Studies on Nitrogen Fixation by Groundnut at ICRISAT

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Symbiotic nitrogen fixation depends on an interaction between the *Rhizobium* strain, host plant genome and environment. We are examining all the three components with the objective of increasing biological nitrogen fixation by groundnut.

Rhizobium: Isolation, Strain Testing and Inoculation Response

Groundnut is promiscuously nodulated by *Rhizobium* of the cowpea miscellany (Fred et al. 1932). When nodulated with effective (nitrogen fixing) rhizobia, groundnut nodules fix most of the nitrogen requirements of the plant (Pettit et al. 1975; Schiftman et al. 1968). Substantial increases in the yield have been obtained following inoculation in fields where peanuts had not been grown before (Burton 1976; Pettit et al. 1975; Schiftman et al. 1968; Sundara Rao 1971). In fields where groundnuts had been grown earlier, inoculation sometimes resulted in increased yield, increase in seed quality, higher protein and oil content (Arora et al. 1970; Chesney 1975). A decrease in yield following inoculation has also been reported (Subba Rao 1976). Allen and Allen (1940) described differences between *Rhizobium* strains in nodulation and nitrogen fixation in groundnut. Surveys in farmers' fields in the southern states of India showed considerable variation in nodulation with 52 out of 96 fields surveyed having poor nodulation. Nodulation and nitrogen fixing activity, as measured by acetylene reduction, was ten times less in some farmers' fields than that observed in fields at ICRISAT Center at the same stage of growth of the plant. These observations indicate that it should be possible to increase biological nitrogen fixation in these fields by inoculating with effective and competitive *Rhizobium* strains.

We collected nodules from farmers' fields in Karnataka and Andhra Pradesh states in India and from them have purified 50 authenticated *Rhizobium* strains. We also maintain a collection of *Rhizobium* strains for groundnut obtained from all other known major collections in the world such as USDA (Beltsville), North Carolina State University (Raleigh), NifTAL (Hawaii), Dept. of Agriculture, Zimbabwe, and the Australian Inoculants Research and Control Service. As a part of our collaborative project with North Carolina State University (NCSU) on biological nitrogen fixation, we are testing the suitability for use as inoculants of *Rhizobium* strains which have been isolated and characterized at NCSU from nodules obtained during *Arachis* germplasm collection trips in South America (Wynne et al. these Proceedings).

Our experiments show that in nitrogen-free sand: vermiculite media in pots, *Rhizobium* strains vary in their ability to nodulate and fix nitrogen with groundnut (Figs. 1, 2). Although the magnitude of the shoot dry weight was often related to nodule dry weight (e.g. strain IC 6006, Figs. 1, 2), this relationship was not consistent with the array of the strains. Because of the variability in germination in Leonard jars, pot culture assembly was used for strain testing. We sterilize by autoclaving or steaming or heating to 150°C the rooting media and apply nitrogen-free nutrient solution through a permanent 6 cm diameter watering tube which is covered with a metal cap when not in use. The pot surface is covered with heat-sterilized gravel (3-6mm) to protect from aerial contamination (Fig, 3). Six seeds are sown per pot and thinned to three plants/pot.

We have observed a relationship between shoot growth, and the amount of inoculum applied up to 10^7 *Rhizobium* per seed when

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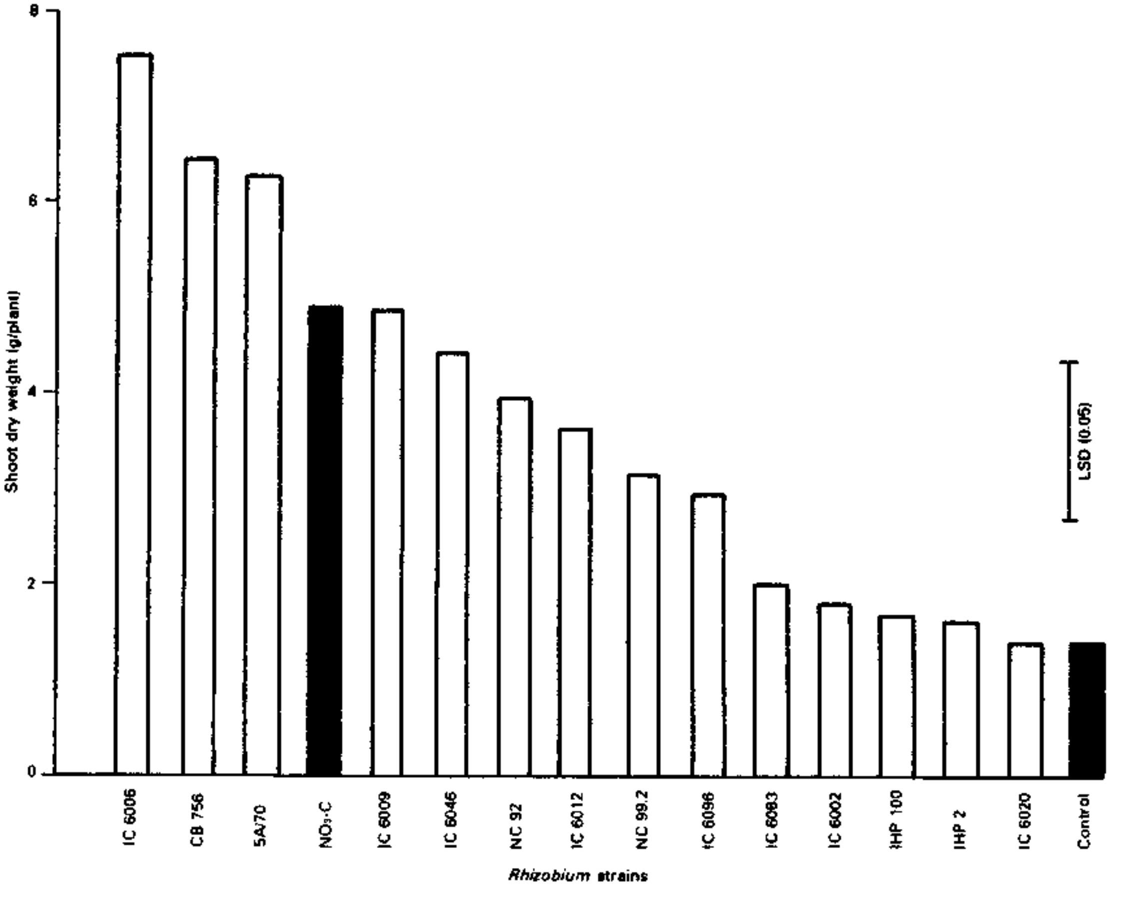

