NITROGEN-FIXING *AZOSPIRILLUM LIPOFERUM* FROM COMMON WEEDS ASSOCIATED WITH RICE AND AQUATIC ECOSYSTEMS

THE participation of *Azospirillum lipoferum* in the nitrogen economy of several crop plants was recognized recently[2,3,6]. This organism has been implicated in associative symbiosis with several grass plants[2–6] and nitrogen fixation in such associations has been confirmed by ^{15}N incorporation[1]. Growth of many grasses and several weeds is perhaps enhanced by such associations because they help to satisfy the plant nitrogen requirements. We report the isolation and evaluation of the N_2-fixing efficiency of *Azospirillum lipoferum* from the roots and phyllosphere of some common weeds of rice fields.

Roots and leaves of the weeds (Table I) were washed with running tap water, surface sterilized with 80% ethanol for 0·5 min, and subsequently washed four times with sterilized phosphate buffer (pH 7·0). Pieces of root weighing 0·1–0·2 g were cut into 0·5–1·0 cm segments and transferred to nitrogen-free malate medium in test-tubes (15 × 150 mm) (Dobeneiner *et al.*[2]). A typical white, dense, undulating, thin pellicle developed a few millimetres below the surface of the medium within 48 h at 30°C. The isolates were purified by serial transfer to fresh semi-solid malate medium (3–4 transfers) and subsequently streaked on agar plates and single colony was picked up for further purification and identification. Microscopic observation revealed the characteristic rods with fat droplets and active spiral movements. Biochemical tests revealed acid production from glucose, manitol, sorbitol, and utilization of sugars, keto-glutarate, manitol, sorbitol, ribose and glucose as sole carbon source, growth on semi-slolid malate medium ⊕ 0·005% yeast extract forming a typical pellicle. All the isolates tested in the present study produced acid from glucose and ribose and required biotin. These isolates thus belonged to *Azospirillum lipoferum* (Group II) as per Tarrand *et al.*[7]. *A. lipoferum* was also isolated and purified from the phyllosphere of the weed plants by transferring the cut leaf bits of uniform weight to the N-free semi-solid malate medium. Nitrogen fixation in these multiple-transfer cultures (72 h old) was

TABLE I

Nitrogen-fixing efficiency of Azospirillum lipoferum *from weed plants associated in the rice fields*

Plant species	Family	Nitrogen-fixing efficiency* of *A. lipoferum* mg N/g malate added	
		Root isolates	Leaf isolates
Boerhaavia repens	Nyctagenaceae	8·55 ± 0·4	..
Phyllanthus niruri	Euphorbiaceae	7·00 ± 0·7	1·32 ± 0·5
Euphorbia hircta	Euphorbiaceae	8·32 ± 0·3	..
Pistia stratiotes	Araceae	10·60 ± 0·2	3·00 ± 0·13
Colocasia anticorum	Araceae	2·37 ± 0·2	4·50 ± 0·4
Lucus aspera	Labiateae	1·00 ± 0·1	..
Eclipta alba	Compositeae	6·27 ± 0·8	8·00 ± 0·16
Cyperus sp.	Cyperaceae	3·64 ± 0·3	..
Commelina benghalensis	Commelinaceae	8·10 ± 0·13	..
Mardamia spirata	Commelinaceae	4·00 ± 0·43	6·60 ± 0·7
Iopomea reptans	Convolvulaceae	4·84 ± 0·23	..
Clerodendron viscosum	Verbeneaceae	5·40 ± 1·2	4·80 ± 0·59
Marsilia quadrifolia	Marsiliaceae	10·00 ± 0·7	2·30 ± 0·39
Eichornia crassipes	Pontederiaceae	..	..
Mimosa pudica	Mimoseae (S.F.)	4·40 ± 0·25	1·60 ± 0·11

– = *Azospirillum* not detected; * Mean N-fixing efficiency of the isolates obtained from 3 plants on one occasion.

