TABLE I

Cross-agglutination reaction among different species of Rhizobium

(Agglutination tubes kept at 70° C for 2 hrs and overnight at 4° C)

Antigen	Highest dilution of antisera showing firm agglutination				
	M I	M 7	SB-16	P-2	G-2
M I	6,400	6,400	200	..	..
M 7	12,800	12,800	400	..	..
SB-16	400	400	12,800	..	..
P-2	..	..	..	12,800	1,600
G-2	..	..	..	1,600	6,400

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1. Dudman, W. F. and Brockwell, J., *Aust. J. Agri. Res.*, 1968, **19**, 439.
2. Vincent, J. M. and Humphry, B. A., *J. Gen. Microbiol.*, 1970, **63**, 379.
3. Skrdleta, V., *Soil Biol, Biochem.*, 1970, **2**, 167.
4. Date, R. A.. *Proc. Ind. Nat. Sci. Acad.* 1975, 40 B.
5. Dadarwal, K. R. and Sen. A. N., *Ibid.*, 1975, 40 B.
6. Gaur, Y. D. and Sen. A. N., *Ibid.*, 1975, 40 B.
7. Graham, P. H., *Ant. V. Leeuwenhoek*, 1963, 29, 281.
8. Dudman, W. F., *J. Bact.*, 1964, **88**, 782.
9. Humphry, B.A. and Vincent, J.M., *J. Gen. Microbiol.*, 1965, **41**, 109.
10. Skrdleta, V. *Ant. V. Leeuwenhoek*, 1969, **35**, 77.
11. Kern, H., *Arch. Mikrobiol.*, 1965, **52**, 206.
12. Holland, A. A., *Ant. V. Leeuwenhoek*, 1966, 32, 400.
13. Drozansks, D., *Acta Microbiol, Polon.*, 1966, 15, 323.
14. Gibbin, L. N., *Can. J. Microbiol.*, 1967, **13**, 1375.
15. Weaver, R.W., *Ph.D. Thesis*, Iowa State Univ. Ames. Iowa, 1970.
16. Parker, C. A. and Grove, P. L., *J. appl. Bact.*, 1970, 33, 248.
17. Vincent, J. M., *Proc. Linnen Soc., N.S.W.*, 1941, 66, 145.
18. —, *IBP Hand book No. 15*, Brockwell Scientific Publication, Oxford, 1970.

NITROGEN FIXATION AND AMMONIA ASSIMILATION IN *AZOTOBACTER CHROOCOCCUM* ISOLATES FROM C_3 AND C_4 PLANTS

THE diazotroph, *Azotobacter*, still holds greater promise not only for fundamental studies on nitrogen fixation but also in the practical use as a biofertilizer[1,2]. Though of wider distribution in the tropical soils, *Azotobacter* colonizing the rhizosphere of certain tropical grasses are significant as they have been found to be more efficient in dinitrogen fixation[3,4]. Recently, from our laboratory it has been brought out that the isolates of *Azotobacter* from the rhizosphere of certain C_4 plants recorded more efficiency in nitrogen fixation than the isolates from C_3 plant species[5]. One of the reasons for the luxuriant and rank growth of the tropical grasses and weed plants is believed to be their asociation in their root system with more efficient nitrogen fixing organisms[6,7]. In the present paper, using the acetylene reduction technique, the N-fixing capacities of *Azotobacter chroococcum* isolates and the pathway of ammonia assimilation are reported.

The cultures of *A. chroococcum* (Table I) were isolated from the rhizosphere of the respective plant species and the pure cultures were maintained on Waksman medium No. 77 agar slopes. The isolates were grown under aerated conditions in N-free liquid medium for ten days at room temperature (28 ± 2° C) and the nitrogen content was estimated in a micro-kjeldhal unit[3].

The enzyme nitrogenase, (nitrogen acceptor oxidoredudtase E.C.1.7·99·27) of the isolates was assayed in a Perkin Elmer Model F-33 gas chromatograph fitted with potapak–N column (80–100 mesh) and flame ionization detector[9]. The assay system consisted of 2·0 ml of the cell suspension, harvested from the early log phase growth of the organism in Waksman No. 77 broth devoid of calcium carbonate and washed in 0·1 M phosphate buffer (pH 7·0) in 20 ml vial. The air in the vessel was driven-off by flushing with a jet of nitrogen. Ten per cent acetylene was injected and the internal oxygen pressute was maintained at 0·2 atm. The vials were incubated for 24 h at 28° C after which 1 ml of the gas sample was analysed. The ethylene peaks were compared with a standard graph prepared with pure ethylene and the quantity of C_2H_4 formed calculated. The ammonia assimilatory pathway predominantly involved the coupled reaction catalysed by the dual enzymes, glutamine synthetase (GS, E.C 6.3.1.2) and glutamate synthase (GOGAT, E.C. 2.6.1.5.3) in most of the nitrogen fixing organisms[10,11]. With a view to estimating the activities of these enzymes early log phase cultures were harvested by centrifugation, washed in buffer, disrupted by sonica-

tion and the cell-free extract was used as enzyme source. GS and GOGAT activites were determined as detailed by Shapiro and Stadtman[12] and Vandecasteele *et al*[13], respectively.