Figure 1. Shoot production by groundnut inoculated by different Rhizobium *strains. Three plants per 20 cm dia pot were grown in a sand.vermiculite (2:1) medium, inoculated with broth culture at the rate of 10^s rhizobialseed, watered with nitrogen-free nutrient solution and harvested 64 days after planting. Controls received no inoculum; NO3-C received 240 ppm N continuously.*

groundnut was grown in pots of sterilized sand: vermiculite. Nodulation followed the same trend (Table 1). This is in marked contrast to the situation in soybeans where nodulation and plant growth was reduced in both field and pot trials only if the inoculum was less than 15 x 10^3 per seed (Burton 1975). This indicates that one has to ensure an adequate *Rhizobium* inoculum size in pot trials measuring nitrogen fixation. It also demonstrates the need to examine the interaction between inoculum size and nodulation response in field experiments.

Groundnut and soybean differ in the infection process in nodule formation (Dart 1977), and this may be the cause of the difference in response to inoculum size.

Table 2 summarizes the results of eight inoculation trials at ICRISAT Center. Although a response was not always obtained, Robut 33-1, a cultivar which is about to be released in Andhra Pradesh, gave substantial increases in pod yield when inoculated with a strain NC 92. Strain NC 92 was obtained from NCSU and isolated from nodules collected in South

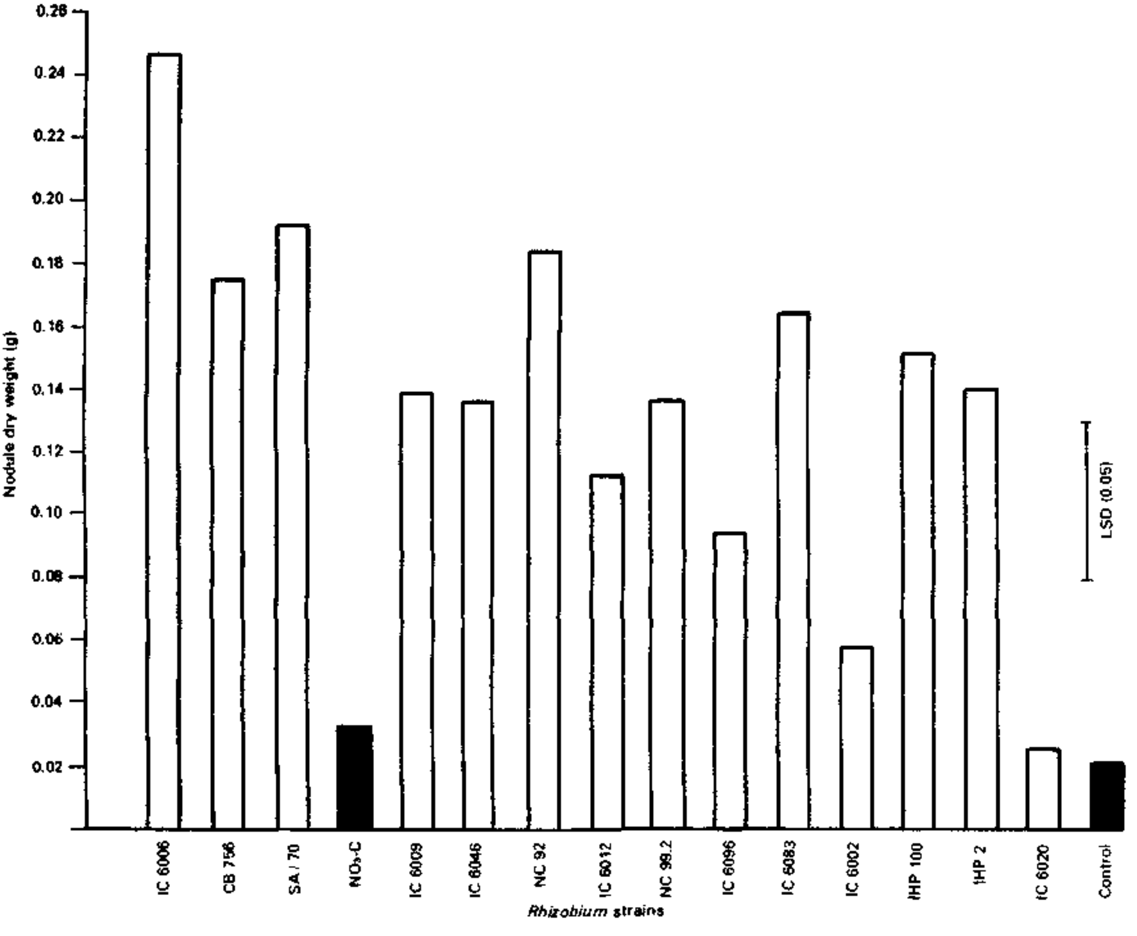

Figure 2. Nodule formation by different Rhizobium *strains. Conditions as in Figure 1.*

America. Robut 33-1 was the variety which most commonly responded to inoculation and in two of the inoculation trials this response was best with strain NC 92. It may be worth developing an inoculum with NC 92 specifically for use with Robut 33-1 provided the evidence of poor competition ability against strain IC 6009 (Table 3) is not found with other *Rhizobium* strains.

Since seed treatment with fungicides is a recommended practice, the inoculum for groundnut needsto beseparated from the seed. We do this by applying a granular inoculum below each seed, the granules being made by inoculating 1-2 mm sand particles with peat inoculum using methyl cellulose as the sticker.

Nitrogen Fixation by Groundnut

Nitrogenase Activity Assay

We have studied several parameters that influence acetylene reduction by groundnut root nodules in our efforts to standardize the assay for measu ring nitrog enase activity of field grown plants. There is a marked diurnal variation in the nitrogenase activity of field grown plants (Fig. 4). The increase in activity after daybreak suggests a close link with photosynthesis. Thus plants which produce more photosynthate are likely to fix more nitrogen. It will be interesting to see if photosynthesis declines in

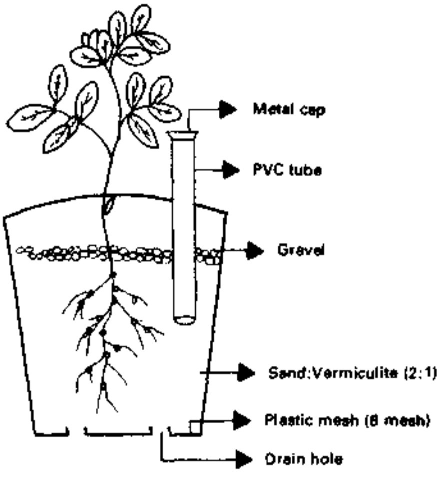

Figure 3. Cross section of a pot system.

