

Short Scientific Reports

Distribution of Nitrogen Fixing *Beijerinckia* in the Rhizosphere of Coconut*

Nitrogen fixation studies assume particular significance for plantation crops like coconut due to the cultivation of these crops in tropical soils where major portion of applied inorganic nitrogenous fertilizers is lost due to leaching, denitrification (Sanchez, 1976) etc. A number of workers have reported non-symbiotic N_2 fixation at magnitudes, that are of agronomic significance under different agro-climatic conditions (Dart and Day, 1975; Charyulu and Rao, 1979; Wani, 1986). *Beijerinckia* is a non-symbiotic N_2 fixing bacterium capable of growing and fixing N_2 at acidic soil conditions in which coconut and other plantation crops are grown (Nair, 1974; Subba Rao, 1983). There have been only very few studies to know the plant-soil nitrogen fixing bacterial interactions in plantation crops. The present investigation has been undertaken with a view to study the extent of association of *Beijerinckia* with the rhizosphere of coconut in two important coconut growing soil types of Kerala viz., laterite and sandy soils and to determine the N_2 fixing potential of the isolates from different locations.

Rhizosphere and non-rhizosphere soil samples of coconut were collected from three locations each in laterite and sandy soil types in coconut growing tracts of Kerala. Farmers' plots having monocropped adult West Coast Tall coconut palms in the age group of 25-40 years were selected. The plots were not receiving inorganic fertilizers and irrigation. The locations selected in

the sandy soil type were Kayangulam (L_1), Thakazhi (L_2) and Quilon (L_3) and those in laterite soil type were Uduma (L_4), Mavelikkara (L_5) and Kasaragod (L_6).

A total of six samples comprising three each from rhizosphere and interspaces were obtained from each location. Blocks of soil containing roots were collected at a distance of one meter from bole of the palms from 0-20 cm depth to represent rhizosphere samples. Samples obtained from three directions in the basin were pooled and composite samples prepared. Non-rhizosphere samples were also obtained at the same depth from three locations in the interspaces and pooled.

Soil samples were collected from four coconut nurseries of Dept. of Agriculture, Govt. of Kerala viz., Nileshwar, Munderi, Parappanangadi and Thikkodi and one nursery of Central State Farm, Aralam. Four replicate samples were collected from the rhizosphere of one year old WCT seedlings from each nursery.

The soil dilution and plate count method (Timonin, 1940) was used to estimate the number of *Beijerinckia* and total bacteria employing Becking's agar and soil extract agar, respectively. Rhizosphere effect was measured in terms of R:S ratio on the basis of the ratio of the number of bacteria per gram of rhizosphere soil to the number per gram of non-rhizosphere soil. The pH of the soil was determined using digital pH meter.

* Contribution No. 754, Central Plantation Crops Research Institute, Kasaragod, Kerala

Bacterial cultures were isolated from rhizosphere and non-rhizosphere soils from different locations using N₂ free Becking's agar. The isolates were purified and characterised as per the methods described in Harrigan and Mc Cance (1966). The cultural characteristics studied included colony characters such as size, shape, elevation, colour, production of gum in Becking's agar and the type of growth in Becking's broth. Morphological tests of bacteria were carried out to study Gram reaction, presence of bipolar lipid bodies and motility. The physiological characters studied included growth on N₂ free media, growth at acidic pH, growth with nitrate

as N source, growth on casein agar and catalase test.

The N₂ fixing efficiency of isolates were estimated in terms of nitrogenase activity by acetylene reduction method. The procedure adopted by Masterson and Murphey (1979) was followed. The culture broth of the isolates were incubated under partial atmosphere (10%) of acetylene and ethylene production was determined using Shimadzu gas chromatograph fitted with flame ionization detector.

The data on the population of *Beijerinckia* in different locations in sandy and laterite soil types are presented in Table I.

