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Selection of Growth Promotory Rhizobia for *Dalbergia sissoo* from Diverse Soil Ecosystems of India

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Abstract

Root nodule bacteria were recovered from the leguminous tree, *Dalbergia sissoo* growing in the states of Jammu and Kashmir, Punjab, eastern Uttar Pradesh and Tarai belt of Uttaranchal (26.45°N to 34.25°N Lat. and 74.57°E to 82.10°E long). Greenhouse trials were carried out on *Dalbergia sissoo* clones PSC-2, PS-31 and D for two consecutive years to screen the bacterial strains (35) for nodulation specificity and improvement of plant growth potential. Considerable strain variation in host genotype specificity with respect to nodulation and nitrogen fixation was observed. During the first year trial involving 35 strains, 19 nodulated *D. sissoo* clone PSC-2. In all the treatments that were positive for nodulation, nitrogen percent per g shoot dry wt. ranged from 0.4–1.8, DSNP2d was an exception where N level was 0.1%. Eighteen bacterial strains which were positive for nodulation in the first year trial were further tested on clone PS-31 and D. Nodulation and effectiveness of nodulation was plant host-genotype dependent. All eighteen strains formed nodules on clone PS-31 whereas only eight nodulated clone D. Bacterial strains DSNPNA13 and DSNJKS7 effectively nodulated clone PS-31 as confirmed by nitrogenase activity (280.6 and 399.5 µmole of ethylene / g nodule fresh wt. / min); they were however ineffective against clone D. The nodule number did not show any correlation with the level of ethylene formed or the plant

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shoot dry weight. The number of nodules per plant in DSNPNJ8 treated clone D and clone PS-31 was 39.4 and 76.6 whereas shoot dry wt. in the corresponding treatments was 3.9 and 2.2 g, respectively. The amount of ethylene formed was 96.4 and 2050.0 µmole, respectively. Considerable variability amongst the strains has been observed at the genetic level based on whole cell protein profiles. This could partly explain variations in nodulation and nitrogenase activity of strains vis-à-vis host genotype. Among *D. sissoo* bacterial strains, 20 nodulated *Sesbania aculeata*, whereas three each nodulated *Leucaena leucocephala* and *Vigna mungo*.

Keywords: Effectiveness, *Dalbergia sissoo*, nodulation, rhizobia

1. Introduction

Occurrence of nodules in different tree legumes was recorded by Allen and Allen (1981). Transplantation of nitrogen fixing leguminous trees (NFLTs) which can establish themselves in barren lands is a key to check further spread of semiarid and arid landscape (Turk and Keyser, 1992). Leguminous trees useful in reclamation of salt affected soils include *Acacia nilotica, A. auriculiformis, Dalbergia sissoo* and *Prosopis juliflora* (Singh et al., 1994; Sun and Dickinson, 1995; Patil et al., 1996). Kabi et al. (1982) observed that use of rhizobial biofertilizers in forestry has considerable potential for nitrogen turn-over from the atmosphere. Several workers have reported that NFLTs accumulate as much as 580 kg N ha^{-1} per annum (Kadiata et al., 1996; Dakora and Keya, 1997). Although biological nitrogen fixation (BNF) is an important attribute of NFLTs, little has been done to delineate their rhizobial specificity in terms of nodulation and nitrogen fixing ability. Such knowledge is a prerequisite to develop effective inoculants for NFLTs, since they are to be introduced in degraded soils, which usually lack compatible rhizobia.

Dalbergia sissoo (vern. shisham) has been identified as one NFLTs species that is suitable for transplantation in a tropical country like India. It is the natural inhabitant of foothills of Himalayas from eastern Afghanistan through Pakistan and India to Nepal. It occurs from sea level to >1500 m (Tewari, 1994). It is also grown in sewage-irrigated greenbelt in Khartoum, Sudan and as shade tree in Arizona and Florida (USA). *Sissoo* is the most useful multipurpose tree of South Asia (NAS, 1979, 1983). It is a primary colonizer of new alluvial soils and often occurs in association with *Acacia catechu* (Khair-*sissoo* forest). *Dalbergia sissoo* is known internationally as a premier timber species, is also an important fuelwood, shade, shelter and fodder tree. With its multipurpose products and ability to tolerate frost as well as long dry seasons, makes this species suitable for agroforestry applications. Being a member of the subfamily Papilionoideae of the family Leguminosae, this species is nitrogen fixing. Mixed cropping with *sissoo* resulted in marked increase in the

