

VARIATION IN NODULE OCCUPANCY OF INOCULATED RHIZOBIAL STRAINS AT DIFFERENT STAGES OF GROWTH IN TWO SPECIES OF *LEUCAENA*

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Nodule occupancy by different rhizobial strains in multi-strain rhizobial inoculants as tested by immunofluorescent technique varied depending upon the species of *Leucaena* and their growth stages. While rhizobial strain TAL 1145 dominated the nodule occupancy during all the growth stages in *L. leucocephala* and *L. diversifolia*, TAL 582 strain was an effective strain only on 50th day and TAL 82 was least effective in all the growth stages in both species of *Leucaena*. Irrespective of the rhizobial strains used in the inoculants, *L. leucocephala* produced cylindrical coralloid and large sized nodules mostly on taproot, while *L. diversifolia* produced small effective spherical nodules mostly on secondary roots. The rhizobial strain TAL 1145 increased the root length three to four folds when compared to control in both the species of *Leucaena*. Irrespective of the rhizobial strains, *L. diversifolia* exhibited higher nitrogenase activity than *L. leucocephala*.

Successful nodulation response of inoculated leguminous plant depends on the competition for nodule sites between the introduced strains and native rhizobia or on the competition between the strains used in the mixed inocula (Skrdleta and Karimova, 1969). *Leucaena* species were known to be infected by fast growing rhizobial strain *Rhizobium loti* (Jarvis *et al.*, 1982). Moawad and Bohlool (1984) reported that the competitive ability of the introduced rhizobial strains of *Leucaena leucocephala* depended upon the soil types; also TAL 1145 strain was found to be the most successful competitor in multistrain inocula irrespective of the soil types. The objective of using multistrain rhizobial inoculant is to enhance the possibility of successful nodulation with any one or more than one of the strains at different growth

periods of the plant. However, no information is available with regard to the nodule occupancy by different rhizobial strain in different *Leucaena* species and hence study was undertaken to determine the variation in nodule occupancy by different strains in single and multistrain rhizobial inoculants in *Leucaena leucocephala* and *L. diversifolia*.

MATERIALS AND METHODS

(i) Rhizobial strain used

Three strains of *Rhizobium* sp. viz., TAL 82, TAL 582 and TAL 1145 obtained from culture collections of NifTAL Project, University of Hawaii, U.S.A., were used in the present studies. Unless otherwise stated, single strain inoculants and multi-strain inoculant prepared out of the above three strains were used throughout the studies.

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(ii) *Preparation of Peat inoculants*

Peat packets of 200 g each sterilized by γ -irradiation (5 mega rads), were injected with 50 ml suspensions containing ca. 10^9 cells/ml and incubated at room temperature (28-30°C) for one week. Following the inoculation the populations of rhizobia were estimated by drop plate method (Miles and Misra, 1938). The required quantity of each single inoculant was mixed in such a way that the population of the strains in the mixture was adjusted to the ratio of 1:1:1.

(iii) *Greenhouse growth experiment*

Experiments were conducted in controlled conditions employing growth pouches (Northrup company, Minnesota, U.S.A.). Seeds of *L. leucocephala* and *L. diversifolia* were acid scarified with conc. sulphuric acid for 5 minutes and washed with 5-6 changes of sterile distilled water. The seeds were then surface sterilized with 0.1% mercuric chloride followed by 5 changes of sterile distilled water and kept immersed in distilled water for 4 hours to imbibe water. The seeds which imbibed water were then planted on water agar in petri plates and incubated for 2 days at 37°C for germination. The germinated seeds were transferred to the top of growth pouches keeping one seed per pouch which contained 30 ml. of nitrogen free nutrient solution (Broughton and Dilworth, 1971). The suspensions of single or multistrain rhizobial inoculant containing 10^9

cells/ml. were added @ 1 ml/pouch. Uninoculated control plants were also maintained. The growth pouches, arranged in racks were kept in greenhouse. Periodically, sterile nitrogen free nutrient solution was added. Five replications were set up for each treatment.

(iv) *Harvest*

Harvest of the plants was made on 30th, 50th day and 70th day. The nodule numbers on the first two stages and the shoot and nodule dry weights and nitrogenase activity of the root samples with nodules on 70th day (Hardy *et al.*, 1968) were recorded.

(v) *Nodule occupancy*

The smears from 20 nodules from each replication were prepared. Fluorescent antibody technique (Date 1974) was followed with fluorescein isothiocyanate as fluorochrome to identify the rhizobial strain in the nodules. The conjugated fluorescent antibodies of TAL 82, 582 and 1145 obtained from the antisera bank of NifTAL, were used to stain the smear and to identify the strain in a fluorescent microscope (Standard 14 with epifluorescence condenser). Cultured cells were used as control. Nodule occupancy was assessed as described by Somasegaran and Hoben (1985).

RESULTS AND DISCUSSION

Population of rhizobia in three single strain inoculants varied from 3.82×10^9 to 11.90×10^9 / g. of inoculant.

(i) *Nodulation*

Nodulation of *Leucaena* species in growth pouches appeared in 15-20 days after planting. Although no variation among the strains in the nodule morphology could be observed, the nodule size and occupancy in the root varied with the *Leucaena* species. While *L. leucocephala* contained larger nodules of cylindrical, and coralloid shape varying from 3-5 mm in length and 1.5 to 3 mm in width located on tap root, *L. diversifolia* contained only smaller nodules, mostly spherical measuring 0.5 to 2.5 mm in width, were present on the secondary roots. Irrespective of the strain, the nodules on *L. leucocephala* were big in size and located in tap root while those on *L. diversifolia* were smaller and distributed throughout the secondary roots although some could be observed in tap root also.