TABLE I

Nitrogen fixation by different* Azotobacter *isolates*

Plant species	Isolate No.	Nitrogen fixed (mg/g of mannitol)
C_3 plants:		
Oryza sativa L.	AB. 1	16·80
	AB. 2	16·00
	AB. 3	17·00
Gossypium hirsutum L.	Gh. 1	7·60
	Gh. 3	8·20
Capsicum annuum L.	Ca. 1	9·50
	Ca. 2	9·00
Lycopersicon esculentum Mill.	Le. 1	6·40
	Le. 2	9·20
C_4 plants:		
Amaranthus viridis L.	Am. v. 4	22·40
Brachiaria mutica Stapf.	Bm. 3	22·00
Cyperus rotundus L.	Cyp. 3	25·20
Cynodon dactylon Pers.	Cyn. 3	22·00
Portulaca tuberosum Roxb.	P. tub. 1	23·60
Zea mays L.	Zm. 1	17·20
Sorghum vulgare Pers.	Sorgh. 1	21·00
Portulaca oleracea L.	P. ole. 3	25·00
Amaranthus pentaphylla L.	Am. P. 1	21·60

* Data represent average of three estimations.

The data in Tables I and II clearly indicate that the isolates of *A. chroococcum* from the rhizosphere of the C_4 plants possess a greater potential for dinitrogen fixation than the C_3 plant isolates. The acetylene-reduction test confirms our earlier results. The greater availability of organic acids and carbon compounds through root-excretion in the rhizosphere of C_4 plant species might be responsible for greater nitrogen fixing activity. Admittedly, this enhanced activity is not only confined to the "microhabitat", rhizosphere, but also traceable under laboratory conditions.

Our earlier attempt to study the ammonia assimilating enzyme in *Azotobacter* revealed[14] comparatively higher activity of GOT (Glutamate oxalocetate transaminase E.C. 2·6·1·1) in C_4 isolates and low GDH (Glutmate dehydrogenase, E.C. 1.4.1.4) in both the groups of isolates.

TABLE II

Activities of Glutamine synthetase (GS) and Glutamate Synthase (GOGAT) in Azotobacter *isolates*

	Nitrogenase activity †	GS activity*	GOGAT activity**
Isolate from C_3 plant species :			
AB, 1	12000	5·80	148·09
AB. 2	9500	5·30	115·43
AB. 3	18200	6·05	206·14
Gh. 1	5000	3·50	144·29
Gh. 2	15000	3·43	65·96
Ca. 1	9300	3·52	116·99
Ca. 2	7000	4·75	161·87
Le. 1	9500	2·16	96·19
Le. 2	9400	2·96	82·45
Isolate from C_4 plant species:			
Am. 4	28000	7·30	150·05
Bm. 3	48000	8·26	194·06
Cyp. 3	11000	9·40	265·00
Cyn. 3	12000	10·00	400·00
P.tub. 1	54000	6·64	180·37
Zm 1	8000	6·05	200·00
Sorgh. 1	25000	12·94	501·03
P.ole. 3	29000	11·42	359·73
Am.p. 1	23000	11·17	330·87

† Expressed as *n* moles of C_2H_4 formed/g of cell/24 hr at 28° C.

* Enzyme activity expressed as μ moles of glutamic acid monohydroxyamate formed/mg protein/30 min.

** μ moles of NADPH oxidised/mg protein/30 min.

The results (Table II) strongly suggest that in *A. chroococcum* ammonia assimilation is chiefly mediated through the coupled reactions of GS and GOGAT. Interestingly, the C_4 plant isolates of *Azotobacter* registered a greater activity of these enzymes than the C_3 plant isolates suggesting that the former isolates not only possess a more efficient N fixing system but also an efficient ammonia assimilating enzyme system.

