Mysore J. agric. Sci., **12** : 568-572

Occurrence of A Symbiotic Nitrogen Fixers in the Phyllosphere of Common Plants and their Efficiency in Nitrogen Fixation

SUHAS P. WANI, B. M. BOPAIAH AND P. V. RAI
Department of Microbiology, University of Agricultural Sciences, G.K.V.K., Bangalore-560065

ABSTRACT

The Phyllosphere microflora of some crop plants, weeds, forest trees and plantation crops have been studied. The presence of asymbiotic nitrogen fixers have been recorded from the phyllosphere of jowar (*Sorghum vulgare* Pers.), sunflower (*Helianthus annuus* L), bean (*Phaseolus lunatus* L), ragi (*Eleusine coracana* Gaertn), sandalwood (*Santalum album* Linn.). teakwood (*Tectona grandis*, Linn.), *Cyperus rotundus* Linn., *Acanthospermum hispidum*, De., *Lantana camera*, *Ageratum conyzoides* Linn. and *Bidens pilosa* Linn. The asymbiotic nitrogen fixers from the phyllosphers of *S. vulgare* and *A hispidum* have been tentatively identified as *Beijerinckia* sp. and those from others as different

Maximum number of asymbiotic nitrogen fixers was recorded in *S. vulgare* amongst crop plants, *C. rotundus* amongst weeds and *T. grandis* amongst forest trees. It was noted that most of these asymbiotic nitrogen fixers were very slow growers *in vitro*.

The ability of these asymbiotic nitrogen fixers for fixing atmospheric nitrogen and to produce the slime *in-vitro* have been evaluated. *In-vitro* these microorganisms vary in their ability to fix nitrogen and the nitrogen fixed per 100 ml of broth medium was in the range of 2.25 to 7.02 mg. It seems that in general, there is no direct correlation between the slime production and nitrogen fixation by these microorganisms.

The leaf surface inhabiting nitrogen fixers play an important role in the fixation of atmospheric nitrogen asymbiotically. They have a clear advantage over other organisms being independent of combined nitrogen and not highly selective in their carbon requirements. The presence of *Azotobacter*, *Aerobacter*, *Desulphovibric*, *Methanobacterium*, *Pseudomonas*, *Chromatium*, *Chlorobium* and *Rhodospirillum* nitrogen fixers on plant leaf surface was recorded by Ruinen (1956), Bond (1959) and Meiklejohn (1962) noted the presence of *Beijerinckia* and *Azotobacter* on leaf surface of forest vegetation, on mulberry by Vasantharajan and Bhat (1968) and on *Santalum* and *Dolichos* by Bhat *et al.* (1971) from India. The reports of phyllosphere nitrogen fixers from crop plants, weeds, forest and plantation crops in India are scanty. The present studies were conducted to examine the phyllosphere microflora of some plants and the results on the occurrence of nitrogen fixers in the phyllosphere of common plants and their efficiency to fix atmospheric nitrogen are presented.

Material and Methods

For the present investigation, in all fifteen plants of different groups *viz.*, crop plants, weeds, forest trees and plantation crops listed in Table I were selected. The leaf samples were collected from the plants grown in G.K.V.K. Campus of the Agricultural University, Bangalore. The samples from plants at different locations and from different plant heights were collected in the month of November-December 1976. The quantitative estimation of phyllosphere nitrogen fixers was done by following two methods (i) leaf surface washing method (Dickinson, 1967) and leaf maceration method. Waksman-77 nitrogen-free agar medium was used for plating, in both the methods. The representative colonies were isoloted, purified and maintained on the same medium.

The nitrogen fixing efficiency of these organisms was evaluated by growing them in N_2-free broth for 21 days at room temperature on a mechanical shaker. At the end of 21 days the population of nitrogen fixers was determined from each sample by following dilulion and plate method. The nitrogen content of the broth culture was determined by microkjeldahl method. Slime producing ability of these organisms was studied and compared by isolating the slime with excess of acetone. The precipitated slime was air-dried and weights of slime from each sample were recorded.

