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Ontogenic variation in nitrogen assimilation, nodulation and nitrogen fixation in groundnut (*Arachis hypogaea*) genotypes*

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SUMMARY

Seasonal pattern of acetylene reduction (AR) and shoot nitrogen accumulation was studied in nine groundnut cultivars. Shoot N accumulation by all the cultivars was maintained until shortly before maturity and it occurred faster over the reproductive growth phase than over the earlier phases. In all cultivars plant AR (PAR) did not reflect this pattern of N accumulation, being greater over the vegetative and pod initiation phases. This suggests that the commonly observed low PAR values for groundnut over the reproductive growth phase may be the result of factors other than sink competition.

There were significant interactions of cultivar with stage of crop growth for PAR, nodule mass, and specific nitrogenase activity (SNA). Virginia types generally showed better nodulation, higher N_2-fixing capacity (both PAR and SNA) than valencias, and significant differences were observed between cultivars within a botanical type.

INTRODUCTION

In many legumes, senescence of nodules has been observed from the early pod-filling stage, this being associated with a marked decline in symbiotic nitrogen fixation (Lawn & Brun, 1974; Hardy & Havelka, 1976). This decline in symbiotic activity has generally been attributed to changes in source-sink relations, and a limitation to nitrogenase activity (NA) imposed by decreased carbohydrate supply to the nodules (Lawn & Brun, 1974; Hardy & Havelka, 1976). Changes in photosynthetic-assimilate supply have been reported to have a drastic effect on N_2 fixation in soybean (Lawn & Brun, 1974) and in groundnut (Osman, Wynne, Elkan & Schneeweis, 1983). Thus a close relationship between root and nodule carbohydrate content and nodule activity is expected. However, the nonstructural carbohydrate content in the roots of groundnut (Williams, 1979*a*) and in the nodules of soybean (Streeter, Mederski & Ahmad, 1979) has not been well correlated with nitrogen accumulation or nodule activity. Additionally, Williams (1979*b*) reported increased nitrogen accumulation at the onset of the reproductive growth in groundnut, which differs from the observation of decreased PAR in soybeans at the same stage of development (Lawn & Brun, 1974).

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Nitrogen fixation is often measured by the acetylene-reduction assay (ARA) of Dart, Day & Harris (1972). This ARA method only measures current PAR, and is influenced by a number of environmental factors (Nambiar & Dart, 1983). Nevertheless this method is widely used for the relative estimation of N_2 fixation because it is simple and quick (Wacek & Brill, 1976). However, the nitrogenase activity has been shown to be influenced by the presence of acetylene (Minchin, Witty, Sheehy & Müller, 1983) and there is clear need to re-examine the value of the ARA method because of its simplicity (Wacek & Brill, 1976) and because of the numerous reports of positive results being achieved using it as an index for N fixation attributes of crops. The technology needed to measure NA despite this effect is sophisticated and may be impractical for application to progeny selection in breeding programmes. Substantial genotypic variability in nitrogenase activity has been reported using the simple method (Wynne *et al.*, 1982; Nambiar, Dart, Nigam & Gibbons, 1982). Arunachalam *et al.*, (1984) have shown that a single ARA estimate of NA was correlated with the total N accumulated by final harvest and was an efficient selection criterion for N fixation attributes.

The objective of our experiment was to study seasonal variation in PAR (as measured by Dart *et al.*, 1972) and N uptake of groundnut cultivars, and the relationship between them.

MATERIALS AND METHODS

Nine cultivars of *Arachis hypogaea* belonging to the ssp. *fastigiata* var. *fastigiata* (valencia cvs MH 2, Gangapuri, and PI 259747), ssp. *fastigiata* var. *vulgaris* (spanish cv. Argentine), and ssp. *hypogaea* var. *hypogaea* (virginia cvs Kadiri 71 - 1, M 13, Florunner (runners), Florigiant and MK 374 (bunch)) were chosen for the study. The crop was grown during the 1979/80 post-rainy season in an Alfisol at ICRISAT Center, Hyderabad. The experimental design was a randomised complete block with four replications. Each plot consisted of six rows of 4-m length. The plots were fertilised at 40 kg P_2O_5 ha^{-1}; no nitrogen was added. The plots were not inoculated with *Rhizobium* as there was a history of groundnut cultivation in the field. Seeds were sown on ridges 75 cm apart, at 15-cm spacing within the row. The crop was irrigated at 7 - 10 day intervals, and plant protection measures were carried out as required. Observations were recorded at 29, 40, 55, 76, 86, 98, 106, and 112 days after sowing (DAS).