production of wheat as compared to monocrop of wheat (Joker, 2002). Preferential nodulation of host genotype with indigenous strains of rhizobia has been reported (Lieven-Antoniou and Whittam, 1997). Moreover, even when roots become nodulated, the process of nitrogen fixation is not always efficient since compatible rhizobia are often not present in soil. Seedling health and its establishment is the key to success of any tree transplantation programme. Application of an effective rhizobial strain during establishment of nursery can result in build up of necessary vigour so that seedlings are able to sustain the transplantation shock.

Barnett and Catt (1991) reported that strains from nodules of Australian *Acacia* species possessed a broad host-range and nodulated members in the families Mimosaceae, (the acacias) and Fabaceae (*Kennedia* and *Macroptilium*). On the other hand, *Gliricidia sepium, Calliandra calothyrus* and *Leucaena leucocephala* required specific strains of rhizobia for establishing effective symbiosis (Turk and Keyser, 1992). Genetically distinct bacteria have been isolated from bean nodules (Segovia et al., 1993; Eardly et al., 1995; Hernàndez-Lucas et al., 1995; Martínez-Romero et al., 1991, 1996), *Acacia, Amorpha fructicosa, L. leucocephala* (de Lajudie et al., 1994) and *Sesbania* (Boivin et al., 1997). In *Prosopis glandulosa*, surface and phreatic roots bear nodules formed by different species of rhizobia (Jenkins et al., 1987).

Considering the importance of *Dalbergia sissoo* in agroforestry and reforestation programmes in the tropics, this study was carried out with *D. sissoo* to assess nodulation and nitrogen fixation efficacy under artificial inoculation based on the screening of a gene pool of bacteria recovered from various genotype and soil types in the country.

2. Materials and Methods

Isolation of bacteria from nodules

Nodules from *Dalbergia sissoo* stands in the states of Jammu and Kashmir, Punjab, eastern Uttar Pradesh and Tarai belt of Uttaranchal (26.45°N to 34.25°N Lat. and 74.57°E to 82.10°E long.) were collected in sterile polyethylene bags and brought to the laboratory. They were washed several times in sterile distilled water (SDW), surface sterilized in 95% ethanol for 1 min and 0.1% $HgCl_2$ acidified with conc. H_2SO_4 for 5 min followed by repeated washing with SDW. Sterile nodules were crushed and the resulting suspension streaked onto yeast extract mannitol agar (YEMA) plates (Somasegaran and Hoben, 1985). Single colonies which did not take up congo red dye were subcultured twice to recover pure cultures and only those showing Gram negative reaction were selected for further study (Table 1). All 35 strains were grouped into slow

growers and fast growers based on acid/alkali production in YEM agar plates supplemented with 25 ppm bromothymol blue dye (Somasegaran and Hoben, 1985). The change of colour from green to blue indicated presence of slow growers (alkali reaction) and to yellow, fast growers (acid production).

Selection of Dalbergia sissoo genotypes

Three clones of *Dalbergia sissoo* were selected based on their performance in agroforestry system. Seeds for clones PSC-2 and PS-31 were obtained from Dr. Salil Tewari, Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture & Technology and those of Clone D, from Dr. T.C. Pokhariyal, Forest Research Institute, Dehradun. Primary screening of all thirty five bacterial strains for nodulation and growth performance was carried out during April–September 1999 on the *Dalbergia* clone PSC-2. Further assessment of select rhizobial strains was conducted during April–September 2000 on two other *Dalbergia* clones PS-31 and D.