± Standard variation of the mean.

determined following the method already described[5]. Three plants belonging to each family were used for isolation and 4–6 isolates from root and leaf pieces were obtained once. The nitrogen-fixing efficiency was expressed as mg N fixed. g^{-1} g malate added.

A. lipoferum is widespread among the weeds investigated and the cultures possessed appreciable nitrogen-fixing efficiency (Table I). Although *A. lipoferum* could be isolated from most of the weed plants considerable variations with regard to N_2-fixing ability was observed. Nitrogen fixation was higher in cultures isolated from the roots of *Pistia stratiotes*, *Marsilia quadrifolia*, *Boerhavia rapens* and *Euphorbia hircta*, while *Lucus aspera*, *Colocasia anticorum* and *Cyperus* sp. harboured *A. lipoferum* cultures with low nitrogen-fixing ability. The phyllosphere isolates were less efficient than the isolates from the roots of same plant in fixing nitrogen except for those from *Eclipta alba*, *Mardamia spirata* and *Colocasia anticorum*. Moreover, *A. lipoferum* was absent from the leaves of some plants, while present in the roots.

A. lipoferum has been isolated from the roots of several plant species, with wide variations in the N_2-fixing ability[2,4,6]. Variation in nitrogen fixation by *A. lipoferum* isolates from the roots of several rice cultivars has also been observed (Nayak and Rao, unpublished). This has been attributed to the differences in the root exudates and the intrinsic ability of the isolates for efficient utilization of the available carbon source. Also, Kavimandan *et al.*[3] observed wide variations in N_2-fixation by *A. lipoferum* depending on the wheat varieties.

Our studies indicate the widespread association of *A. lipoferum* with the roots of weeds in rice fields.

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Laboratory of Soil Microbiology,
Central Rice Research Institute,
Cuttack 753 006
(Orissa) India, *April* 30, 1979.

D. N. NAYAK.
A. SWAIN.
V. RAJARAMAMOHAN RAO.

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CARBONIC ANHYDRASE ACTIVITY AS AN INDEX OF ZINC NUTRITION IN CABBAGE

ZINC nutrition has assumed considerable importance in Indian Agriculture as its widespread deficiency has been reported in a variety of crops. Among the various functions of zinc in plants, its association with carbonic anhydrase (CA) (EC 4·2·1·1) activity was one of the earliest to be detected. CA is a zinc containing enzyme occurring mostly in the chloroplast in plants. It catalyses the hydration of CO_2 reversibly and carries out CO_2 fixation according to Calvin cycle where ribulose diphosphate is carboxylated[4]. In recent years attempts have been made to relate the enzyme activity in plants with their zinc content and yield, and it has been suggested that the activity of the enzyme could be used as an index of zinc nutrition in crop plants[1,2,8]. However, there has been no study so far, on the activity of the enzyme in vegetable crops. An attempt was, therefore, made to study the relationship among CA activity, zinc content of the leaf and the yield of cabbage.

For this study samples were collected from zinc-treated plots of a micronutrient experiment on cabbage (Cv. Pride of India) conducted at the Indian Institute of Horticultural Research Experimental Farm, Hessaraghatta, Bangalore. The details are furnished in Table I. The experiment was conducted on red sandy loam soil (Typic Haplustalf) with low organic matter,

TABLE I

Effect of soil and foliar application of zinc on yield, leaf zinc and CA activity in cabbage
(Means of 3 replications data of two seasons)

Treatment	Yield kg/plot	Leaf Zn ppm	CA activity *
N, P, K only	7·367	23	13·8
N, P, K + zinc soil application(10 kg/ha)	11·280	260	24·8
N, P, K + Foliar** application of zinc	11·633	246	36·3
C.D. (5%)	3·325	64·7	7·3

* CO_2 evolved/mg protein/hour at 25° C ± 2.

** Foliar application of zinc was given twice at fortnightly intervals during the active growth period with a solution of 0·25% solution of zinc sulphate.