Acetylene reduction assay

A nitrogen fixation index was based on the ARA, conducted essentially as described by Dart *et al.* (1972). Four plants were dug out for each replication, the tops cut and soil adhering to the roots removed by shaking. The nodulated roots were placed in a 800 ml bottle fitted with a Suba seal. Acetylene was injected through the Suba seals into the bottles to give a final concentration of 10%. After injecting acetylene the gas pressure was returned to atmospheric pressure by venting through a needle for about 2 min. Gas samples were taken in pre-evacuated 10 ml vacutainers after 30 min incubation, and later analysed by gas chromatography. Although it was not possible to maintain uniform incubation temperatures under field conditions, excessive heating of the bottles was prevented by placing them under wet jute bags. Assays were carried out between 0930 and 1400 h on the third or fourth day after irrigation.

At each sampling dry mass of nodules and plant biomass were measured. The nitrogen content (percent dry weight) of the plant parts was determined by microkjeldahl analysis with a Technicon Autoanalyzer (11 Industrial Method No. 218 - 72 AA 11).

Specific nodule activity (SNA) was calculated as the amount of acetylene reduced g^{-1} dry nodule mass h^{-1}.

Statistical analysis was conducted using standard procedures for analysis of variance. The values of PAR at sample dates as determinants of final N yield were evaluated by simple and stepwise regression and correlation across sample dates.

RESULTS AND DISCUSSION

The virginia 'runner' cultivars did not vary significantly in their growth, PAR, and nodule mass plant^{-1}. Similarly, two valencia cultivars had similar growth patterns. To simplify data presentation, these groups have each been represented by a single cultivar; Gangapuri for the similar valencias and Kadiri 71 - 1 for the virginia runners. However, the full treatment sets were used for statistical analysis. Dry matter continued to increase in all cultivars until the final harvest, and in only MH 2 and Gangapuri was there evidence of pod growth ceasing before 112 DAS (Figs 1 and 2). Shoot-N content decreased as the season progressed (Fig. 3), although Kadiri 71 - 1 tended to maintain N contents higher than other cultivars. The shell-N content also decreased with time once the kernels were large enough to shell out. Kernel-N content tended to increase, or remained constant, over the last three samplings (Fig. 4). Contrary to the report of Tonn & Weaver (1981), differences were present for seed-N content

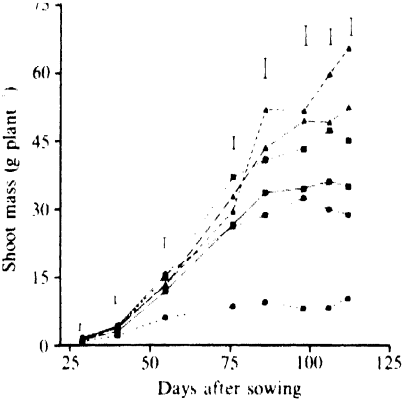

Fig. 1. Changes with time in the shoot mass (g plant^{-1}) of six groundnut cultivars grown at ICRISAT Center. (● ---●) MH 2; (● —— ●) Gangapuri; (■ -- ■) Argentine; (▲ ---▲) Kadiri 71-1; (▲— - —▲) MK 374; (■ - ■) Florigiant. Bars represent standard errors.

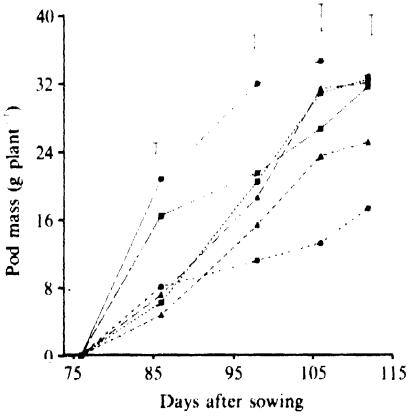

Fig. 2. Changes with time in the pod mass (g plant^{-1}) of six groundnut cultivars grown at ICRISAT Center. (●---●) MH2; (● —— ●) Gangapuri; (■ - - - - ■) Argentine; (▲---▲) Kadiri 71-1; (▲— - —▲) MK 374; (■ - - ■) Florigiant. Bars represent standard errors.