Growth systems and experimental design

For nodulation trial, seedlings were raised in 1 l plastic pots (6×6″) containing 900 g sterile potting mixture which comprised of soil:sand in a ratio of 2:1 (w/w). Three plants were grown in each pot. Plants were watered regularly with SDW. Half strength nitrogen free Hoagland's solution (Somasegaran and Hoben, 1985) was provided to the seedlings at 15 d interval. Pots were covered with sterile gravel following inoculation to help prevent rhizobial contamination. Pot experiment was set up in a greenhouse in completely randomized design and each treatment was replicated 3 times.

Seedling preparation, inoculum preparation and inoculation

Seeds were separated from pods and surface sterilized by soaking in 0.1% $HgCl_2$ acidified with H_2SO_4 for 5 min, followed by 5 rinses in SDW. Surface sterilized seeds were soaked in SDW for 2 h prior to sowing. Seedlings were germinated in a nursery bed of sterile soil:sand mixture (2:1, w/w) and transplanted into pots after 21 d.

Rhizobial strains were grown in 250 ml Erlenmeyer flasks containing 100 ml YEM broth (Somasegaran and Hoben, 1985) shaken at 130 rpm, at 28±1°C for 5 d. One ml of undiluted rhizobial broth containing approx. 10^9 cells ml^{-1} was applied directly to roots of the seedlings within 24 h of transplantation. A similar booster dose of the inoculum was also applied directly to the roots of seedlings 21 d after 1st inoculation.

Table 1. List of bacterial strains used in the study.

S. No.	Isolate code	Location	Latitude/longitude
1.	DSNPN15	Kansapur, Punjab	31.0°N Lat.–76°E Long.
2.	DSNPNA12		
	DSNPNA13	Rajpura, Ambala	30.4°N Lat.–76.6°E Long.
3.	DSNPNA14,		
	DSNPNJ8	Amritsar	31.35°N Lat.–74.57°E Long.
4.	DSNJKS7, DSNPNH16	Haathikunda, J&K	34.25°N Lat.–77.0°W Long.
5.	DSNF10	Kumarganj, Faizabad	26.45°N Lat.–82.10°E Long.
6.	DSNP1a, DSNP1b	Pantnagar	29.0°N Lat.–79.30°E Long.
	DSNP2a, DSNP2b		
	DSNP2d, DSNP3		
	DSNP5a, DSNP6a, DSNP7a		
	DSNP8Bcx, DSNP9c		
	DSNP11a, DSNP10Ba		
	DSNP10Bc, DSNP14a		
	DSNP15bM, DSNP15bW		
	DSNP16a, DSNP16e		
	DSNP18b, DSNP21a		
	DSNP22cx, DSNP26b		
	DSNP27b, DSNP27c		
	DSNP28a, DSNP28b		

Bacterial strains, DSNP7a, DSNP26b, DSNPNJ8, DSNP1a, DSNP27b, DSNP28b, DSNP2b, DSNJKS7 and DSNP11a have been deposited in the MTCC culture collection, Chandigarh, India under the accession no. MTCC 4186 to MTCC 4194.

Harvest of plants

Plants were uprooted carefully to record nodulation, shoot dry wt. per plant, nitrogen content in shoot and *in situ* nitrogenase activity to measure effectiveness of symbiosis. Nitrogen content in shoot was analysed using Kjeldahl digestion according to Jackson (1958).

Nitrogenase activity was measured by the acetylene reduction assay (ARA) according to Hardy et al. (1968) where the amount of ethylene formed was calculated by the formula:

$$\mu\text{mole of } C_2H_4 \text{ formed/g/min} = \frac{\text{Peak height (mm)} \times \text{gas phase (ml)}}{\text{wt. of nodule (g)} \times \text{C2H4 factor} \times \text{time of incubation (min)}}$$

Analysis of data

The data from the greenhouse trials carried out during 1st year (April–September 1999) and 2nd year (April–September 2000) was statistically analyzed separately. In greenhouse trials, the performance of strains was evaluated using analysis of variance (ANOVA).

