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REGULAR ARTICLE

DETERMINATION OF THE HOST RANGE OF TREE AND HERB RHIZOBIA FOR THEIR ALTERNATIVE LEGUMES

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SUMMARY

A series of experiments were conducted in the green house and in growth chamber to test the rhizobia of *Leucaena leucocephala* (Lam) de Wit, *Sesbania sesban* (L) Merr. *Tephrosia purpurea* (L) Pers. and *Crotalaria medicaginea* (Lam) to determine their host range in other tree and herb legumes as alternative host. Other tree legumes under this study included *Albezzia lebbeck* (L.) Benth., *Prosopis juliflora* (Sw.) DC, *Acacia nilotica* (L.) Willd, *Acacia senegal* (L.) Willd, *Acacia leucophloea* and herb legumes included *Vigna radiata* (L.) R. Wilczek, *Vigna mungo* (L.) Hepper, *Dolichos lablab* (L.) Sweet, *Pisum sativum* (L.) and *Indigofera tinctoria* (L.). Promiscuity of *L. leucocephala* was observed for both tree and herb rhizobia but *S. sesban* and *T. purpuria* were very specific in their rhizobial requirement and could be nodulated only by their own rhizobia. However, *Acacia* spp. were not found be nodulated by any of the studied isolates. Ineffective nodules have been observed in *D. lablab*. The cross infection of agriculturally important legumes with isolates from wild legumes may prove a useful mean of increasing nitrogen contents within these plants.

Key words: Host range, Rhizobia, Nodulation, Legumes

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1. Introduction

Rhizobia are, by definition, bacteria that establish symbioses with legumes, forming root or stem nodules on the host and fixing atmospheric nitrogen (N_2). An important characteristic of the legume rhizobia symbiotic interaction is host specificity or host range, where defined species of rhizobia forms nodules on specific legumes. Some legume- *Rhizobium* associations can be highly specific while others are less (Dommergues, *et al.,* 1984). All legume plants susceptible to nodulation by a *Rhizobium* species constitute a "cross inoculation group". Although it quickly became obvious that nodulation ability overlaps across inoculation groups, this concept has been retained in a modified form because of its practical significance in inoculant use (Giller, 2001). Moreover, awareness of the benefits of cross inoculation as a means of symbiotic effectiveness of wild rhizobial strains in cultivated crop legumes has increased in the past (Lewin *et al.,* 1987; Zhang *et al.,* 1991; Amarger, 2001). Therefore, attempts have been made to examine the host range of rhizobia isolated from nodules of *Tephrosia purpurea, Crotolaria medicaginea, Leucaena leucocephala a*nd *Sesbania sesban* in other legumes grown in Ajmer and Bikaner regions of Rajasthan.

2. Materials and Methods

Experimental site

Two regions (A-Ajmer and B-Bikaner) of Rajasthan were selected representing the typical semi-arid and arid zone, respectively with sparse vegetation.

Region A: Ajmer

Ajmer district occuping an area of 8481 km^2 is located in the centre of Rajasthan state at 26°27' North latitudes and 72° 42' East longitudes (Source: www.mapsofindioa.com/maps/rajasthan). The average maximum temperature recorded is 46.0°C. The annual rainfall is below 500 mm, showing a semi-arid climate

(Khan, 1999). The northwestern part is covered with sand dunes and rest of the area is generally flat. Hydrogeologically, the major part of the region is occupied by crystalline rocks comprising of calc-schist, amphibolite/calc-gneiss and biotite schist (all Precambrian); sand and alluvium of younger age are other important formations (GSI, 1977; Srivastava, 2001).

Region B: Bikaner

Bikaner district lies in the north-west of Rajasthan in heart of 'Thar' desert at 28°01' North latitude and 73° 22' East longitude (Source: www.mapsofindioa.com/maps/rajasthan) comprising a total geographical area of 27, 244 sq km (CAZRI, 1990). Climate of the district ranges from arid in the east to extremely arid in the west. The mean rainfall of the district is 247 mm varying from 300mm in the east to 180mm in the west. The annual potential evapo-transpiration is 1770 mm (Gheesa, 1999). The mean maximum temperature ranges from 24.4 to 43.80°C and mean minimum for 7.3 to 31.0°C. Frequent drought once in 2.5 years is a common phenomenon. Soils of this district are predominately light textured, weak structured and well drained. All the soils are calcareous, amount of calcium carbonate increases with depth merging at lower depths with lime concretionary zone particularly in the flat aggraded older alluvial plains and the flat interdunal plains (CAZRI, 1974).