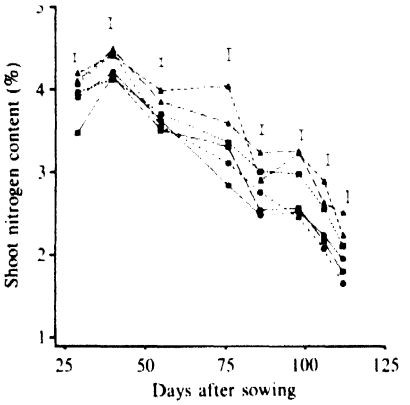

Fig. 3. Changes with time in the shoot nitrogen content (%) of six groundnut cultivars grown at ICRISAT Center. (●------●) MH 2; (●——●) Gangapuri; (■- - -■) Argentine; (▲—·—▲) Kadiri 71-1; (▲·····▲) MK 374; (■- - -■) Florigiant. Bars represent standard errors.

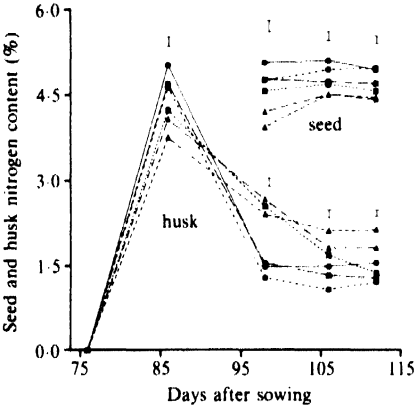

Fig. 4. Changes with time in the seed and husk nitrogen content (%) of six groundnut cultivars grown at ICRISAT Center. (●----●) MH 2; (●——●) Gangapuri; (■----■) Argentine; (▲---▲) Kadiri 71-1; (▲---▲) MK 374; (■----■) Florigiant. Bars represent standard errors.

within botanical groups, particularly so for virginia bunch cultivars.

Nitrogen accumulation and PAR of these cultivars at each sampling are shown in Fig. 5. The PAR of cultivars generally increased with time over the vegetative and flowering stage, reaching a maximum activity at 76 or 86 DAS; however for MH 2 and Argentine maximum activity occurred by 40 DAS. At 86 DAS, PAR was greatly decreased. The low PAR over the pod growth phase was associated with a marked decrease in SNA 96 DAS (Table 3). In all cultivars except Gangapuri, PAR increased at the last sampling. Major differences in PAR over the first half of crop life existed between the cultivars. The virginia cultivars had significantly more PAR than the valencia and spanish cultivars. MH 2 had very low PAR. Nodule mass generally increased up to 76 DAS, after which it remained relatively constant until 112 DAS (Fig. 6).