Cross nodulation studies with bacterial strains

All 35 bacterial strains were tested for cross nodulation on *Sesbania aculeata, Leucaena leucocephala* and *Vigna mungo* variety PU-35. Seeds of these three legumes were obtained from Crop Research Centre (CRC), G.B. Pant University of Agriculture & Technology. Experimental design, potting mixture, inoculum preparation and inoculation procedure were same as those with nodulation trial on homologous host (*Dalbergia sissoo*).

Phylogenetic relationship among the bacterial strains based on whole cell protein profiles

Whole cell protein extracts were prepared according to Laemmli (1970). SDS solubilized whole cell protein extracts were analyzed by one-dimensional discontinuous vertical SDS-PAGE with 12/4% gel system consisting of separating-stacking gels using TRIS glycine electrode buffer (Hames, 1990). Coomassie Brilliant Blue stained gels were observed on a UVP gel documentation system. The protein profiles from different gels were normalized using protein molecular weight marker (Medium range) supplied by Bangalore Genei, India. The profiles between 14 and 93 KDa were used for constructing UPGMA dendrogram, calculating dice coefficient with QUANTITY ONE software in GS-710 calibrated Imaging densitometer (Bio-Rad).

3. Results

Primary screening of rhizobial strains on Dalbergia sissoo clone PSC-2

Based on acid/alkali reaction all strains except DSNP8Bcx were fast growing. Of the thirty-five bacterial strains screened during April–September 1999, only nineteen, all of which were fast growing, nodulated clone PSC-2. Strains, which formed nodules, produced relatively effective symbiosis as evident by shoot dry weight and nitrogen content (Table 2). Amongst nodulating strains there was no significant relationship between the shoot dry weight of strains DSNF10 and the uninoculated controls whereas, highly significant in

Table 2. Screening of rhizobial strains on *D. sissoo* clone PSC-2 for growth performance in nursery conditions.

Treatment	Shoot dry wt.	Nodule No.	Nitrogen %
Control	0.2±Nil^{ab}	0.0^{a}	0.6±Nil^{c}
Kansapur, Punjab			
DSNPN15	0.6±Nil^{bc}	10.33±0.5^{d}	0.9±Nil^{d}
Rajpura, Ambala			
DSNPNA12	0.5±0.1^{bc}	0.0^{a}	1.4±Nil^{f}
DSNPNA13	0.9±0.1^{cd}	23.67±1.2^{g}	0.9±Nil^{d}
Amritsar			
DSNPNA14	0.8±0.1^{cd}	0.0^{a}	0.4±Nil^{b}
DSNPNJ8	0.7±0.1^{c}	23.33±1.9^{g}	1.4±Nil^{f}
Haathi Kunda, Jammu & Kashmir			
DSNJKS7	0.4±0.1^{b}	9.33±0.5^{cd}	0.9±Nil^{d}
DSNPNH16	0.5±Nil^{bc}	7.33±1.2^{c}	0.5±Nil^{bc}
Kumarganj, Faizabad			
DSNF10	0.3±Nil^{ab}	8.67±0.5^{cd}	1.1±0.1^{e}
Pantnagar			
DSNP1a	0.2±0.1^{a}	0.0^{a}	1.2±Nil^{e}
DSNP1b	0.6±Nil^{bc}	0.0^{a}	1.2±0.2^{e}
DSNP2a	0.6±0.1^{bc}	0.0^{a}	1.7±0.1^{gh}
DSNP2b	0.9±Nil^{cd}	30±1.6^{h}	0.4±Nil^{b}
DSNP2d	0.4±Nil^{b}	13.33±2.5^{e}	0.1±Nil^{a}
DSNP3	0.3±0.1^{ab}	0.0^{a}	1.1±Nil^{e}
DSNP5a	0.3±0.1^{ab}	0.0^{a}	0.8±Nil^{d}
DSNP6a	0.4±Nil^{b}	0.0^{a}	1.4±Nil^{f}
DSNP7a	0.5±0.1^{bc}	7.67±0.5^{c}	0.9±Nil^{d}
DSNP8Bcx	0.4±Nil^{b}	0.0^{a}	1.5±Nil^{fg}
DSNP9c	0.5±Nil^{bc}	2.7±0.5^{b}	0.7±Nil^{cd}
DSNP10Ba	0.4±Nil^{b}	0.0^{a}	0.8±0.1^{d}
DSNP10Bc	1.0±0.2^{d}	25.3±0.5^{g}	1.5±Nil^{fg}
DSNP11a	0.7±Nil^{c}	12.7±0.5^{e}	1.8±Nil^{h}
DSNP14a	0.5±0.1^{bc}	0.0^{a}	0.1±Nil^{a}
DSNP15bM	0.4±Nil^{b}	0.0^{a}	0.7±Nil^{cd}
DSNP15bW	1.4±0.1^{ef}	20.3±1.7^{f}	0.9±Nil^{d}
DSNP16a	0.7±Nil^{c}	10.0±1.4^{d}	1.8±Nil^{h}
DSNP16c	0.4±Nil^{b}	0.0^{a}	0.6±Nil^{c}
DSNP18b	0.3±Nil^{ab}	0.0^{a}	1.1±Nil^{e}
DSNP21a	0.1±0.2^{a}	0.0^{a}	0.4±Nil^{b}
DSNP22cx	0.5±0.1^{b}	0.0^{a}	0.9±Nil^{d}
DSNP26b	1.3±0.1^{e}	30.3±2.4^{h}	1.1±Nil^{e}
DSNP27b	2.2±0.1^{g}	29.7±2.6^{h}	1.6±Nil^{g}
DSNP27c	1.2±0.3^{de}	28.7±2.0^{h}	0.9±Nil^{d}
DSNP28a	1.6±0.1^{f}	23.3±1.2^{g}	1.7±Nil^{gh}
DSNP28b	1.7±0.2^{f}	31.3±0.5^{i}	1.2±Nil^{e}

