis: Nodulation pattern and cross inoculation trials with the tree legumes and cultivated legumes. Ann. Arid Zone, 9, 427-431.

Dahiya, J. S.; Khurana, A. L., (1981). Chillum Jar, a better technique for screening of rhizobia under summer condition. Plant and Soil, 63, 299-302.

Dommergues, Y. R.; Diem, H. G.; Gauthier, D. L.; Dreyfus, B. L.; Cornet, F., (1984). Nitrogen-fixing trees in the tropics: Potentials and limitations, In: Advances in nitrogen fixation research, C. Veeger and W. E. Newton (Ed.), Martinus Nijhoff/Dr. W. Junk Publishers, Wageningen, The Netherlands.

Duhoux, E.; Dommergues, Y. R., (1985). The use of nitrogen-fixing trees in forest and soil restoration in the tropics: In: Biological Nitrogen Fixation in Africa. H. Sali and S. O. Keya, (Eds.), Matianum Press Consultants, Nairobi.

Gour, Y. D., (1993). Microbiology, physiology and agronomy of nitrogen fixation: Legume-*Rhizobium* symbiosis. Proc. Indian Nat. Sci. Acad., B., 59, 333-358.

Herrera, A. M.; Bedmar, E. J.; Olivars, J., (1985). Host specificity of rhizobium strains isolated from nitrogen-fixing trees and nitrogenase activities of strain GRH2 in symbiosis with *Prosopis chilensis*. Plant Sci., 42, 177-182.

Hernandez, B. S.; Focht, D. D., (1984). Invalidity of the concept of slow growth and alkali production in cowpea rhizobia. Appl. Environ. Microbiol., 48, 206-210.

Iqbal, R.; Mahmood, A., (1992). Response of *Leucaena leucocephala* to inoculation with rhizobia from tropical legumes. Pak. J. Bot., 24, 153-156.

Javid, Z.; Fisher, R., (1989). Dinitrogen fixation (C_2H_2 reduction) by *Dalbergia sissoo* and *Leucaena leucocephala* with native rhizobial strains. Arid Soil Res. Rehab., 34, 385-390.

Lalani Wijesundara, T. I.; Van Holm, L. H. J.; Kulasooriya, S. A., (2000). Rhizobiology and nitrogen fixation of some tree legumes native to Sri Lanka. Biol. Fertil. Soils, 30, 535-543.

Moerira, F. M. S.; Gillis, M.; Port, B.; Kerstens, K.; Franco, A. A., (1993). Characterization of rhizobia isolated from different divergence groups of tropical Leguminosae by comparative polyacrylamide gel electrophoresis of their total proteins. Syst. Appl. Microbiol., 16, 135-146.

Moerira, F. M. S.; Hauska, K.; Young, J. P. W., (1998). Biodiversity of rhizobia isolated from a wide range of forest legumes in Brazil. Mol. Ecol., 7, 889-895.

Nelson, D. W.; Sommers, L. E., (1980). Determination of total nitrogen in plant material. Agron. J., 65, 109-112.

Padmanabhan, S.; Hirtz, R. D.; Broughton, W. J., (1990). Rhizobia in tropical legumes. Cultural characteristics of *Bradyrhizobium* and *Rhizobium* species. Soil Biol. Biochem., 22, 23-28.

Somasegaran, P.; Hoben, H. J., (1994). Handbook of Rhizobia: Methods in Legume *Rhizobium* Technology. Springer-Verlag, New York.

Trinick, M. J., (1980). Relationships amongst the fast-growing Rhizobia of *Lablab purpureus*, *Leucaena leucocephala*, *Mimosa* spp., *Acacia farnesiana* and *Sesbania grandiflora* and their affinities with other rhizobial groups. J. Appl. Bacterial., 49, 39-53.

Turk, D.; Keyser, H. H., (1992). Rhizobia that nodulate tree legumes: Specificity of the host for nodulation and effectiveness. Can. J. Microbiol., 38, 451-460.

Vessey, J. K.; Pawlowski, K.; Bergman, B., (2004). Root-based N_2-fixing symbiosis: Legumes, actinorhizal plants, *Parasponia* sp. and cycads. Plant and Soil. 266, 205-230.

Wange, S. S., (1989). Response of groundnut (*Arachis hypogaea* L.) to inoculation with strains isolated from wild arboreal legumes. J. Appl. Microbiol. Biotech., 5, 135-141.

Zahran, H. H., (2001). Rhizobia from wild legumes: diversity, taxonomy, ecology, nitrogen fixation and biotechnology. J. Biotech., 91, 143-153.

Zahran, H. H.; Ahmad, M. S.; Abdel-Fatteh, M.; Zaki, A. Y., (1999). Phenotypic characteristics, cross nodulation and nitrogen fixation of root nodule bacteria isolated from wild leguminous plants in Egypt. Proc. Int. Symp. Biol. Nit. Fix. and Crop Prod., 77-90.

Zhang, X.; Harper, R.; Karisto, M.; Lindstrom, K., (1991). Diversity of *Rhizobium* bacteria isolated from the root nodules of leguminous trees. Int. J. Systm. Bacteriol., 41, 104-113.

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This article should be referenced as follows:

Mahmood, A.; Athar, M. (2008). Cross inoculation studies: Response of Vigna mungo to inoculation with rhizobia from tree legumes growing under arid Environment. Int. J. Environ. Sci. Tech., 5(1), 135-139.