Table 1. Influanca of *RMxoblum* Inoculum level *on* nodulation and nltrogan fixation by groundnut.

Level of *Rhizobium* applied as broth (number/seed)	Shoot dry wt* (g/plant)	Nodule dry wt* (g/plant)
3.2×10^9	3.38*	0.13[a]
5.5×10^7	2.38*	0.12[a]
4.8×10^4	1.08*	0.03*
6.1×10^2	0.97*	0.02*
Nitrate control	4.34	0

* Data in each column followed by the same letter are not significantly different at the 0.05 level.

Note: Kadlrl 71-1 plants inoculated with strain NC-92 were grown under semlsterile conditions watered with nitrogen-free nutrient solution and harvested 57 days after planting.

Table 2. Summary of Inoculation trials conducted at ICRISAT Center.

Year/ Season	Soil type	Cultivars	Strain	Pod yield response
Rainy season 1977	HF, Alfisol	TMV-2 Kadiri 71-1	5a/70	Nil
Rainy season 1977	LF, Alfisol	Kadiri 71-1 Robut 33-1, TMV-2	5a/70	TMV-2, 25%, Robut 33-1, 32%
Rainy season 1977	HF, Vertisol	Kadiri 71-1 TMV-2	5a/70 IC 6006	Nil
Rainy season 1978	HF, Alfisol	Robut 33-1 Argentine AH-8189	5a/70 ICG-60 IC6006 Mixture	Nil
Rainy season 1978	LF, Alfisol	MH-2 Argentine Robut 33-1	5a/70 ICG-60 6S Mixture	Robut 33-1, 26% (NS)
Postrainy season 1979	HF, Alfisol	MH-2 Robut 33-1 AH-8189	NC92 IC6009 Mixture	Robut 33-1, 28.5% (NC 92)
Rainy season 1979	HF, Alfisol	Kadiri 71-1 Robut 33-1 AH-8189	5a /70 IC6006 NC 43.3 NC7.2 NC92	Robut 33-1, 25.7% (NC 92)
Postrainy season 1980	HF, Alfisol	Robut 33-1	NC92	Nil

HF - High Fertility LF = Low Fertility

Table 3. Response of groundnut to *Rhixobium* Inoculation In an Alfisol (1978-79 postrainy season).

	Pod w light (kg/ha)			
Cultivars	Uninoculated	IC-6009	NC 92	Mixture (IC 6009 + NC 92)
MH-2	2222	1888	1944	2027
Robut 33-1	3500	3333	4500**	2805*
AH-8189	2833	2861	2694	2805

CV (%) 15.51 **SIg nilficant at 1% level *Significant at 5% level

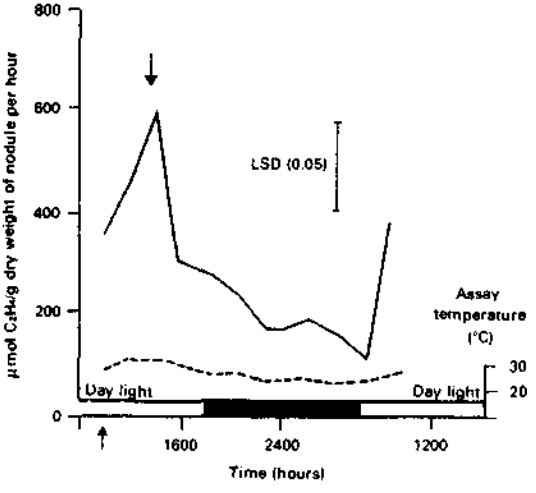

Figure 4, Nitrogenase activity (µmol C2H4/g dry weight of nodule per hour) in groundnut cultivar Kadiri 71-1. after 81 days of planting, ICRISA T Center, postrainy season 1976.

Table 4. Mean squares from Anova for nltrogenase activity (µ moles C2H4/ plant par hr) of selected germplasm linea assayed during day and again at night.

Source of variation	df	Mean square
Replication	3	5500**
Time of assay	1	374254**
Germplasm lines	13	16314**
Interaction	13	5659**
Experimental error	81	1321

** Significant at 1% level

Table 5. Effect of incubation temperature on acetylene reduction by peanut roots.

Incubation temperature	µmoles C2H4/ plant per hr
25°C	46.34
30°C	33.97
35°C	32.52
CV (%) 42	
LSD (0.05) 9.8	

the same way as nitrogenase activity in the early afternoon, when leaf vapor pressure deficits are likely to be greater. A preliminary acetylene reduction assay of 14 groundnut lines selected for variability in foliage production, showed significant interaction in acetylene reduction between lines and time of measurement of nitrogenase activity — day time assay at 0900-1100 hr and night time assay at 2100-2300 hr (Table 4).

Temperatures in the assay bottle greater than 25° C decreased nitrogenase activity of nodulated roots of groundnut cv Kadiri 71-1 (Table 5). Excess or insufficient moisture also decreased acetylene reduction activity (Fig. 5). We have observed that shading causes a rapid decrease in nitrogenase activity. When 109-day old Kadiri 71-1 plants were shaded to 60% of ambient light intensity, nitrogenase activity was reduced within a day by 30% (Fig. 6). Plants grown during the dry season, which received fewer irrigations but were not allowed to wilt, produced about half the pod yield of plants irrigated every 7-10 days. There were indica-