Values are mean of three replicates ±SD; letters in superscript showing overlap of significance cd at 5%: shoot dry weight, 0.2; nodule no., 2.1; nitrogen % , 0.1.

treatments with strains DSNP27b, DSNP28a, DSNP28b (Table 2). Nodule number and nitrogen content of shoot were not related. This is evident from great variation in the nitrogen content in shoot for bacterial treatments forming no nodules. In DSNP14a it was 0.1% whereas in DSNP1a, DSNP1b and DSNP2a was as high as 1.2, 1.1 and 1.7%, respectively. Dry weight of shoot was maximum (2.2 g) in treatment with DSNP 27b whereas nitrogen was maximum (1.8) in case of strains DSNP11a and DSNP16a.

Assessment of select rhizobial strains on Dalbergia sissoo clones PS-31 and D

All eighteen rhizobial strains based on first selection were able to nodulate clone PS-31, however only eight i.e., DSNPN15, DSNPNA13, DSNP9c, DSNP2b, DSNP26b, DSNPNJ8, DSNP7a and DSNJKS7 formed nodules on clone D (Table 3). Shoot dry weight was not directly related to the number of nodules per plant. For example, nodule number in DSNPNJ8 treated clones PS-31 and D was 76.6 and 39.4, whereas shoot dry weight in the corresponding treatments was 2.2 and 3.9 g, respectively. Similarly, in treatment with bacterial strain DSNP2b, nodule numbers were 57.2 and 86.6 for the two clones and dry weight of shoot 2.1 and 1.8 g, respectively (Table 3).

Symbiotic effectiveness was measured on the basis of ethylene formed in the nodules. However, nodulation of plants did not always result in ethylene formation. Nodulation of clone PS-31 by strains DSNPNH16 and DSNP7a was ineffective since no ethylene was detected (Table 3). Effectiveness of nodulation was host-genotype dependent. For example, bacterial treatments DSNPNA13 and DSNJKS7 effectively nodulated PS-31 and released 280.6 and 399.5 µmole of ethylene but they were ineffective against clone D. On the contrary, treatment with bacterium DSNP7a was effective in clone D and ineffective in clone PS-31. Genotype dependent change was also observed in levels of ethylene detected. For example, treatment of clones D and PS-31 with DSNPNJ8 resulted in formation of 96.4 and 2050.0 µmole of ethylene, respectively.