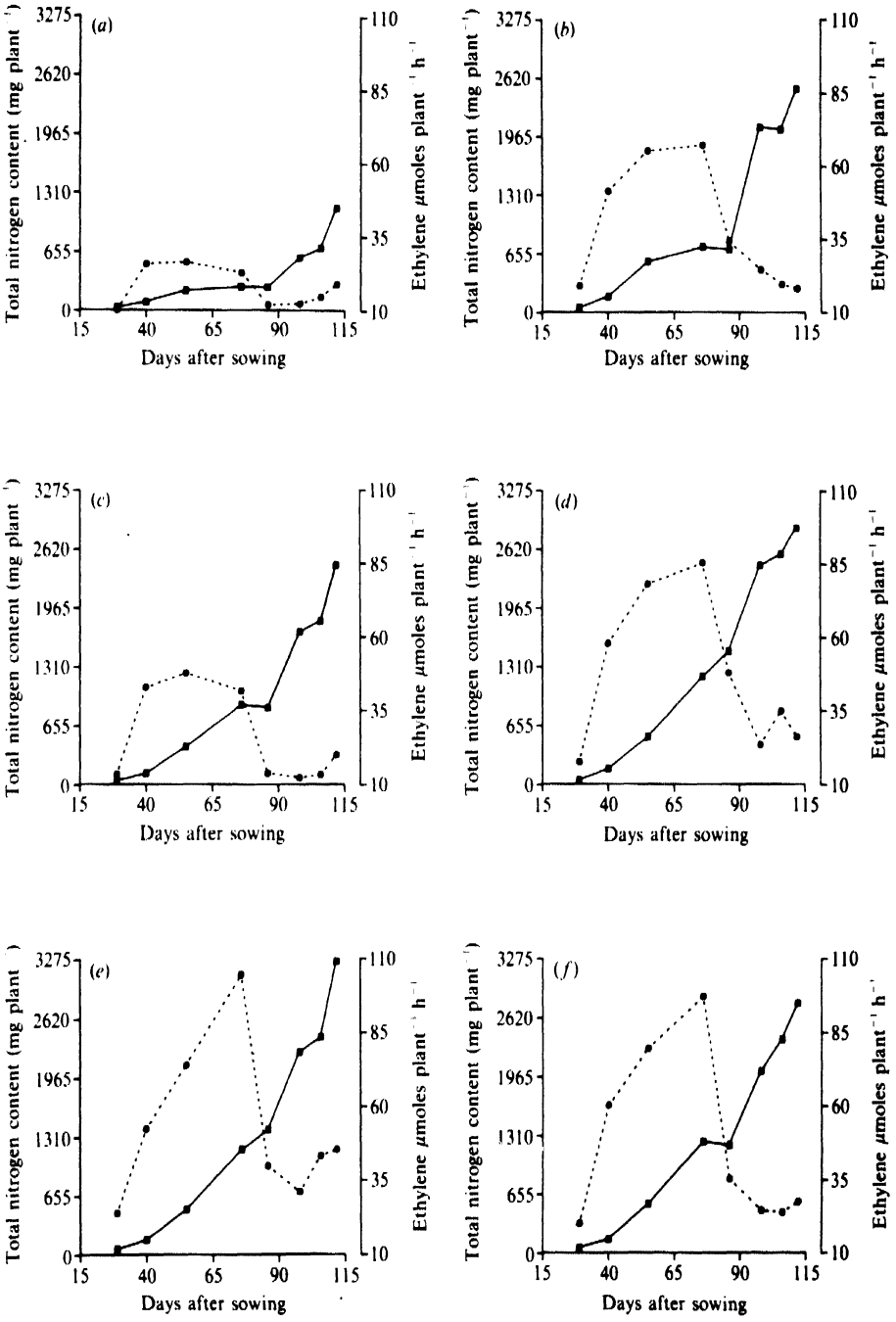

Fig. 5. Changes with time in the total nitrogen content (mg plant^{-1}) (■——■); and nitrogenase activity (μmoles ethylene plant^{-1} h^{-1}) (●----●) of six groundnut cultivars. (A) MH 2; (B) Gangapuri; (C) Argentine; (D) Kadiri 71-1; (E) MK 374; (F) Florigiant.

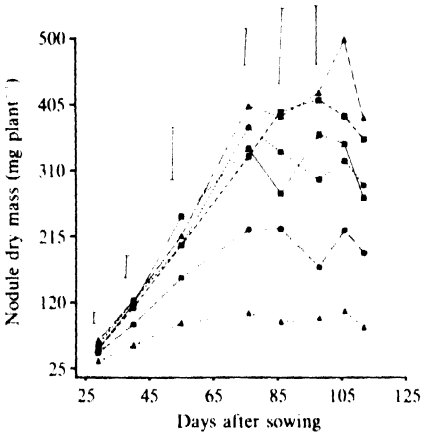

Fig. 6. Changes with time in the nodule mass (mg plant^{-1}) of six cultivars. (▲ — ▲) MH 2; (■ — ■) Gangapuri; (● — ●) Argentine; (■ — ■) Kadiri 71-1; (▲ — ▲) MK 374; (● — ●) Florigiant. Bars represent standard errors.

In an earlier study with 21 cultivars, it was observed that PAR peaked between 60 and 70 DAS during the rainy season (Arunachalam *et al.*, 1984). The discrepancy in the pattern of activity is probably due to the differences in temperature between the growing seasons. In the post-rainy season, plants flower 7 - 10 days later than in the rainy season. However, in Israel the maximum PAR occurred 90 to 100 DAS (Ratner, Lobel, Feldhay & Hartzook, 1979). This might be due in part to the maturity differences of the cultivars used, and in part to different temperatures and daylengths associated with the different geographical locations.