Table I. Population of *Beijerinckia* and pH in rhizosphere and non-rhizosphere soils of coconut in different locations

Soil type/ location	Population of <i>Beijerinckia</i> 10 ⁸ g ⁻¹ dry soil		pH	
	Rhizosphere	Non-rhizosphere	Rhizosphere	Non-rhizosphere
I. Sandy				
L ₁	4.57	1.04	5.05	4.93
L ₂	4.93	3.44	5.78	5.15
L ₃	8.77	1.72	6.05	5.63
Mean	6.09	2.07	5.63	5.24
II. Laterite				
L ₄	0.46	0.25	5.22	4.72
L ₅	1.00	0.82	5.33	5.23
L ₆	2.68	2.40	6.98	5.73
Mean	1.48	1.16	5.84	5.23
General Mean	3.73	1.61	5.74	5.23
LSD (P = 0.05) for comparison of rhizosphere vs. non-rhizosphere			Population	pH
			1.69	NS
LSD (P = 0.05) for comparison of locations			2.92	NS
LSD (P = 0.05) for comparison of soil types			1.69	NS

Beijerinckia colonies were observed when soil samples from all six locations were analysed using N_2 free Becking's medium. The rhizosphere samples harboured significantly higher populations than non-rhizosphere samples. The average population was $3.73 \times 10^3 \text{ g}^{-1}$ soil in rhizosphere compared to $1.61 \times 10^3 \text{ g}^{-1}$ soil in non-rhizosphere. Comparative study of the populations in the two soil types revealed significantly more number of *Beijerinckia* in sandy soils when compared to laterite soils. It was interesting to note that the rhizosphere soils had a higher pH than non-rhizosphere soils. The occurrence of a relatively high population of *Beijerinckia* in certain locations such as Kayangulam and Thakazhi could be due to the high organic carbon content of those soils. Organic matter serve as carbon and energy source for the growth and multiplication of micro-organisms in soil.

The proportion of *Beijerinckia* population to the total bacterial population was higher

in rhizosphere samples than in non-rhizosphere sample (Fig. 1). Among the six locations studied, rhizosphere samples from four locations yielded greater proportion of *Beijerinckia* in the total bacterial population than that in the corresponding non-rhizosphere samples.

The R:S ratio for *Beijerinckia* was higher than that of total bacteria in majority of locations (Fig. 2.) The mean R:S ratio for *Beijerinckia* and total bacteria were 2.65 and 1.87, respectively.

Analysis of soil samples from five coconut nurseries also revealed the occurrence of *Beijerinckia* in all nurseries (Table II). There was significant difference in the population in different nurseries. Soil samples from Aralam nursery harboured very high population of *Beijerinckia* when compared to those of other nurseries. *Beijerinckia* constituted 0.04 to 16.19% of total bacteria in the different nurseries.