Phylogenetic relationships amongst bacterial strains based on analysis of whole cell protein profiles

SDS-PAGE profiles of whole cell protein extract showed high level of variability amongst the bacterial strains. Two highly variable regions were in the range, 14.3–20.0 kDa and 43–66 kDa. Several workers have used this technique to estimate genetic diversity (Versalovic et al., 1994; Laguerre et al., 1997). Phylogenetic relationships from UPGMA derived dendrogram showed that the level of similarity between the bacterial strains varied between 50 to 85% (Fig. 1). The difference among the strains was not only at the species but also at the generic level.

Table 3. Effect of inoculation with rhizobia on nitrogenase activity, nodulation, shoot dry weight of two clones of *Dalbergia sissoo*.

Treatment	Nitrogenase activity (µmole/g nodule fresh weight/min)		Nodule No.		Shoot dry weight (g)	
	Clone PS-31	Clone D	Clone PS-31	Clone D	Clone PS-31	Clone D
Control	2.0^{a}	0.0^{a}	1.4±0.5^{a}	0.0^{a}	1.1±Nil^{a}	1.6±0.1^{bc}
DSNP26 b	44.1±2.6^{a}	615.1±8.3^{d}	70.8±10.4^{e}	14.4±2.7^{bc}	3.3±0.3^{de}	2.5±0.4^{c}
DSNPNJ 8	96.4±2.2^{b}	2050.0±1.2^{f}	76.6±2.2^{e}	39.4±7.1^{e}	2.2±0.5^{cd}	3.9±0.6^{e}
DSNP28b	103.2±1.4^{b}	0.0^{a}	48.8±9.4^{cd}	0.0^{a}	1.9±0.5^{b}	0.0^{a}
DSNF10	120.4±2.1^{bc}	0.0^{a}	11.8±2.6^{a}	0.0^{a}	1.3±0.4^{b}	0.0^{a}
DSNP10Bc	131.4±2.7^{bc}	0.0^{a}	35±6.9b^{c}	0.0^{a}	2.1±0.4^{c}	0.0^{a}
DSNP16a	170.9±1.2^{c}	0.0^{a}	27.6±7.2^{bc}	0.0^{a}	2.6±0.2^{cd}	0.0^{a}
DSNP15bW	180.6±2.2^{c}	0.0^{a}	20.6±4.9^{b}	0.0^{a}	2.3±0.7^{cd}	0.0^{a}
DSNP2b	183.4±2.9^{c}	251.1±2.6^{b}	46.2±22.5^{cd}	86.6±11.8^{f}	2.1±0.4^{c}	1.8±0.3^{bc}
DSNP27b	263.6±2.6^{d}	0.0^{a}	77.8±13.8^{e}	0.0^{a}	3.7±0.2^{e}	0.0^{a}
DSNPNA13	280.6±3.3^{d}	0.0^{a}	54.4±8.0^{d}	42.4±2.9^{e}	2.0±0.4^{c}	2.2±0.5^{cd}
DSNPN15	297.7±2.4^{d}	0.0^{a}	47.4±5.0^{cd}	18.4±1.4^{bc}	2.0±0.3^{c}	2.7±0.9^{d}
DSNP9c	122.4±3.2^{bc}	412.8±7.8^{c}	67.8±8.7^{de}	31.0±4.1^{d}	2.1±0.4^{c}	2.2±0.3^{cd}
DSNJKS 7	399.5±7.0^{e}	0.0^{a}	37.0±6.2^{c}	21.2±1.6^{c}	1.9±0.5^{bc}	2.3±0.2^{cd}
DSNP28a	585.2±1.4^{f}	0.0^{a}	47.4±13.4^{cd}	0.0^{a}	2.4±0.6^{cd}	0.0^{a}
DSNP27c	825.0±3.6^{g}	0.0^{a}	44.2±3.0^{cd}	0.0^{a}	1.9±0.4^{bc}	0.0^{a}
DSNP11a	613.1±0.1^{f}	0.0^{a}	94.8±15.8^{f}	0.0^{a}	3.3±0.3^{de}	0.0^{a}
DSNP7a	0.0^{a}	670.7±6.9^{e}	47.4±12.3^{cd}	35.2±7.2^{d}	0.6±0.2^{a}	2.0±0.2^{c}
DSNPNH16	0.0^{a}	0.0^{a}	26.0±6.4^{bc}	0.0^{a}	2.8±0.2^{d}	0.0^{a}