However, N accumulation by these crops followed the pattern observed by Williams (1979*a*) in that the rate of N assimilation was, in all cultivars, faster during reproductive growth than at earlier stages. Study of the pattern of PAR shows that some 70 - 80% of the total (integrated) PAR occurred before 76 DAS, while the plant N accumulated over the same period represented only 40 - 50% of the total N accumulation, leaving a large gap in the N budget. Clearly, the estimation of nitrogenase activity during pod growth is a poor reflection of N accumulation during this phase of crop growth; and the use of the observed low PAR to hypothesise energy limited nodule activity in groundnuts may be erroneous.

These data are unable to explain this discrepancy, and we may only speculate as to possible reasons. This situation could arise if the plants depended mainly on mineral nitrogen sources over the reproductive stage. However, this is unlikely since, for groundnuts grown at ICRISAT Center, the contribution of mineral N to the total N economy has been estimated to be only 10 - 20% (Giller *et al.*, 1987). Even if all the mineral N assimilated was taken up after seed-fill started, we are still unable to account for the difference.

It is possible that redistribution of the N accumulated in the roots could provide a source of N to bridge the gap between that suggested by PAR and N accumulated in the shoot. We have been able to examine this possibility by using a well verified model of growth and N distribution (Boote, Jones & Hoogenboom, 1987) for Florunner. This simulation model indicates that the increased rate of N accumulation over the pod growth phase is due to

Table 1. *Specific nodule activity (μmoles C_2H_4 g^{-1} nodule dry mass h^{-1}) of groundnut cultivars at various sampling dates.*

Cultivar	Days after sowing							
	29	40	55	76	86	98	106	112
Florunner	77	568	363	273	133	74	60	69
Kadiri 71-1	70	528	396	260	122	58	94	74
Florigiant	74	502	411	266	100	82	82	119
Argentine	23	489	309	188	65	48	31	115
MH 2	37	453	299	218	134	140	136	226
MK 374	93	446	349	263	109	74	114	124
M 13	59	442	318	262	114	59	63	80
Gangapuri	74	438	270	199	103	70	59	68
PI 259747	37	389	305	203	63	45	48	68
SE ±	9·7	41·1	30·2	19·6	13·9	13·8	7·7	35·8
CV%	32	17	17	16	26	38	20	68

decreased root growth, but that the remobilisation of root N does not adequately explain the differences between PAR and N assimilation patterns.

Another possible explanation for the discrepancy between the two measurements is the sampling process, which may no longer recover the later-formed nodules on the periphery of the root system. These may be contributing most of the N_2 fixed by groundnuts during the reproductive phase. This possibility is supported by the fact that SNA decreased progressively after 40 DAS while major reproductive sink competition only started at 96 DAS.

Associated with the genotypic and ontogenic variations in PAR were variations in both the nodule masses and in their SNAs. The SNAs also varied with time and cultivar (Table 1), the interaction being significant ($P < 0{\cdot}001$). In all cultivars the specific activity was greatest at 40 DAS and declined thereafter.

At 40 DAS, Florigiant, Florunner and Kadiri 71 - 1 had consistently higher SNA, and Gangapuri and PI 259747 low SNA. MH 2 maintained a higher SNA than the other cultivar during reproductive growth. However, no consistent pattern was observed with respect to botanical groups.

While the reasons for this phenomenon are not clear, the results have several important implications for other fields of research. Many researchers have observed correlations of reproductive growth with decreased PAR, and the resulting hypothesis of sink competition for carbohydrates as a limiting factor to N_2 fixation by legumes needs to be critically examined for each species. It is possible that many of the data used to support this hypothesis may be artifacts of the sample methods, as has been suggested by these results.

Because the acetylene used in the assay has been shown to influence the nitrogenase activity under assay conditions (Minchin *et al.*, 1983) the ARA used here cannot be expected to provide absolute measurements of N fixation. However, for breeding selection purposes relative estimates of PAR have value if they correlate with final N accumulation. The importance of growth stage in assessing N_2 fixation indicated in an earlier study (Arunachalam *et al.*, 1984), is confirmed here. Across all genotypes the PAR measured before 86 DAS was significantly correlated with the total N accumulated at the final harvest (Table 2). However, MH 2 data had a major impact on the relationship because effectively this was an out-lier to the main data set and dominated the relationship. But although these data provide a limited sample of genotypes this relationship is consistent with other studies. A linear relationship between PAR at a given specific stage and final N accumulated has also been observed in soybeans (Bello *et al.*, 1980).