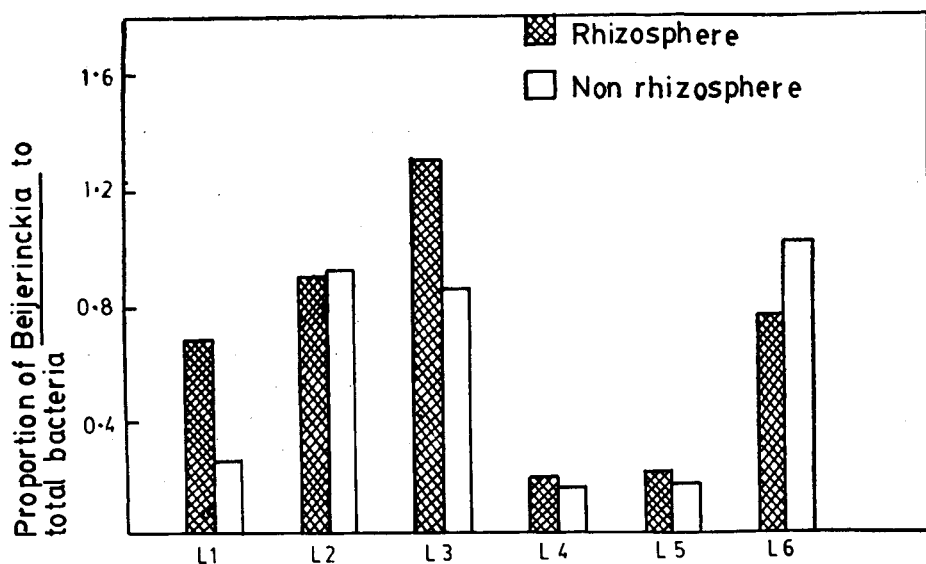


Fig. 1. Proportion of *Beijerinckia* to total bacteria in different locations
L1 - Kayangulam, L2 - Thakazhi, L3 - Quilon, L4 - Uduma, L5 - Mavelikkara, L6 - Kasaragod

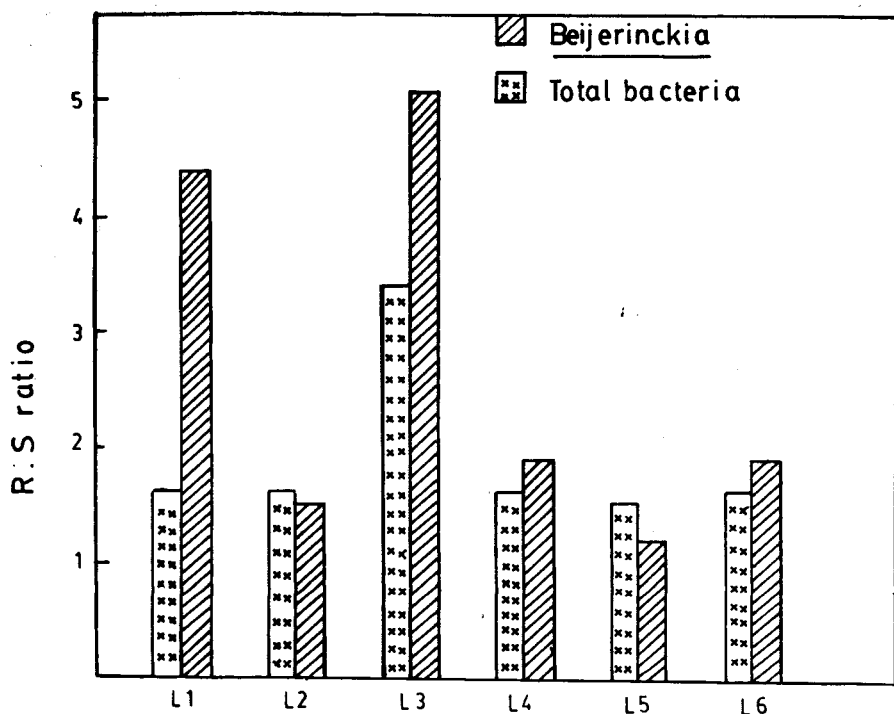


Fig. 2. R:S ratio of *Beijerinckia* and total bacteria in different locations
L1 - Kayangulam, L2 - Thakazhi, L3 - Quilon, L4 - Uduma, L5 - Mavelikkara, L6 - Kasaragod

Table II. Population of *Beijerinckia* and total bacteria in different coconut nurseries

Nursery	<i>Beijerinckia</i> population 10^8g^{-1} dry soil	Proportion of <i>Beijerinckia</i> to total bacteria
Thikkodi	2.41	1.67
Munderi	3.75	0.74
Parappanangadi	0.84	0.42
Nileshwar	5.83	0.64
Aralam	16.35	16.19
Mean	5.83	3.86
LSD ($P=0.05$) for comparison of nurseries	9.09	—

Investigations on the population levels of *Beijerinckia* in coconut gardens from different locations in the two soil types revealed selective stimulation of *Beijerinckia* in coconut rhizosphere. This was evidenced by higher R:S ratio obtained for *Beijerinckia* in comparison with that of total bacteria and higher proportion of *Beijerinckia* to total bacteria in coconut rhizosphere when compared to non-rhizosphere. Similar rhizosphere effect on *Beijerinckia* has been reported for other crops also (Dobereiner, 1961; Ruschel and Dobereiner, 1966; Subba Rao, 1983). The root exudates of coconut may be providing a better micro-environment for proliferation of *Beijerinckia* in coconut rhizosphere. It is also possible that low nitrogen environment available