Results are mean of five replicates ±SD; letters in superscript show overlap of significance.

cd at 5%:	Clone D	PS-31
Nitrogenase activity	6.3	60.8
Nodule no.	5.4	15.5
Shoot dry weight	0.4	0.6

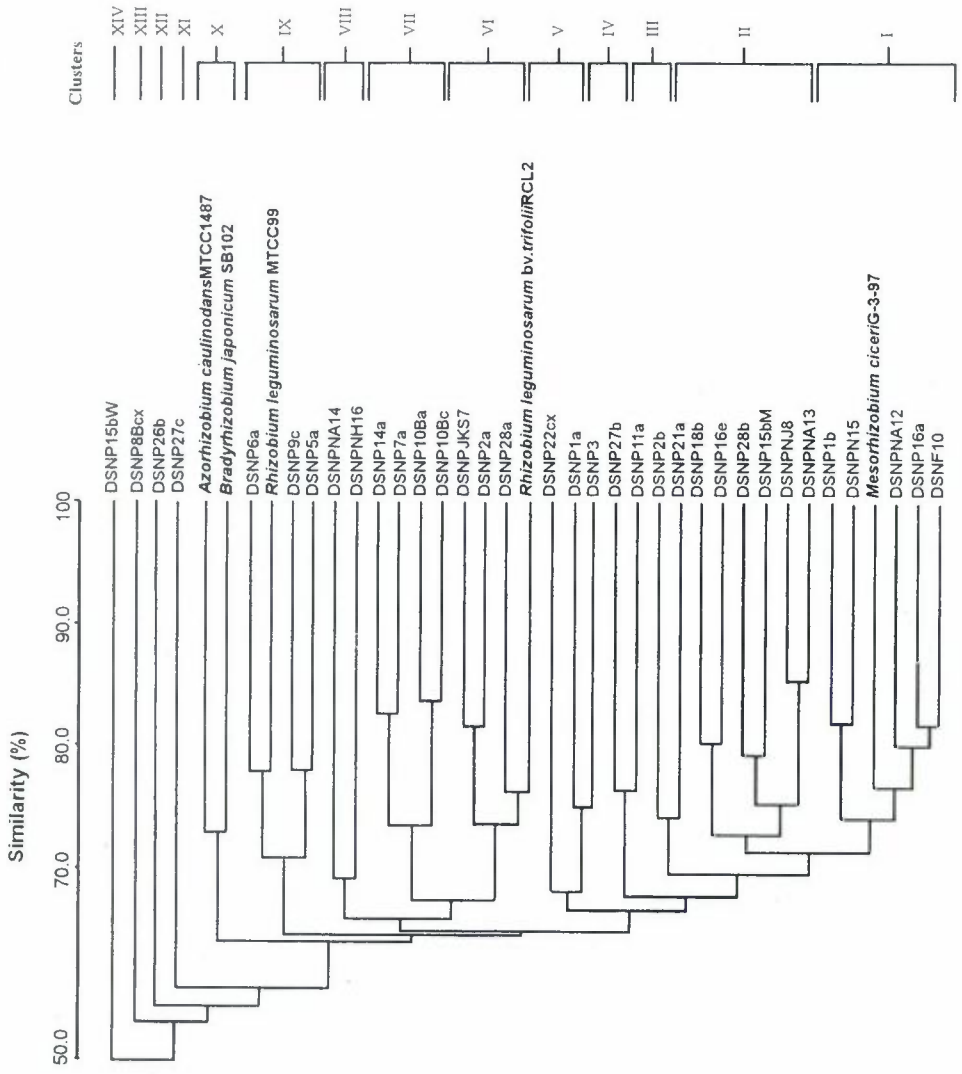

Figure 1. Phylogenetic relationships derived from the unweighted pair group average linkage of dice correlation coefficients between the whole-cell protein patterns of *Dalbergia sissoo* isolates along with reference strains. The correlation coefficient is expressed as percentage similarity.