Table 2. *Correlation between total N content at 112 DAS and N_2-ase activity (μmoles C_2H_4 plant^{-1} h^{-1}) on each sampling date.*

Days after sowing	Correlation coefficients Including MH 2	Excluding MH 2
29	0·88**	0·84**
40	0·78**	0·59
55	0·91**	0·89**
76	0·85**	0·62
86	0·85**	0·79*
98	0·65	0·65
106	0·49	0·85**
112	0·63	0·65
**P > 0·01% *P > 0·05%	df = 7	df = 6

Table 3. *Standard error of means for the parameters presented in figures 1 to 6.*

DAS	Nodule g p^{-1}	μmC_2H_4 p^{-1} h^{-1}	Shoot g p^{-1}	Pod g p^{-1}	Shoot N%	Seed N%	Shell N%
29	0·014	2·27	0·14	–	0·08	–	–
40	0·039	4·97	0·32	–	0·15	–	–
55	0·073	7·09	1·19	–	0·07	–	–
76	0·052	7·19	1·89	–	0·14	–	–
86	0·106	4·38	3·21	–	0·11	–	–
98	0·089	3·25	3·88	1·71	0·10	0·126	0·013
106	0·148	2·38	4·20	3·03	0·11	0·095	0·013
112	0·085	6·44	3·95	2·90	0·11	0·105	0·030

This study clearly shows that any evaluation of the PAR of a range of genotypes must occur before reproductive growth occurs. The PAR measured at 98, 106, and 112 DAS were poorly correlated with both current N accumulation rate and final N. A single assay for nitrogenase activity conducted before 86 DAS would seem the best to differentiate cultivars for N_2 fixation attributes. However, this assay would not detect those situations where the duration of N_2 fixation may vary, and total N accumulated must be more reliable than nitrogenase-based estimates of fixation in these cases.

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Detection and assay of siderophores in cowpea rhizobia (*Bradyrhizobium*) using radioactive Fe (^{59}Fe)

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NAMBIAR, P.T.C. & SIVARAMAKRISHNAN, S. 1987. Detection and assay of siderophores in cowpea rhizobia (*Bradyrhizobium*) using radioactive Fe (^{59}Fe). *Letters in Applied Microbiology* **4**, 37–40.

In this paper we describe a method for the detection and assay of siderophore (catechol type) using radioactive ^{59}Fe. Using this method we have found that cowpea rhizobia (*Bradyrhizobium*) differ in their ability to produce this type of siderophore.

Although iron is abundant in soils (1–6%), it is often unavailable to plants because its solubility is dependent on pH, and under natural aerobic soil conditions most of the iron exists in the insoluble ferric form, which is not available to plants. Iron availability to plant roots may be modified by organic chelators (Powell *et al.* 1980; Neilands 1981). When grown under conditions of iron deprivation micro-organisms secrete ferric-specific ligands called siderophores (Neilands 1981). Most of the siderophores can be classified into two types: the catechol-like compounds found only in bacteria, and the hydroxamate-like compounds found in fungi, yeasts and bacteria (Neilands 1981; Miller *et al.* 1985). Powell *et al.* (1980) demonstrated that hydroxamate siderophores are widely distributed in a variety of soils in sufficient concentrations to contribute to the iron nutrition of higher plants. Currently used methods for the detection of siderophores include (1) chemical methods (Neilands 1981) and (2) bioassays (Powell *et al.* 1980). It is impossible to quantify the iron-binding capacity of the ligands using the former. Siderophore-bound iron uptake is strain-specific, and utilization of siderophores from different sources may vary depending on the test strains (Smith & Neilands 1984). Hence bioassays may not be very effective for comparative purposes. In this paper we describe a method for detecting and quantifying the iron-binding capacity of catechol type siderophores using ^{59}Fe, and demonstrate that cowpea rhizobia (*Bradyrhizobium*, Jordan 1984) differ in their ability to produce these siderophores.