Collection of nodules and isolation of rhizobia

Nodules were collected from tree and herb legumes growing in Ajmer and Bikaner regions under arid and semiarid environment. *L. leucocephala, S. sesban, T. purpurea,* and *C. medicaginea* were selected due to their importance in agroforestry, reforestation, and reclamation programs.

Rhizobia were isolated from the nodules as described by Somasegaran and Hoben (1994). Bacterial isolates were examined for their phenotypic and biochemical characteristics followed by the plant assay test with parental host legume.

Legume species and rhizobial strains used for cross inoculation study

Seven tree legumes {*L. leucocephala, S. sesban, A. senegal, A. leucophloea, A. lebbeck, P. juliflora* and *A. nilotica*} as well as seven herb legumes {*V. radiata, D. lablab, C. medicagenia, I. tingtoria, P. sativum* and *T. purpurea* were used for cross inoculation study. These legume species were selected on the basis of their importance as medicinal legumes, grain legume and major agroforestry species.

Eight rhizobial strains from tree legumes (five from *L. leucocephala* and rest three from *S. sesban*) and seven from herb legumes (Four from *T. purpurea* and three from *C. medicaginea*) were used in the study. Studied rhizobial strains were randomly selected from a collection of over 30 isolates obtained from Ajmer and Bikaner regions of Rajasthan.

Seedling preparation and inoculation

Scarification and sterilization of hard-coated seeds were achieved by treatment with concentrated H_2SO_4 for varied periods of time depending on the seed (Somasegaran and Hoben, 1994). Seeds that required no scarification were soaked in 3% (v/v) sodium hypochlorite for 5 min for sterilization. All treated seeds were thoroughly rinsed with sterile distilled water until all traces of acid or hypochlorite were removed. *V. radiata* and *V. mungo* seeds were germinated on 1% (w/v) water agar at 28°C and transferred in growth pouches after 2 – 4 days. Whereas rhizobial inoculum were prepared for other species (*L. leucocephala, S. sesban, A. senegal, A. leucophloea, A. lebbeck, P. juliflora, A. nilotica, D. lablab, C. medicagenia, I. tingtoria, P. sativum* and *T. purpurea*)

Authenticated pure rhizobial strains were grown in YEM (Yeast Extract Mannitol) broth for 3-5 days until CFUs reached 10^9 to 10^{11} cells ml^{-1} and seedlings were inoculated by applying 1 ml of undiluted rhizobial culture directly on the roots of seedlings. Thereafter, seedlings were transferred into growth pouches. Uninoculated seedlings served as control for both the legumes.

A mixture of phosphorus free sterilized activated charcoal (pH 6.8) and sand (3:1) was used as carrier for rhizobial inoculants. Carrier inoculant having around 10^9 to 10^{11}

bacterial cells g^{-1} was applied to surface sterilized legume seeds before showing by using 10% sugar (Jaggery) solution (Ndoye et al., 1990) as a sticker material for proper seed pelleting. Seeds without bacterial treatment served as control.

Growth conditions

Seedlings of *V. radiata* and *V. mungo* were grown in growth pouches (Mega Int., Minneapolis, MN, USA) under growth chamber's controlled conditions (14 h light, 12,000 lux value, 28-30°C temperature and 70 - 80% humidity) and watered with sterile N-free Jensons media (Moreira *et al.,* 1998) while rhizobial inoculants were prepared for other legume seeds which were grown in sterilized soil filled in pots under green house conditions (30-35°C temperature and 65-80 % humidity and light 1200 to 16000 lux value).

For herbaceous and woody species, respectively, nodule assessment and harvest of plants were carried out 6 week after inoculation. Plants were scored for nodulation as: (1) positive nodulation (+) when plants nodulated and were greener than uninoculated plants (2) ineffective nodulation ($+^{b}$) when nodules were white and same as uninoculated plants and (3) Negative nodulation (-) when no nodulation were observed in plant roots.