Results and Discussion

It is evident from the results presented in Table I that presence of nitrogen fixers in the phyllosphere of plants vary with plant species. It was observed that the leaf surfaces of all crop plants and weeds surveyed showed the presence of nitrogen fixers. Amongst forest trees and plantation crops only *T. grandis* and *S. album* showed the presence of asymbiotic nitrogen fixers in the phyllosphere.

The results indicated that not only the occurrence but also the number of nitrogen fixers vary with plant species. In general, the leaf surfaces of crop plants have more

TABLE I

Qualitative and quantitative estimation of asymbiotic nitrogen fixers from the Phyllosphere of some important plants

Sl.No.	Name of plant	Leaf surface wash method No. of m.o./sq cm × 10^4	Leaf maceration method No. of m.o/g of leaf × 10^4	Name of the organism
I	Crop plants			
1.	*Cajanus cajan*	0. 096	0 524	*Azotobacter* sp
2.	*Eleucine coracana*	0. 041	0.462	*Azotobacter* sp
3.	*Helianthus annus*	0. 088	0.484	*A. vinelendii*
4.	*Phaseolus lunatus*	0. 079	0.382	*Azotobacter* sp
5.	*Sorghum vulgare*	0. 108	0.687	*Azotobacter* sp & *Beijerinckia*
II.	Forest trees and plantation crops			
6.	*Anacardium occidentale*	—	—	—
7.	*Eucalyptus* sp (Mysore gum)	—	—	—
8.	*Santalum album*	0. 003	0.080	*Azotobacter* sp
9.	*Syzygium cumini*	—	—	—
10.	*Tectona grandis*	0. 0002	0.013	*Azotobacter* sp
III.	Weeds			
11.	*Acanthospermum hispidum*	0. 047	0.256	*Azotobacter* sp
12.	*Ageratum conyzoides*	0. 003	0.103	*Azotobacter* sp *Beijerinckia*
13.	*Bidens pilosa*	0. 020	0.122	*A. vinelendii*
14.	*Cyperus rotundus*	0. 010	0.678	*Beijerincktia* & *Azotobacter*
15.	*Lantana camera*	0. 010	0.505	*Azotobacter* sp

number of nitrogen fixers, than those of weeds, forest trees and plantation crops. Amongst crop plants the maximum population of 0.108×10^4/ cm^2 was recorded from *S. vulgare* phyllosphere. In the case of weeds *A. hispidum* phyllosphere showed maximum population (0.047×10^4). However the numbers differed with plant species and also depended greatly on the time of sampling and on the age of the leaves (Ruinen, 1970; Bessems, 1973; Becking, 1975). The results of quantitative analysis by leaf surface washing method and leaf maceration method showed the same trend except in the case of weeds. Such disparity might be due to the presence or absence of more sticky organisms on the particular leaf surface. By using selective nitrogen free medium either of the methods can be successfully used for qualitative and/or quantitative survey of phyllosphere nitrogen fixers.

The nitrogen fixers from the leaf surface of *S. vulgare* and *A. hispidum* were identified as *Beilerinckia* sp. and *Azotobacter* sp. The nitrogen fixers from other plants were identified as different species of *Azotobacter*. The presence of *Azotobacter* on the leaf surface of *C. cajan*, *E. coracana* and *Santalum* confirmed the reports of earlier workers (Moore, 1963; Vasantharajan and Bhat, 1968). The presence of *Beijerinckia* sp. and *Azotobacter* on *S. vulgare* leaf surface and of *Azotobacter* sp on *H. annuus* and *P. lunatus* seems to be the first record.