Materials and Methods

CHEMICALS

^{59}Fe as ferric chloride (specific activity, 920 mCi/g) was obtained from the Bhaba Atomic Research Center (Bombay, India). Instagel was purchased from Packard Instrument Co. (USA). Other chemicals used were of analytical grade.

STRAINS AND CULTURE CONDITIONS

The origin and details of the test strains of *Bradyrhizobium* have been described previously (Nambiar *et al.* 1984). The strains were grown in 1 l media as described by Modi *et al.* (1985). The cultures were harvested during the late logarithmic phase of growth and centrifuged at 6000 g for 15 min. Cell pellets were dried at 80°C for 48 h and their dry weights recorded.

ASSAY PROCEDURE

The pH of the culture supernatant fluid was adjusted to 3·0 with 0·1 N HCl, and 500

ethyl acetate added. The mixture was then stirred for 1 h at room temperature (27° ± 2°C). The ethyl acetate fraction was collected and evaluated by flash evaporation at 45°C. The residue was then dissolved in 4 ml of ethyl alcohol and stored at −4°C.

Freshly prepared ferric chloride (in 0·5 ml dilute HCl, 5×10^{-4} N) solutions were used. The optimum concentration of cold Fe required for the assay was determined by varying the concentration of cold Fe (as ferric chloride) whilst maintaining constant amounts of radioactive Fe (0·2 μg/ml). In later experiments, 0·2 μg radioactive Fe was mixed with 6 μg cold Fe in 0·5 ml dilute HCl. To this mixture, 0·1 ml of ethanol extract from the test sample was added, stirred well and the mixture incubated for 3 h at room temperature (27°C). At the end of the incubation period, the siderophore–Fe complex was extracted in 2 ml of ethyl acetate and 0·5 ml of the ethyl acetate layer was added to a vial containing Instagel scintillation fluid. The β emission was counted in a Beckman (5801) counter and the concentration of radioactive Fe calculated after correcting for counting efficiency and half-life. In the control experiments, pure ethyl alcohol was substituted for the test sample. The concentration of siderophore-bound ^{59}Fe was calculated after subtracting the values obtained for the control. All experiments were replicated four times.

Effect of EDTA (ethylenediamine tetraacetic acid) on Fe-siderophore

The effect of EDTA on Fe–siderophore was tested as follows. Six ml of Fe–siderophore (strain NC 92) were extracted in ethyl acetate as described above, the ethyl acetate was evaporated in a stream of nitrogen and the residue was dissolved in 2 ml ethyl alcohol. The above extract (0·1 ml) was incubated with varying concentrations of EDTA in 0·5 ml HCl (5×10^{-4} N) for 30 min. The Fe–siderophore was re-extracted in 2 ml ethyl acetate and the radioactivity was measured. Other assay conditions were the same as described earlier.

Results and Discussion

The optimum concentration for binding was 2–6 μg Fe/ml (Fig. 1). The ethyl alcohol extract

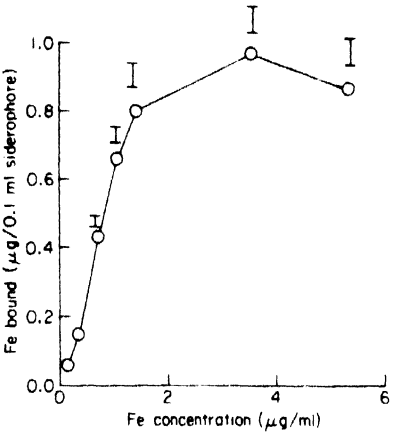

Fig. 1. Effect of iron concentration (as ferric chloride) on siderophore–Fe binding. Siderophore (ethyl alcohol extract) from strain NC 92 (0·1 ml) was added to a mixture of varying concentrations of cold iron and radioactive iron (0·2 μg Fe/ml) and incubated for 3 h. Siderophore-bound Fe was calculated as described in Materials and Methods. Bars represent ± S.E.

of strain NC 92 was yellow in colour, but extracts of other strains were colourless. The standard test for siderophores, i.e. addition of an equal volume of $FeCl_3$ (0·1 mmol/l $FeCl_3$) to the ethyl alcohol extract (Neilands 1981), resulted in the appearance of a brick red colour (a positive result) for two of the seven test strains (NC 92 and 5a/70). However, using the radioactive Fe binding method, Fe-binding chelants were detected in the ethyl acetate extract of all strains tested, values being highest in strains NC 92 and 5a/70 (Table 1). The results indicate that the method described here may be a more sensitive assay for the Fe-binding siderophores that can be selectively extracted in ethyl acetate. Interference due to unbound $FeCl_3$ is minimal ($FeCl_3$ is insoluble in ethyl acetate, Stecher 1968).