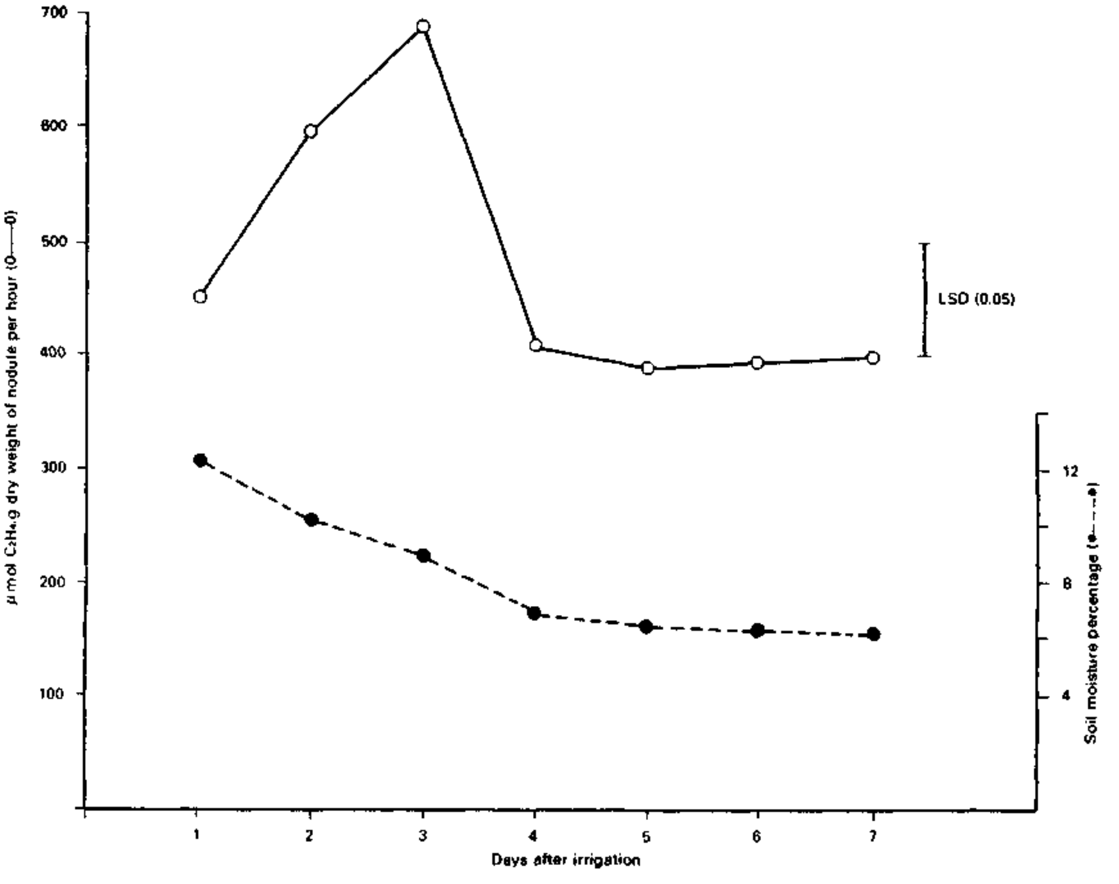

Figure 5. Effect of soil moisture on nitrogenase activity. Sixty days old Kadiri 71-1 plants were assayed on different days after an irrigation.

tions of differences between varieties in response to water stress. Nodule development and nitrogenase activity were much reduced by the water stress. Nitrogenase activity recovered rapidly after an irrigation.

Seasonal and Cultivar Differences

Figure 7 shows the nodulation and nitrogen fixing activity of cv Kadiri 71-1 (A *hypogaea* subsp *hypogaea,* a long-season runner cultivar) and of cv Comet (A *hypogaea* subsp *fastigiata,* a short duration erect-bunch cultivar) when grown in the rainy season 1976 and under irrigation in the postrainy season 1977. In 1976, a 57 day dry period beginning 39 days after planting had an overriding effect on nodule formation and nitrogenase activity. For the rainy season planting, nodules formed rapidly during the first 25 days, but the drought restricted further nodule formation with little difference between cultivars. For Comet, nodules changed little in size after 25 days but for Kadiri 71-1, nodules continued to grow so that by 75days nodule mass per plant was twice that of Comet.

In the postrainy season, nodule formation was slower to start but then increased until 80 days after planting when three times as many nodules had formed as in the rainy season. New nodules were still forming on Kadiri 71-1 at 128 days. Nodule dry weight per plant reflected the pattern for nodule number.

Nitrogenase activity per plant and the efficiency of the nodules (nitrogenase/g nodule tissue) differed significantly between cultivars