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1. Rubenchik, L. I., *Azotobacter and Its Use in Agriculture* Natl. Sci. Found., Washington D.C. and Israel Program for Scientific Translation Ltd., 1963, p. 278.
2. Mulder, E. G. and Brotonegoro, S. In: A. Quispel, (ed.), *The Biology of Nitrogen Fixation,* North Holland Publ. Co., Amsterdam, Oxford, 1974, p. 37.
3. Dobereiner, J., Day, J. M. and Dart, P. J., *Plant Soil,* 1972, 37, 191.
4. Neyra, A. C. and Dobereiner, J. *Advan, Agron.* 1977, 29, 1.
5. Purushothaman, D., Kasirajan, C. and Rangaswami, G. *Curr. Sci.,* 1976, **45**, 807.
6. Day, J. M. Neves, M. C. P. and Dobereiner, J. *Soil. Biol. Biochem.* 1975, **7**, 107.
7. Tjepkema, J. D. and Burris, R. H., *Plant Soil,* 1976, **45**, 81.
8. Bremner, J. M., J. *Agri. Res. Sci.,* 1960, **55**, 11.
9. Hardy, R. W. F., Holsten, R. D., Jackson, E. K. and Burns, R. H., *Plant Physiol.* 1968, **43**, 1185.
10. Nagatani, H., Shimizu, M. and Valentine, R. C., *Arch. Mikrobiol.,* 1971, **79**, 164.
11. Brill, W. J., *Annu. Rev. Microbiol.* 1975, 29, 109.
12. Shapiro, B. M. and Stadtman, E. R., In: Tabor, H. and Tabor, C.W. (eds.), *Methods in Enzymology,* Vol. XVII. Part A, 1970, p. 910.
13. Vandecasteele, J. P., Lemel, J. and Coudert, M., *J. Gen. Microbiol.,* 1975, 90, 178.
14. Sadasivam, S., Pandian, S. and Purushothaman, D., *Mysore J. Agri. Res.,* 1978 (In press).

RAPID CLONAL PROPAGATION OF *DIOSCOREA FLORIBUNDA* BY *IN VITRO* CULTURE OF EXCISED LEAVES

DEVELOPED excised leaves devoid of axillant buds of *Dioscorea floribunda* were induced to differentiate multiple shoots from their pulvinus in a treatment containing 0·5 mg/l BA + 50 mg/l AdS + 0·1 mg/l NAA under aseptic cultures. Single-node leaf cuttings taken from such aseptically growing plants were rooted 100% in the presence of 0·1 mg/l 2,4-D, resulting in the formation of plantlets, 100% of which were successfully transplanted in soil where they grew normally and vigorously and produced tubers.

The possibility of mass multiplication of *D oscorea floribunda,* a commercially important medicinal plant, through Tissue Culture was first demonstrated by Chaturvedi[1] followed by confirmatory reports by Lakshmi Sital *et al.*[2] for *D. floribunda* and Chaturvedi *et al.*[3] for *D. deltoidea.* In such studies, the single-node leaf cuttings of aseptically-grown plants were used for rooting. It was noticed[1] that after repeatedly obtaining numerous single-node leaf cuttings (*ca* 40) from a single culture of aseptically-grown plant of *D. floribunda* a good number of excised leaves were wasted every time. It was, therefore, thought more advantageous to utilize these leaves so as to further augment the rate of propagation than was possible through only single-node leaf cuttings[1]. There is no previous report of propagation of *Dioscorea* by aseptic culture of leaf cuttings. We now report the results of such an investigation.

The basal medium used, procedure adopted for establishing the plants of *Dioscorea floribunda* Mart. and Gal. in aseptic cultures and other cultural conditions were reported earlier[1]. Fully developed leaves 6–8 cm in length and devoid of axillant buds were excised from aseptically-grown plants and inoculated, one per culture tube, with their pulvinus base inserted in the nutrient agar (Fig. 1). The cultures were incubated under 3 kl fluorescent light for 14 hr daily and at a temperature of about 27° C. Ae the preliminary experiments showed kinetin (Kn) to be ineffective for shoot bud induction in excised leaves, the morphogenetic effects of different concns. (0·25, 0·5, 1·0, 1·5 and 2·0 mg/1) of 6-benzyladenine (BA), or, 6-isopentenyladenine (IPA), or, 25, 50 and 100 mg/1 of thiourea along with 0·1 mg/1 α-naphthaleneacetic acid (NAA) were studied. 2,4-Dichlorophenoxyacetic acid (2,4-D) was used for induction of roots in single-node leaf cuttings and indoleacetic acid (IAA) in excised leaves.

In the control, an excised leaf remained green and healthy throughout the incubation period of 50 days, but did not produce callus from its pulvinus base nor any organs (Fig. 1), whereas, several thin roots were formed in the treatment containing 1 mg/l IAA (Fig. 2). BA was more effective than IPA, whereas,