3. Results and Discussion

In the nodulation tests, each strains elicited nodules on the roots of its homologous legume host, and all uninoculated control plants were without nodules. The host range was reported of a genetically diverse group of rhizobia isolated from nodules of *L. leucocephala, S. sesban, T. purpurea,* and *C. medicaginea.* Promiscuity of *L. leucocephala* was observed for both tree and herb rhizobia but *S. sesban* was very specific in their rhizobial requirement and could nodulate only by its own rhizobia. This finding was supported by Ndoye *et al.* (1990) who reported that *S. sesban* is more specific with its rhizobia being compatible with few species outside the genus *Sesbania*. Moreover, Cummings *et al.,* (2009) reported that *Sesbania* formed effective symbiosis with only genera *Rhizobium* or *Ensifer*. The result of the study indicated that selected eight rhizobial strains could nodulated *A. lebbeck,* 2 from *Leucaena (*ALL-5 & BLL-7), 1 from *Sesbania* (ASS-1), 3 from *Tephrosia* (ATP-2, ATP-3, ATP-4) and 2 from *Crotalaria* (ACM-1 & ACM-6). In the current study, *Acacia* spp. were not found be nodulate by any of the studied isolates although they were capable of forming nitrogen fixing nodules by a wide variety of rhizobia (Räsänen and Lindström, 2003; Ngom et al., 2004; Amrani et al., 2010). The failure of nodulation in these legumes might be explained by loss of sym plasmid (Kucuk et al., 2006) or lateral gene transfer (Haukka et al., 1998) or by changes in Hsn (host specific nod) gene (Han et al., 2008), which are responsible for host specificity. *P. vulgaris* is a promiscuous host and is nodulated by a range of rhizobia including isolates taken from *Leucaena* sp. (Mhamdi et al., 1999; Aguilar et al., 2004) as also observed in the current study. The above mentioned phenomenon probably arises from the fact that the *Nod* factors, which play an important role in the nodulation process, may be very similar in both the legumes (Räsänen and Lindström, 2003). Three tree rhizobial isolates viz., ALL-5, BLL-7 and ASS-1 could nodulate *V. mungo*. Similar results were reported by Mahmood and Akthar (2008), when *V. mungo* was successfully cross inoculated with the rhizobial isolates from tree legume. However, in the research herb rhizobia was also able to nodulate *V. mungo.* Like *Sesbania, T. purpuria* was very specific in their rhizobial requirement and could nodulate only by their own rhizobia. Almost similar specificity has also been observed by *C. medicaginea*. However, *P. sativum* could not nodulated by any isolates. While ineffective nodules have been observed in *D. lablab* by 1 tree (BLL-1) and 3 herbs (ATP-4, ATP-9 & ACM-1) rhizobia. As formation of ineffective nodules is so common among woody and cultivated legumes, it might be possible that ineffective nodules have some importance for rhizobia or plants (Räsänen and Lindström, 2003). Furthermore, Sprent (2001) suggested that at the community level both plants and bacteria may gain advantage from the formation of ineffective nodules. Like,

Acacia spp. studied some herbaceous legumes could not be nodulated by any of the studied rhizobial isolates. It is possible that plants used in the assay relatively insensitive to certain type of symbiotic effects. Alternatively, it is possible that the mutations are critical for the nodulation of some cultivars of plants not yet used in the assays.

This study would, therefore, form the basis for field trial experiments to test the effectiveness of the cross infected rhizobial isolates and the effective isolates may be used as inoculums in cross infected legumes to increase their productivity under legumes growing area of the tropical country.