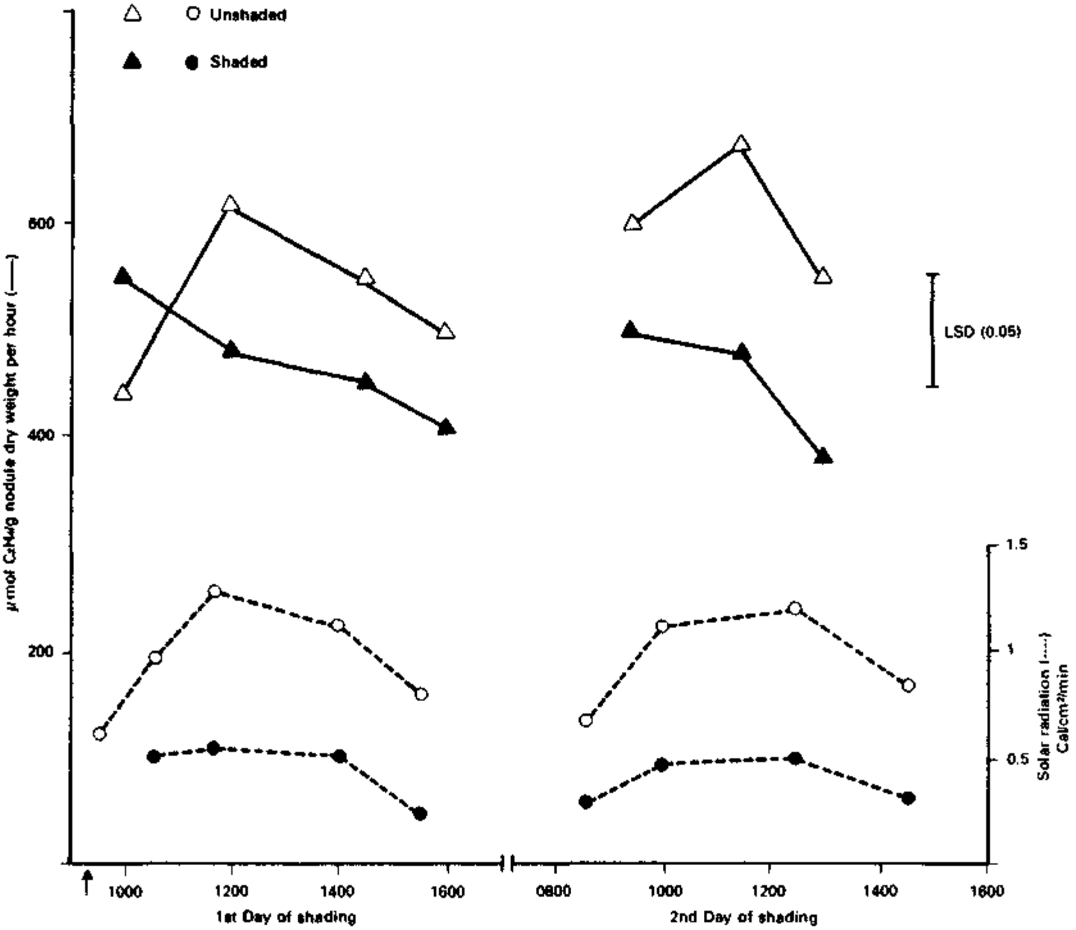

Figure 6. Effect of shading on nitrogenase activity of groundnut. Plants grown as an irrigated crop were shaded in replicated plots at 109 days after planting. Acetylene reduction assays were carried out on the same day and on a subsequent day. | indicates the start of shading treatment.

and seasons. In rainy season 1976, nitrogenase activity was at a maximum by 25 days, but from about 40-70 days, Kadiri 71-1 nodules were more active than those of Comet. It was only after 40 days without appreciable rainfall that the nitrogenase activity of Kadiri 71-1 nodules decreased. The pattern of nitrogen fixation in the irrigated season was quite different, increasing until about 75 days, then decreasing rapidly, with differences developing between cultivars. Kadiri 71-1 nodules at 128 days were still more active than at any stage during the rainy season. Peak activity per plant during the irrigated postrainy season was more than twice that of the rainy season.

The difference in symbiotic performance of Kadiri 71-1 and Comet under the drought stress of 1976, as well as between seasons, suggests that we can select cultivars which are better adapted to fix nitrogen under stress conditions.

Nodulation and nitrogen fixation of two cultivars MH-2, a dwarf mutant, and Kadiri 71-1 was followed throughout the postrainy season (Figs. 8, 9). There were marked differences in the weight of the nodules per plant and nitrogenase activity per plant of these two cultivars. Except for a short period, however, nitrogenase activity per gram of shoot weight was very similar for the two cultivars (Fig. 10). This may indicate that in dwarf cultivars such as

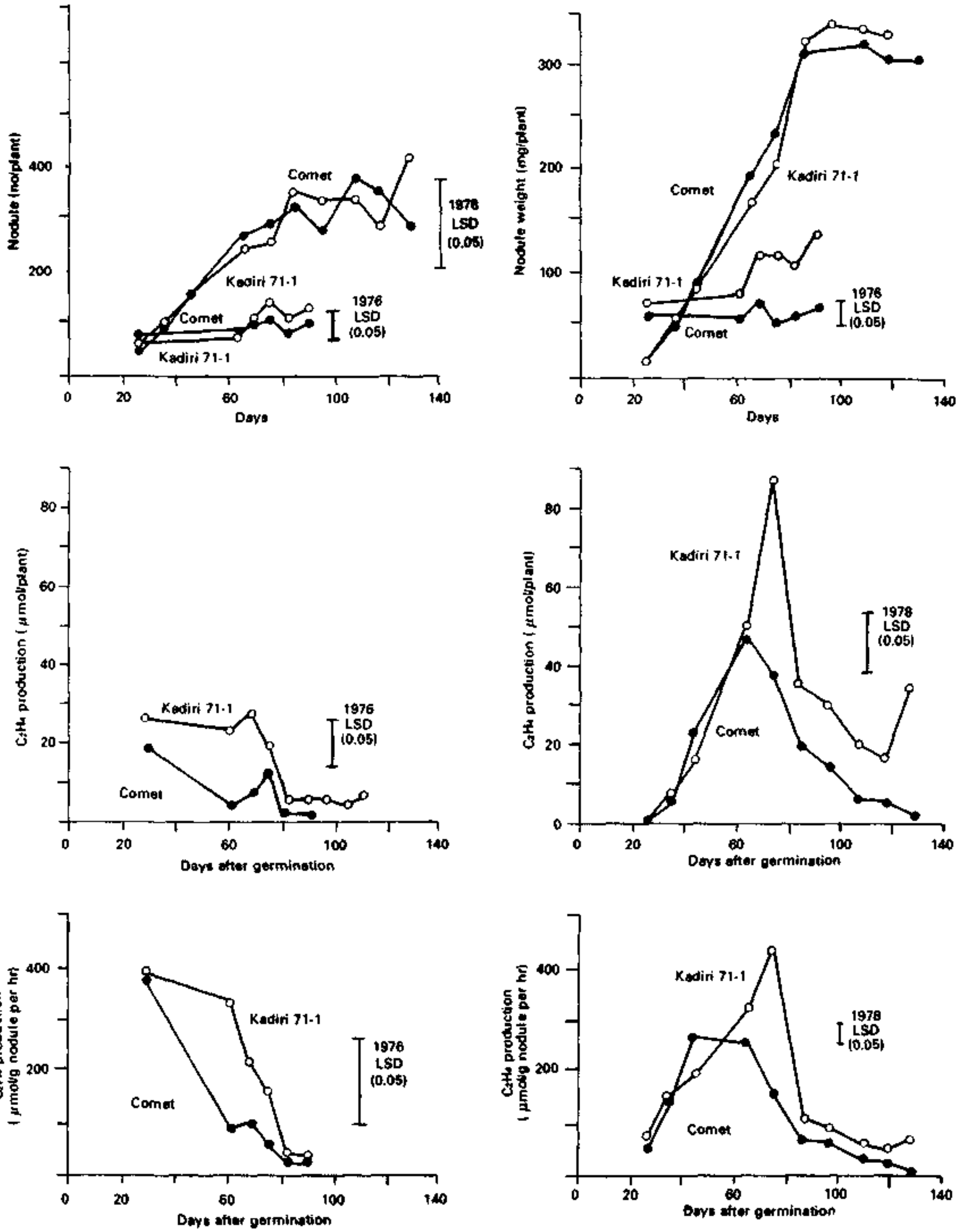

Figure 7. Seasonal variation in nodule number and nodule weight per plant and nitrogenase activity per gram nodule weight and per plant in cv Kadiri 71-1 and Comet grown during rainy season 1976 and postrainy season 1977 at ICRISAT Center. The postrainy season planting was irrigated.