Short scientific reports

Table III. *Nitrogenase activity of Beijerinckia isolates from different locations in coconut soils*

S.No.	Culture number	Source of isolation	Nitrogen activity nM C ₂ H ₄ tube ⁻¹ h ⁻¹
1.	SKR-1	Rhizosphere	33.26
2.	SKN-1	Non-rhizosphere	29.67
3.	SKN-2	"	37.93
4.	SKN-3	"	3.65
5.	SKN-4	"	10.63
6.	STR-1	Rhizosphere	51.48
7.	STR-2	"	78.94
8.	STR-3	"	24.70
9.	STN-1	Non-rhizosphere	13.05
10.	STN-2	"	23.48
11.	SQR-1	Rhizosphere	72.09
12.	SQR-2	"	75.44
13.	SQN-1	Non-rhizosphere	4.31
14.	SQN-2	"	27.62
15.	SQN-3	"	39.43
16.	LCR-1	Rhizosphere	17.32
17.	LCR-2	"	15.93
18.	LCN-1	Non-rhizosphere	2.88
19.	LCN-2	"	2.71
20.	LCN-3	"	43.66
21.	LUR-1	Rhizosphere	33.26
22.	LUR-2	"	42.74
23.	LUR-3	"	12.79
24.	LUN-1	Non-rhizosphere	29.67
25.	LUN-2	"	37.93
26.	LUN-3	"	10.03
27.	LMR-1	Rhizosphere	10.16
28.	LMN-1	Non-rhizosphere	7.62
29.	LMN-2	"	12.61
30.	LMN-3	"	9.84

in the root zone enable N_2 fixing bacteria to compete with great bulk of soil micro-organisms in soils where availability of N_2 is less. Another reason for the high incidence of *Beijerinckia* could be the favourable pH of 5.0 to 6.0 found in most of the coconut soils. Other workers also reported greater proportion of *Beijerinckia* positive samples in this pH range (Becking, 1961; Thompson, 1968).

It was interesting to note that the population of *Beijerinckia* was very high in the Aralam nursery. This study has indicated the possibility for increasing the population of *Beijerinckia* in other coconut nurseries by inoculation with efficient strains of *Beijerinckia*.

The present investigation showed higher populations of *Beijerinckia* in sandy soils compared to that of laterite soils. Some workers have reported that greater percentage of laterite soils contained *Beijerinckia* than the other soils (Becking, 1961; Thompson, 1968). Such a relation between the occurrence of *Beijerinckia* and lateritisation was not corroborated in other investigations (Dobereiner, 1959; Meiklejohn, 1968).

Thirty bacterial cultures were isolated from the different locations and purified. Studies on the morphological, cultural and physiological characteristics of the isolates confirmed the identity of the cultures as *Beijerinckia* as per the descriptions in the VIIIth edition of the Bergey's Manual of Determinative Bacteriology. The cultures were Gram negative short rods and possessed motility. They produced large raised colonies with smooth or plicated surface on Becking's agar. The cultures produced large quantities of slime on agar media which was either tough and tenacious or granular and viscous. The cultures failed to grow when potassium

nitrate was supplemented as N_2 source in Becking's broth. These characteristics revealed the identity of the bacteria as *Beijerinckia*.