Figure 1: Gram's negative bacterial cells

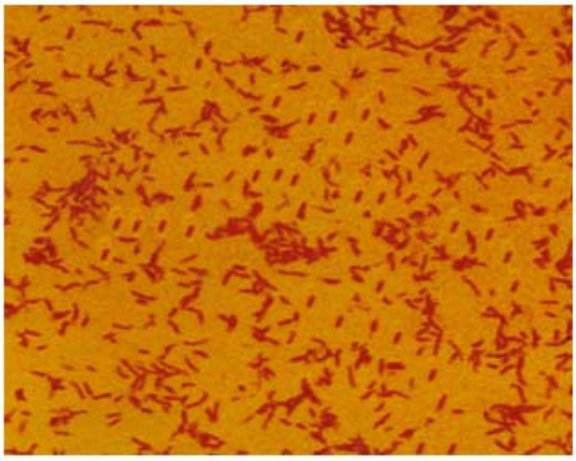

Figure 2: T.S. of Fix+ve nodules of *L. leucocephala* and *S. sesban*

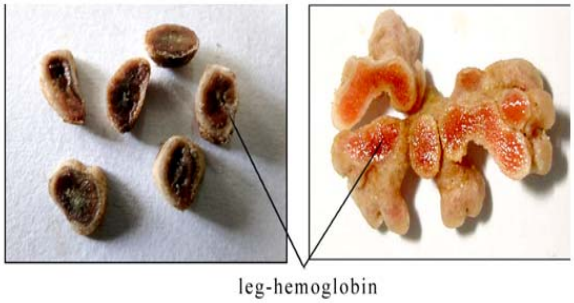

Figure 3: Nodulation in different host legumes

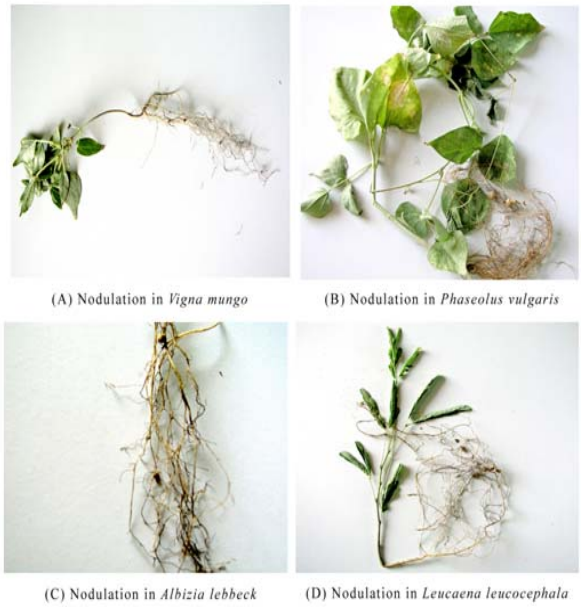

Figure 4: Pot experiment for determination of host range for rhizobial isolates in a mist house

Table 1: Cross inoculation studies using the strains from *L. leucocephala, S. sesban, T. purpuria* and *C. medicagenia*

Tree / Herb	Host Legumes	Rhizobial isolates														
		ALL-1	ALL-2	ALL-5	BLL-1	BLL-7	ASS-1	ASS-4	ASS-7	ATP-2	ATP-3	ATP-4	ATP-9	ACM-1	ACM-2	ACM-6
Tree	L. leucocephala	[+]	[+]	[+]	[+]	[+]	[+]	[-]	[+]	[-]	[+b]	[-]	[-]	[-]	[+]	[+]
Tree	*A. lebbeck*	[-]	[-]	[+]	[-]	[+]	[+]	[-]	[-]	[+]	[+]	[+]	[-]	[+]	[-]	[+b]
Tree	*P. juliflora*	[+]	[-]	[+]	[-]	[-]	[-]	[-]	[+]	[-]	[-]	[-]	[-]	[+]	[-]	[-]
Tree	*A. nilotica*	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]
Tree	S. sesban	[-]	[-]	[-]	[-]	[+]	[+]	[+]	[+]	[-]	[-]	[-]	[-]	[-]	[-]	[-]
Tree	A. senegal	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]
Tree	A. leucophloea	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]
Herb	*V. radiata*	[-]	[-]	[-]	[-]	[-]	[-]	[+b]	[-]	[+]	[-]	[+]	[+]	[+]	[-]	[+]
Herb	*V. mungo*	[-]	[-]	[+]	[-]	[+]	[+]	[-]	[-]	[-]	[-]	[-]	[+]	[+]	[-]	[+b]
Herb	*D. lablab*	[-]	[-]	[-]	[+b]	[-]	[-]	[-]	[-]	[-]	[-]	[+b]	[+b]	[+b]	[-]	[-]
Herb	*P. sativum*	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]
Herb	T. purpuria	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[+]	[+]	[+]	[+]	[-]	[-]	[-]
Herb	C. medicagenia	[-]	[-]	[-]	[-]	[-]	[-]	[+]	[-]	[-]	[-]	[-]	[-]	[+]	[+]	[+]
Herb	I. tinctoria	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]	[-]

[+] Positive Nodulation; [+b] Nodulation but not effective; [-] No nodulation

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