TABLE II

Amount of nitrogen fixed and slime produced by asymbiotic nitrogen-fixers from phyllosphere of different crops in-vitro

SL. No	Organisms	Population ml × 10^6	Amount of nitrogen fixed (mg/100 ml broth)	Amount of slime produced (mg/100 ml broth)
1.	*Azotobacter vinelendii*	0.06	5.000	44.00
2.	*Azotobacter* sp	0.09	3.45	40.00
3.	*A. beijerinckia*	0.10	2.50	74.00
4.	*Azotobacter* sp	0.12	4.60	88.00
5.	*Azotobacter* sp	0.13	2.25	19.00
6.	*Beijerinckia* sp	0.13	3.20	2.00
7.	*Azotobacter* sp	0.32	3.10	98.00
8.	*Azotobacter* sp	0.35	2.60	7.00
9.	*Azotobacter* sp	0.48	2.30	43.00
10.	*Azotobacter* sp	0.55	7.02	320.00

The occurrence of nitrogen fixers in the phyllosphere of weeds and forest trees confirmed the reports of Ruinen (1961) and Meiklejohn (1962).

Experiments on nitrogen fixing efficiency of different isolates *in vitro* clearly indicated that all isolates differ in their capacity to fix nitrogen. The amount of nitrogen fixed per 100 ml of broth medium was varying in the range of 2.25 to 7.02 mg. In general, species of *Azotobacter* have better efficiency to fix atmospheric nitrogen than *Beijerinckia* sp. Becking (1971) noted 2.8 to 50.00 mg of nitrogen fixed by phyllosphere organisms per gram of glucose. As that of nitrogen fixation, the species of *Azotobacter* are superior over *Beijerinckia* in slime production also. *Beijerinckia* produced 2.0 mg of slime per ml of broth culture whereas the species of *Azotobacter* produced the slime in the range of 7.00 to 320.00 mg per 100 ml of broth culture. The maximum amount of slime was produced by that species of *Azotobacter* which fixed highest amount of nitrogen. It seems that in the case of this particular isolate the correlation between the amount of slime produced and amount of nitrogen fixed holds good, whereas in the case of other isolates it does not hold true. The population in broth and the amount of nitrogen fixed also does not show any relationship except the isolate which fixed highest amount of nitrogen and also produced highest amount of the slime. In some cases although the count of viable cells was lower, the nitrogen fixed was highest, and this may be due to higher nitrogen fixing ability.

The further studies on host specificity of these isolates, establishment after inoculation and relationship between slime production and nitrogen fixation are in progress.

REFERENCES

BECKING, J. M., 1975, Nitrogen fixation in some natural ecosystems in Indonesia. In symbiotic nitrogen fixation in plants. (Ed. Nutman, P. S.), pp. 539–550. Cambridge University Press.

BESSEMS, E. P. M., 1973, Nitrogen fixation in the Phyllosphere of Gramineae. *Agric. Res. Rep.*, **780**. 1–88.

BHAT, J. V., LIMAYE, K. N. AND VASANTHARAJAN, V. N., 1971, The effect of the leaf surface microflora on the growth and root exudation of plants. *In Ecology of the leaf surface microorganisms* (Eds. Preece, T. F. and Dickinson, C. H.), Section V. pp. 581–595, Academic Press, London, New York.

BOND, G., 1959, Adv. Sci. 15 : 382. cited from Preece, T. F. and Dickinson, C. H., 1971.

DICKINSON, C. H., 1967, Ephyphytic micro-organisms on leaf surface. *Can. J. Bot.*, 45 : 915–927.

MEIKLEJOHN, J., 1962, Microbilogy of the nitrogen cycle in some Ghana Soils. *Emp. J. exp. Agric.*, 30 : 115–126.

MOORE, A. W., 1963, Nitrogen fixation in latosolic soil under grass. *Pl. Soil.*, 19 1217–1238.

RUINEN, J., 1956. Occurrence of *Beijerinckia* species in the Phyllosphere. *Nature, Lond.*, 117 : 220–122.

RUINEN, J., 1961, The Phyllosphere, I. An Ecologically neglected Milieu. *Pl. Soil.*, 15 : 81–109.

RUINEN, J., 1970, The Phyllosphere V. The grass Sheath, a habitat for nitrogen-fixing micro-organisms. *Pl. Soil.*, 33 : 661–671.

VASANTHARAJAN, V. N. AND BHAT, J. V., 1968, Interrelations of microorganisms and mulberry. II. Phyllosphere microflora and nitrogen fixation in leaf and root surface. *Pl. Soil.*, 28 : 258–267