The possibility of non-specific binding of iron to other molecules due to weak interactions was tested by incubating the Fe–siderophore (strain NC 92) complex with EDTA. Even at a ratio of 1 : 16 molar concentration of bound Fe : EDTA, 50% of the siderophore-bound iron was retained in the siderophore (Table 2). Moreover, when the ethanol extract of strain NC 92 was loaded on a lipophilic Sephadex (LH-20-100, Sigma, St Louis, USA) column (1 cm diameter,

Table 1. Estimation of siderophore production in the culture supernatant fluid of the *Bradyrhizobium* test strains

Bradyrhizobium strain	Fe bound (μg g dry cells)
NC 92	370
5a 70	312
NC 6	207
NC 43.3	128
NC 70.1	114
47 A1	93
IC 6001	93
± S.E.	19·7

Siderophore (0·1 ml ethyl alcohol extract) was added to a mixture of cold iron (6 μg ml as ferric chloride) and radioactive iron (0·2 μg ml) and incubated for 3 h at room temperature (27 ± 2 C) and siderophore–Fe complex was extracted into ethyl acetate and the radioactivity counted. Siderophore-bound iron was estimated as described in the text.

Table 2. Effect of ethylenediaminetetraacetic acid (EDTA) on Fe–siderophore (strain NC 92)

Molar ratio of Fe : EDTA in the incubation medium	Percentage radioactivity retained in the ethyl acetate
1 : 0	100
1 : 1	85
1 : 8	78
1 : 16	52

Fe–siderophore complex (strain NC 92) extracted in ethyl alcohol was incubated with varying concentrations of EDTA for 30 min. The Fe–siderophore complex was re-extracted in ethyl acetate and the radioactivity measured. Other assay conditions are as described in Table 1.

35 cm long), three coloured fractions were separated, and the iron binding ability was associated with only one of the fractions. These results indicate that iron binding ability is specific to certain molecules.

Extraction of the culture filtrate into ethyl acetate is an effective purification step for catechol siderophores, since both salts and macromolecules are excluded by this step (Neilands 1981). By definition, siderophores include all types of small molecular weight, low iron inducible, iron-binding agents regardless of biological activity (Neilands 1981). If the above definition is accepted, then the method described above should assay siderophores alone.

The importance of siderophores produced by micro-organisms in supplying Fe to plants has been suggested by many workers (Powell *et al.* 1980; Miller *et al.* 1985). Apart from iron supply to the plants, siderophore-producing micro-organisms are also reported to help overcome certain root diseases caused by fungi. Enhanced plant growth has been observed following the addition of a siderophore from *Pseudomonas* to soil containing fungal pathogens, and it was suggested that the siderophore binds iron so tightly that it is unavailable to the fungal pathogens (Kloepper *et al.* 1980).

Siderophore production by a strain of *Rhizobium meliloti* (Smith & Neilands 1984) and by a cowpea *Rhizobium* strain (Modi *et al.* 1985) has been reported. Inoculation with strain NC 92 increased the yield of a few groundnut cultivars in India, China and Cameroon (Nambiar 1985). Inoculation with strain 5a/70 increased groundnut yields in a single trial at the ACRISAT Center, whilst inoculation with other strains did not (Nambiar *et al.* 1984), despite some of the strains (for example, NC 43.3) being equally efficient in nitrogen fixation in pot experiments and competitive in nodule formation in the field. Hence it was suggested that the effect of strain NC 92 on groundnut yield may not be due entirely to its symbiotic nitrogen-fixing ability (Nambiar 1985). However, it remains to be seen if these strains produce siderophores in the rhizosphere, or in the nodules of the plant, and if siderophore production by strains NC 92 and 5a/70 has any effect on the growth and yield of groundnuts.

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