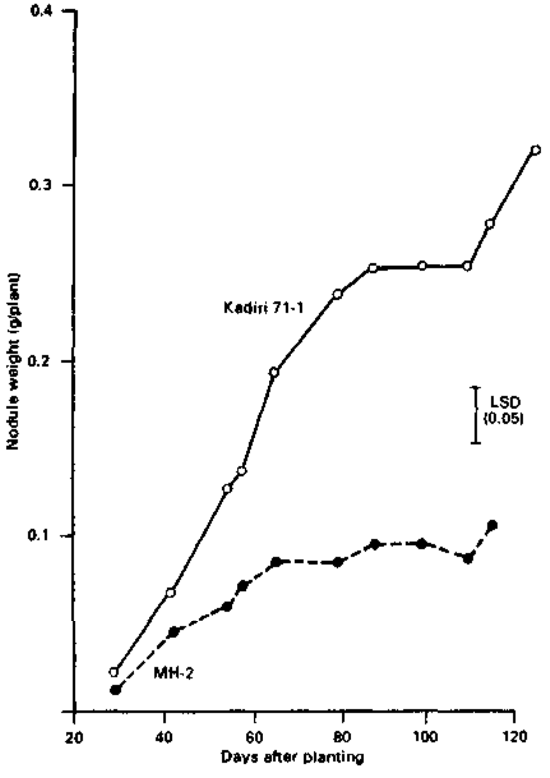

Figure 8. Nodulation of Kadiri 71-1 and MH-2 during irrigated postrainy season.

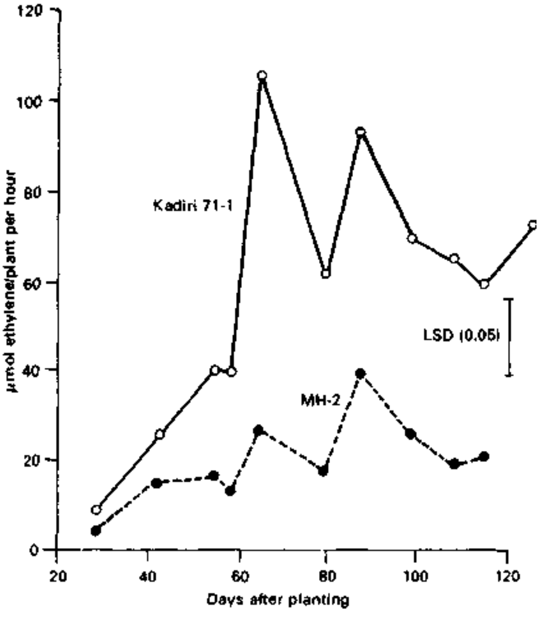

Figure 9. Nitrogenase activity of Kadiri 71-1 and MH-2 during irrigated postrainy season.

MH-2, nitrogen fixation is limited by photosynthate supply, if we assume that net photosynthesis and plant top dry weight are correlated.

Effect of Intercropping

During the 1978 rainy season, we observed that groundnuts when intercropped with pearl millet, nodulated poorly and fixed less nitrogen than the sole crop (Fig. 11). Three rows of groundnut were intercropped with one row of millet which, as commonly practiced, received N fertilizer at the rate of 80 kg N/ha, a level giving near optimum intercrop advantage. During the 1979 rainy season (Table 6) we observed a similar trend in groundnut intercropped in a normally spaced maize stand (two rows of groundnut between maize rows). Interestingly, sole groundnut and groundnut intercropped with maize which received no N fertilizer, had similar nodule number and nitrogenase activity.

The decrease in nitrogenase activity in intercropped groundnut could be due to: (1) the inhibition of nodulation by the nitrogen fertilizer added to the cereal crop (we have observed that fertilizer nitrogen reduces nodulation and nitrogen fixation), and/or (2) the light available to the groundnut in the intercrop decreases as more N fertilizer is added to the cereal.

Observations from an experiment in groundnut/sorghum intercropping where different shading intensities were created by graded defoliation of the sorghum support this (Table 7).

We plan to study the intercropping system more carefully. It may be possible to increase the nodulation and nitrogen fixation of intercropped groundnut by selecting cereal cultivars which allow more light to the groundnut. It may also be possible to select groundnut cultivars more tolerant of reduced light availability. An ideal cereal/legume intercropping situation would utilize the maximum nitrogen fixation ability of the legume while minimizing nitrogen fertilizer addition to the cereal (for other details of these intercropping experiments, see Reddy et al. these Proceedings).

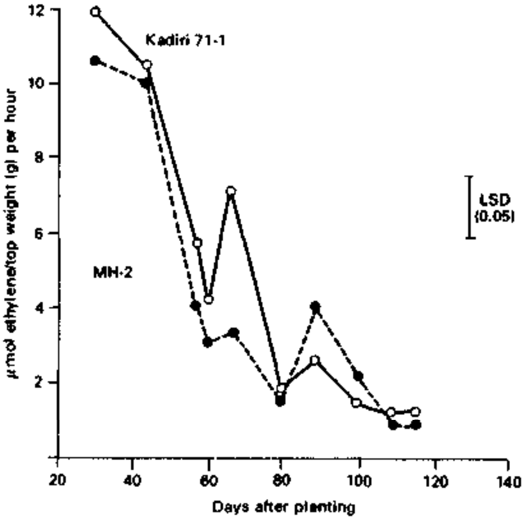

Figure 10. Nitrogenase activity per gram of top dry weight for Kadiri 71-1 and MH-2 during irrigated postrainy season.

We have not observed any interaction among five groundnut cultivars for nodulation and nitrogen fixation in intercrop and sole crop (Table 7). In soybeans it has been suggested that urea has a less harmful effect on nodulation than nitrate (Harper 1975). We plan to study the effect of different sources of N fertilizer applied to the cereal crop and their effect on nodulation and nitrogen fixation of groundnut as the intercrop.

Residual Effects

Rainy season groundnut, when compared with maize, had a large positive residual effect on growth and yield of millet in the subsequent, irrigated postrainy season with an increase in yield of 650 kg/ha, i.e., 45%. All groundnut and above-ground maize material and the groundnut main roots were removed from the field prior to the millet planting. This seems to be an effect of groundnut on N uptake by the millet, and would be consistent with the extremely high nitrogen fixation rates associated

Table 6. Nodulation and nltrogen fixation by groundnut intercropped with maize.