4. Discussion

In a greenhouse trial for selection of promising rhizobial isolates for growth of *Dalbergia sissoo* seedlings, 19 of the 35 bacteria could nodulate PSC-2. This is likely to be on account of heterogeneity of bacterial strains that were recovered from genetically heterogenous clones of *D. sissoo*. The data on shoot dry weight and nitrogen content supports the view that nodulation was effective.

Genetic heterogeneity amongst the bacterial strains is evident from the whole cell protein profiles on 12% SDS-PAGE. Phylogenies drawn based on whole cell SDS-PAGE profiles reveal that the bacterial strains were genetically diverse with their relationships intertwined, within genera *Azorhizobium, Bradyrhizobium, Rhizobium* and *Mesorhizobium*. Further analysis of these bacterial strains based on ARDRA and outer membrane LPS profiles has confirmed this assumption (Sahgal, 2002). Lindström and Zahran (1993) earlier reported similar observations.

Analysis of the data on nodulation and amount of ethylene detected in the nodules during greenhouse trial with select bacterial strains (April–September 2000) showed that *D. sissoo* clones have highly specific rhizobial requirements. This observation receives support from the findings of Herrera et al. (1985) for *Prosopis chilensis* and Abdel Magid et al. (1988) and Ndoye et al. (1990) for *Sesbania grandiflora*.

There is considerable rhizobial specificity for nodulation vis-à-vis host genotype as is evident from reports on *Acacia* spp., *Albizzia lebbeck, Leucaena leucocephala, Robinia pseudoacacia* and *Sesbania grandiflora* (Trinick, 1968; Galiana et al., 1990; Ndoye et al., 1990; Turk and Keyser, 1992).

In our study, nineteen bacterial strains among a total of 35 nodulated clone PSC-2. Possible reason could be that isolation of bacteria was from genetically diverse *D. sissoo* clones whereas nodulation efficacy was tested on clones that were different. All eighteen strains nodulating PSC-2 formed nodules on PS-31 also whereas, only eight nodulated clone D. This reflects rhizobial specificity for nodulation vis-à-vis host genotype and receives support from previous studies, which confirm that some strains have a narrow host range (Herrera et al., 1985). Cross-nodulation studies revealed that strains from *D. sissoo* nodulated *Sesbania aculeata*; 20 of 35 were found nodulating whereas they did not nodulate *Leucaena leucocephala* and *Vigna mungo*. Our results agree with those of previous studies, confirming that the nodulation capabilities of the tree rhizobia are diverse (Habish and Khairi, 1968, 1970; Dreyfus and Dommergues, 1981; Zhang et al., 1991).

The dendrogram in Fig. 1 shows that there is high level of diversity amongst *D. sissoo* rhizobia. All the strains were grouped in 14 clusters. However, all the strains except DSNP8Bcx were fast growing. Earlier also fast growing tree rhizobia from nodules of 11 tree species including *Acacia* spp., *Albizzia falcata,*

Centrosema plumieri, Leucaena leucocephala, Prosopis chilensis and *Sesbania punctata* were placed in 19 clusters (Zhang et al., 1993). They report that isolates nodulating *Acacia senegal* and *Prosopis chilensis* were diverse, spread in 11 of the nineteen clusters and these clusters were not related to each other. In the present study Cluster no. I was related to *Mesorhizobium ciceri* strain G-3-97, those of cluster no. VI related to *Rhizobium leguminosarum* biovar *trifolii* RCL2. Cluster no. IX related to *Rhizobium leguminosarum* MTCC 99, *Azorhizobium caulinodans* MTCC 1487 and *Bradyrhizobium japonicum* SB 102 were placed in the same cluster. Four strains DSNP27c, DSNP26b, DSNP8Bcx and DSNP15bW were placed distantly to all the other strains.

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