All the cultures exhibited nitrogenase activity when tested by acetylene reduction method (Table III). There was large variation in the acetylene reduction activity (ARA) of different isolates. Majority of isolates showed low ARA while only a small percentage exhibited relatively higher ARA. Fifty three per cent of the isolates showed ARA of less than 25 nM, 33% showed ARA in the range of 25-50 nM while remaining 13% showed ARA of more than 50 nM C_2H_4 tube⁻¹h⁻¹. Isolates obtained from rhizosphere soil had better N_2 -fixing efficiency than those isolated from non-rhizosphere soil. The mean ARA of rhizosphere isolates was 35.86 nM whereas the non-rhizosphere isolates showed ARA of 19.14 nM only. This has indicated that root exudates of coconut stimulated the proliferation of efficient strains of *Beijerinckia* in its rhizosphere. It is possible that continuous availability of energy source in root exudate might have helped in maintaining an active group of N_2 fixing bacteria in its rhizosphere. The occurrence of *Beijerinckia* in coconut soils, proliferation of the diazotroph in the rhizosphere of coconut and nitrogen fixing potential of the isolates were brought out in this study. It will be worthwhile to undertake further detailed investigations to augment N_2 fixation by *Beijerinckia* in coconut soils by introduction of efficient strains and by manipulation of various inputs and agronomic practices.

ACKNOWLEDGEMENT

The first author is grateful to Indian Society for Plantation Crops for providing Fellowship during this study.

REFERENCES

- BECKING, J. H. 1961. Studies on nitrogen fixing bacteria of the genus *Beijerinckia* 1. Geographical and ecological distribution in soils. *Plant. Soil*. **14**: 49-81
- CHARYULU, P. B. B. N. and RAO, V. R. 1979. Nitrogen fixation in some Indian rice soils. *Soil Sci*. **128**: 86-89.
- DART, P. J. and DAY, J. M. 1975. Non symbiotic nitrogen in soils; In *Soil Microbiology A Critical Review* Walker, N (ed) Butterworths, London pp. 225-252.
- DOBEREINER, J. 1959. Sobre a ocorrência de *Beijerinckia* em alguns estados do; *Rev. Brasil. Biol.* **19**: 151-160.
- DOBEREINER, J. 1961. Nitrogen fixing bacteria of the genus of *Beijerinckia dextr* in the rhizosphere of sugarcane. *Plant. Soil* **15** : 211-216.
- HARIGAN, W. F. and McCANCE, M. E. 1966. *Laboratory Methods in Microbiology* Academic Press, London pp 362.
- MASTERSON, C. L. and MURPHY, P. M. 1979. The acetylene reduction technique. In *Recent Advances in Biological Nitrogen Fixation* (ed) Subba Rao N. S., Oxford and IBH Publishing Co., New Delhi, India pp 8-33.
- MEIKLEJOHN, J. 1968. New nitrogen fixers from Rhodesian soils. *Trans. 9th Intern. Congr. Soil Sci. Adelaide* **2**: 141-149.
- NAIR, S. K. 1974. *Studies on the Microflora of the Root Region of Plantation Crops - Coconut and Cacao*. Ph. D. thesis. Indian Agricultural Research Institute, New Delhi.
- RUSCHEL, A. P. and DOBEREINER, J. 1966. Nitrogen fixing asymbiotic bacteria in the rhizosphere of graminaceous forage plants. *Proc. Ninth. Intl. Grass. Conf.* pp. 1103-1107.
- SANCHEZ, P. A. 1976. *Properties and Management of Soils in the Tropics*. John Wiley & Sons, Inc., New York pp. 618.
- SUBBA RAO, N. S. 1983. Nitrogen fixing bacteria associated with plantation crops and orchard plants. *Can. J. Microbiol.* **29**: 863-866.
- THOMPSON, J. P. 1968. The occurrence of nitrogen fixing bacteria of the genus *Beijerinckia* in Australia outside the tropical zone. *Trans. 9th Intern. Congr. Soil Sci. Adelaide* **2**: 129-139.
- TIMMONIN M. I. 1940. The interaction of higher plants and soil micro organisms. I Microbial population of rhizosphere of seedlings of certain cultivated plants. *Can. J. Res.* **18**: 307-317.
- WANI, S. P. 1986. Cereal nitrogen fixation; problems and potentialities in cereal nitrogen fixation, *Proc. Working Group Meeting*. Oct. 9-12-1984, ICRISAT Centre, Patancheru, India pp 7-21.

Central Plantation Crops Research Institute
Kasaragod 671 124, Kerala, India

V. J. MERILYN
GEORGE V. THOMAS