Treatment	Nodule Number/plant	μmoles C_2H_4/ plant per hr
Sole groundnut	171	21.3
Intercropped groundnut Nitrogen added to maize (kg/ha)		
0	164.70	20.10
50	159.50	9.36
100	150.0	7.00
150	134.15	3.52
CV (%)	19.71	30.30
LSD (0.05)	18.90	5.75

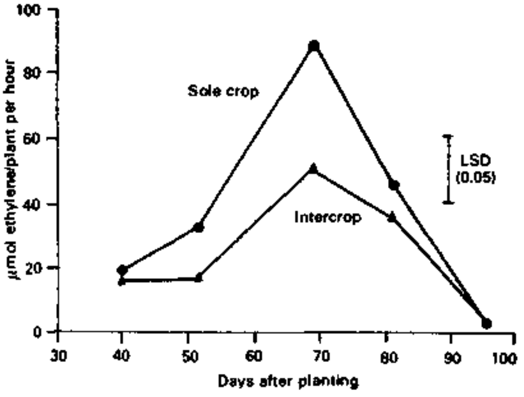

Figure 11. Nitrogenase activity of sole and intercropped groundnut.

Table 7. Nitrogenase activity of sola and Intercropped groundnut.

	Nitrogenase activity[a] (µmoles CzH4/plant per hr)		
Cultivars	Sole crop	Intercrop (low density)	Intercrop (high density)
Chico-17200	15.2	11.8	6.8
TMV-2	18.1	12.6	8.3
MK-374	25.8	23.6	12.2
MH-2	15.4	7.9	9.1
Gangapuri	15.7	10.6	6.5
CV (%) 42			

a. Intercrop treatment effects ere significantly different for all cultivars.

Note: Groundnut end sorghum were planted in a ratio of 2:1 in the Intercrop. Low density Intercrop wes obtained by removing alternate pairs of leaves of sorghum. Plants were hervested 70 days after planting. Sorghum was fertilized with 80 kg N/ha.

with groundnut. The effect could be due to the N left in fine roots in the soil or due to exudation of N into the soil or due to less removal of available soil N by groundnut when compared with maize (Table 8).

Genetic Variability in Groundnut Germplasm Lines for Nodulation

Varietal differences in nodulation among groundnut were reported by Duggar as early as 1935(Duggar 1935). We found large differences among germplasm lines for nodulation (nodule dry weight) and nitrogenase activity during the 1977-78 postrainy season and the 1978 rainy season. The data on nodule weight and nitrogenase activity were analyzed by the Scott-Knott procedure (Gates et al. 1978). The clusters formed were classified into low, medium, and high nodulating and nitrogen fixing (as measured by acetylene reduction) lines (Tables 9, 10). The comparison over season indicated an interaction between cultivar and season for nodulation and nitrogen fixation.

Similar host plant differences in nodulation and N_2 (C_2H_2) reduction have been documented in North Carolina peanut fields containing native rhizobia (Wynne et al. these Proceedings). From the variation present in the germplasm lines, it seems possible to develop genotypes with greater nitrogen fixing ability by selecting parents that consistently have high nitrogen fixing ability over seasons. Isleib et al. (1978) from a 10 x 10 diallel study indicated significant additive gene action for nodulation, nitrogenase activity and plant weight.

We have also found groundnut cultivars which consistently nodulate on the hypocotyl, often with a subtending lateral root, whereas others form few or no nodules in this region (Fig. 12). For example, during the 1977 rainy season, cv NC Acc 10 formed 175 nodules per plant on the hypocotyl (23% of the total nodules formed) whereas cv NC Acc 770 formed only 12 nodules (2% of the total) in this region. Some cultivars such as MK-374, nodulate further up the stem, beyond the crown of the plant. We have observed that cultivars belonging to the botanical variety *hypogaea* nodulated better in the hypocotyl region than those from *fastigiata* and *vulgaris.* We are presently studying the heritability of this location difference in nodule formation.

Table 8. Residual effect of groundnut and maize on millet grain yield in an Alfisol.[a]

First crop	Yield (kg/ha)
Groundnut	1980
Maize unfertilized	1325
Maize 20 kg N/ha	1456
LSD (0.01)	360

a Groundnut and maize grown In rainy season 1977 at ICRISAT Center, followed by irrigated millet, In dry winter season 1977-78.

Table 9. Symbiotic characters in 48 groundnut germplasm entries (85 days after planting).

	Range
Nodule number	247-628
Nodule weight	0.30-0.75 g/plant-'
Nitrogenase activity	
μ mol C_2H_4 plant-'h-[1]	36-176
μ mol C_2H_4/gdry wt nodule/hr	95-386

Non-Nodulating Groundnut

The *host-Rhizobium* interaction in legumes is well documented. The genetic basis for non-nodulation has been described in soybeans, red clover and peas (Williams et al. 1954; Caldwell 1966; Nutman 1949; Holl 1975). Recently Gorbetand Burton reported non-nodulating lines of *Arachis hypogaea* (L) in the progenies of a cross 48 7 A - 4 - 1 - 2 X PI 262090.

During the 1978 rainy season we observed that F2 plants in the rust screening nursery were segregating for non-nodulation. All the parents of the crosses were found to nodulate normally. Later during the rainy season in 1979, non-nodulating lines were found in 14 additional crosses (Table 11). All these crosses have a rust resistant, Valencia groundnut as one of the parents [PI 259747; NC Acc 17090, EC 76446

Table 10. Nodulation and acetylene reduction of groundnut cultivars.