The nodulation data in Table 1 indicated that the strain TAL 1145 was more competitive in causing nodulation on 30th and 50th day of planting in both species of *Leucaena*. The data on the shoot and nodule dry weights and nitrogenase activity of the two *Leucaena* species (Table 2) on 70th day of planting clearly indicated that TAL 1145 and mixed inoculant were very effective in increasing the shoot dry weight and nodule dry weight. The nitrogenase activity of the nodules revealed that TAL 1145 was found to be superior over other strains. Recently, Somasegaran and Martin (1986) also

reported that mixed strain inoculant was inferior to TAL 1145 alone in a hybrid of *L. leucocephala* and *L. diversifolia*.

(ii) *Effect of inoculation on root length*

Rhizobia were reported to produce growth promoting substances such as indole acetic acid, gibberellic acid, cytokinins etc., in infection processes (Vincent, 1977). Growth of root may also be influenced by the appropriate effective rhizobial strains. The results on the root length (Table 2) of *Leucaena* species clearly indicated that all the single or multistrain inoculants increased the root length over control. However, TAL 1145 increased the root length three folds over uninoculated control.

(iii) *Nodule occupancy by different rhizobial strains*

In all the growth periods of the plants, the inoculated single strains occupied 100% nodules irrespective of the species of *Leucaena*. But in respect of mixed inoculant during 30th day, *L. leucocephala* contained all the nodules occupied by TAL 1145, while *L. diversifolia* had 80% of the nodules occupied by TAL 1145 (Table 3). Nodule occupancy varied on the 50th day. In *L. leucocephala* 60% of the nodules was occupied by TAL 1145, 30% by TAL 82 with 10% of mixed infections. Similarly, in *L. diversifolia*, 50% of the nodules was occupied by TAL 1145 and 30% by TAL 582 with 20% by mixed infections. During 70th day of planting the 75% nodules of *L. leucocephala*

Table -1. Effect of single and multi-strain rhizobial inoculants on the nodulation of *Leucaena* spp.

Rhizobial Strains	Nodule numbers per plant (mean of 5 replications)			
	<i>L. leucocephala</i>		<i>L. diversifolia</i>	
	30th day	50th day	30th day	50th day
TAL 82	10.3	12.3	8.5	17.3
TAL 582	11.0	16.3	15.1	17.0
TAL 1145	12.2	18.2	18.6	22.0
Mixed inoculant (1:1:1 of 82+582+1145)	13.2	14.8	17.0	19.5
Control (Uninoculated)	—	—	—	—
CD (P = 0.05)	N'S	2.14	5.02	2.07

Table -2 Influence of single and multi strain rhizobial inoculants on the shoot dry weight, nodule dry weight, and nitrogenase activity of two *Leucaena* species*

Rhizobial Strain	Root length (in cm)		Shoot dry weight (g/pl)		Nodule dry weight (g/pl)		Nitrogenase activity / μ Mole C ₂ H ₂ /g dry wt. root/hr.	
	L. l**		L. d		L. l		L. d	
	L. l	L. d	L. l	L. d	L. l	L. d	L. l	L. d
TAL 82	41.0	24.0	0.980	0.570	0.074	0.051	14.35	10.50
TAL 582	42.3	28.8	1.270	0.650	0.086	0.052	18.62	13.13
TAL 1145	74.0	68.0	1.420	0.730	0.088	0.071	27.26	22.68
Mixed Inoculant	44.0	26.5	1.570	0.750	0.096	0.094	19.16	9.52
Control	25.0	21.0	0.1760	0.490	—	—	—	—
CD (P=0.05)	12.08	NS	0.212	0.130	0.011	0.017	3.13	NS

* Measurements were recorded on 70th day of planting

** L. l: *L. leucocephala*; L. d. *L. diversifolia*

were occupied by TAL 1145 and 25% by mixed infections. But in the case of *L. diversifolia* 80% of the nodules were occupied by TAL 1145 and 5% by TAL 582 and 15% by mixed infection.

These results suggested that in the competition among the three strains, TAL 1145 dominated in infection at all the growth stages. Also TAL 582 was found to be a good competitor in both the species of

Leucaena. Materon and Vincent (1980), while reporting the interstrain competition with soybean rhizobia observed that the root colonization of *R. japonicum* NU 150 dramatically increased from 29% on the 7th day to 50% on the 14th day in Lee variety of soybean with simultaneous reduction on another strain NU 248 from 18% to 5%. Mixed infections of nodules were also recorded by them.

Table-3 Nodule occupancy in two *Leucaena* species by different rhizobial strains

Host/period of Sampling	Percent Strain representation*						
	TAL 82	TAL 582	TAL 1145	TAL 82+ TAL 582	TAL 82 + TAL 1145	TAL 582 + TAL 1145	TAL 82 + TAL 582 + TAL 1145
<i>L. leucocephala</i>							
30th day	—	—	100 (20)	—	—	—	—
50th day	—	30(6)	60 (12)	—	—	5(1)	5(1)
70th day	—	—	75 (15)	—	5(1)	20(4)	—
<i>L. diversifolia</i>							
30th day	10(2)	10(2)	80(16)	—	—	—	—
50th day	—	30(6)	50(10)	—	10(2)	5(1)	5(1)
75th day	—	5(1)	80(16)	5(1)	—	5(1)	5(1)

* The figures in parentheses indicate the number of smears out of twenty nodule smears prepared from the treatments

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