Cultivar	ICG No.	Botanical type	Postrainy season 1977-78				Rainy sceason 1978	
			1st sampling		2nd sampling			
			Nodu-lation	Nitro-genase	Nod il-lation	Nitro-genase	Nodu-lation	Nitro-genase
Ah 3277	1218	Spanish	L	L	L	L	L	L
Ah 3275	1216	Spanish	L	L	L	L	L	L
No. 421	3158	Valencia	L	L	L	L	-	-
Ah 39	1161	Spanish	L	L	M	H	L	L
Ah 5144	1235	Spanish	L	L	M	M	M	-
NC Acc 888	359	Spanish	L	L	L	L	M	L
Ah 61	1173	Spanish	L	L	L	M	L	M
Ah 3272	1213	Spanish	L	L	L	M	L	M
No. 3527	1524	Spanish	L	-	L	M	L	-
Faizpur-1-5	1102	Spanish	L	M	L	L	M	M
No. 418	1500,2202	Spanish	L	L	L	M	-	-
NC Acc 1337	358	Valencia	L	L	M	M	M	L
NC Acc 516	279	Valencia	L	-	M	M	L	-
NC Acc 945	366	Valencia	L	L	M	M	M	-
NC Acc 699	1630	Spanish	L	L	L	M	L	M
148-7-4-3-12-B	1573	Spanish	L	-	L	M	-	-
No. 1780	1508	Spanish	L	L	M	L	-	-
NC Acc 738	331	Valencia	L	-	M	M	M	-
TG 17	2976	Spanish	L	L	L	M	L	L
No. 3270	1489	Spanish	L	L	L	L	-	-
NC Acc 51	263	Valencia	L	M	L	L	L	-
TG 8	95	Valencia	L	L	M	M	L	L
Ah 42	1163	Valencia	L	-	M	L	M	-
NC Acc 2651	402	Spanish	L	L	L	M	M	-
NC Acc 1002	380	Valencia	L	-	M	M	M	-
NC Acc 524	283	Valencia	L	M	M	M	M	M
GAUG 1	-	Spanish	L	-	M	M	L	L
NC Acc 2734	420	Valencia	L	L	*M*	M	M	L
NC Acc 495	1623	Spanish	M	M	L	M	L	L
Spancross	3472	Spanish	M	-	M	M	M	L
NC Acc 1286	389	Valencia	M	L	M	M	M	L
NC Acc 17149	475	Valencia	M	L	M	M	M	M
Ah 1069	1196	Spanish	M	M	M	M	L	L
Kadiri 71-1		Virginia runner	M	M	M	M	L	L
Ah 6279	2983	Spanish	M	-	M	M	-	-
NC Acc 2600	400	Virginia Bunch	M	L	M	M	L	M
POL 2	154	Spanish	M	M	M	M	L	L
JH 171	3375	Spanish	M	M	M	M	L	L
NC Acc 1303	393	Spanish	M	L	M	M	M	M
NC Acc 975	376	Valencia	M	M	M	M	M	*M*
Sm-5	2956	Spanish	M	M	M	*M*	L	L

Continued

Table 10. *Continued*

			Postrainy season 1977--78					
			1st sampling		2nd sampling		Rainy season 1978	
Cultivar	ICG No.	Botanical type	Nodu-lation	Nitro-genase	Nodu-lation	Nitro-genase	Nodu-lation	Nitro-genase
Argentine	3150	Spanish	M	M	M	L	L	L
Tifspan	3495	Spanish	M	M	M	L	L	L
Robut 33-1	799	Virginia Bunch	M	M	M	M	M	"
Pollachi 1	127	Spanish	M	L	M	M	M	M
NC Acc 17113	1699	Spanish	M	M	M	M	M	M
Ah 8254	2962	Spanish	M	M	M	M	M	L
Ah 7436	1547	Spanish	M	M	M	M	M	-
NC Acc 490	274	Valencia	M	M	M	M	M	M
X-14-4-B-19-B	1561	Spanish	H	M	M	M	M	-
NC Ace 2821	2405	Virginia	H	M	H	M	M	M
NC Acc 2654	404	Valencia	H	M	M	H	M	-

Range of nodulation and nitrogenase activity for the clusters formed

	Nodulation (g nodule/plant)			Nitrogen fixation (µ moles C2H4/plant per hr)		
Season	Low (L	Medium (M)	High (H)	Low (L)	Medium (M)	High (H)
Postrainy season 1978						
1 Sampling	0.08-0.11	0.11-0.16	0.188-0.19	16-28	30-44	-
2 Sampling	0.3 -0.38	0.38-0.6	0.6 -0.75	36-64	65-132	166
Rainy season 1979	0.11-0.14	0.14-0.17	-	68-92	93-117	-

Table 11. Crosses In which non-nodulatIng progenies were observed.

Shantung Ku No. 203 x NC Acc 17142
NC Acc 2731 x NC Acc 17090
NC Acc 2731 x EC 76446 (292)

NC Acc 2768 x NC Acc 17090
NC-17 x NCAcc 17090
Shantung Ku No. 203 x NC Acc 17090

Shantung Ku No. 203 x EC 76446 (292)
Shantung Ku No. 203 x PI 259747
NC-17 x EC 76446 (292)
NC-F1a-14 x NC Acc 17090

Rs-114 x NC Acc 17090
NC-17 x PI 259747
NC Acc 2731 x PI 259747
Shantung Ku No. 203 x PI 259747

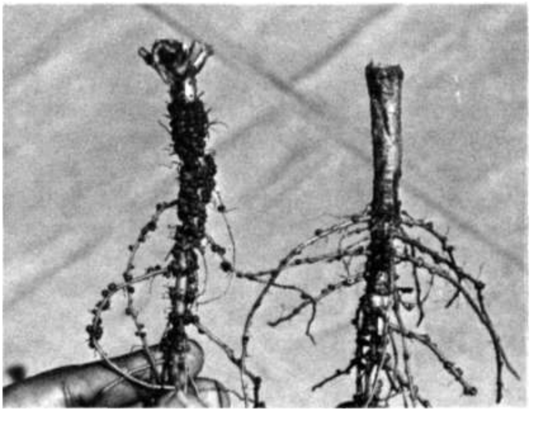

Figure 12. Differential distribution of nodules on groundnut root. Left, cv NC Acc 10 has many nodules on the hypocotyl. Right, cv NC Acc 770 has only few nodules.

(292]. Interestingly some segregants formed only a few, very large nodules, much larger than the parents or normally nodulating F2 plants. Acetylene reduction assays showed that their nitrogen fixing activity on a nodule weight basis was similar to that of normal nodules on the parents. A preliminary genetic analysis based on segregation for nodulation vs no nodulation showed that a pair of independent duplicate genes control nodulation and that non-nodulation is governed by recessive genes (Nigam et al. 1980)

Summary

1. Groundnut yield can be increased by inoculating with *Rhizobium.*
2. Nodulation and nitrogen fixation increased with increase in the number of *Rhizobium* inoculum cells per seed.
3. Nodulation and nitrogen fixation decreased when groundnut was intercropped with millet, maize or sorghum.
4. Photosynthesis is one of the major limiting factors in nitrogen fixation as evidenced by diurnal variation in nitrogenase activity, reduction of nitrogenase activity on shading groundnuts, and reduction of nitrogenase to different degrees when groundnut is grown in an intercrop with variable leaf area index of the companion crop.
5. There is genotypic variation in nodulation and nitrogen fixation.
6. Non-nodulating lines observed in the F2 populations of some crosses have been purified and advanced to Fe.

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