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Response of groundnut (*Arachis hypogaea* L.) to *Rhizobium* inoculation in the field: problems and prospects*

P. T. C. Nambiar

Groundnut Improvement Program, ICRISAT, Patancheru P.O. 502 324, Andhra Pradesh, India

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Introduction

Rhizobium inoculation is a cheaper and usually more effective agronomic practice for ensuring adequate nitrogen nutrition of legumes than the application of fertilizer nitrogen. Development of an inoculant industry in many countries has been largely motivated by the desire to introduce legume species to new areas (Burton 1982). *Rhizobium* inoculation of newly introduced crops has resulted in dramatic increases in yields in several countries (Burton 1976). In the USA, 80% of the total inoculants produced are for soybeans and alfalfa, which are introduced crop species (Burton 1982). However, results of inoculation trials on many other legume crops have not been consistent or encouraging (Subba Rao 1976; Lopes 1977; Graham 1981; Hegde 1982; Hadad *et al.* 1982). Reviewing the prospects for inoculating groundnut, Lopes (1977) observed "since advantages from seed inoculation of peanuts are not clearly established, the practice of inoculating this legume is not usual". The objective of this article is to examine this issue using primarily the data obtained from seven years of research at the ICRISAT Center, Patancheru, near Hyderabad, India.

Rhizobia nodulating groundnut

Groundnut is nodulated by rhizobia that also nodulate many species of tropical leguminous plants and these rhizobia are classified as the cowpea miscellany (Allen & Allen 1981). Most cultivated soils of the tropics appear to have relatively large populations ($>10^2$/g dry soil) of these rhizobia. Rhizobia differ in their ability to fix N_2 however, and the presence of nodules on roots of the groundnut plant does not necessarily mean that sufficient N_2 is being fixed for maximum growth of the host plant (Weaver 1974; Nambiar *et al.* 1982a). Hence it may be necessary to introduce superior strains of *Rhizobium*, to ensure adequate N_2 fixation for maximum growth and yield of the host plant.

Assessing the need to inoculate

The following factors are generally considered while assessing the need to inoculate with *Rhizobium.*

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Cropping history

Inoculation with efficient rhizobia in fields where no crop had previously been grown resulted in increased yields at several locations (Seeger 1961; Shimshi *et al.* 1967; Schiffmann & Alper 1968; Chesney 1975; Pettit *et al.* 1975; Burton 1976). Absence of *Rhizobium* strains which nodulate groundnut could be a major constraint to crop growth and yield in these fields. Hence, an introduced *Rhizobium* inoculant for groundnut, once established, does not have to compete with other *Rhizobium* strains for nodule formation.

Rhizobium *population*

Low numbers of appropriate rhizobia can lead to poor nodulation and N_2 fixation. Many workers advocate enumeration of the soil population to assess the need to inoculate (e.g. Hadad *et al.* 1982) but, as the *Rhizobium* population varies during crop growth and season (Kumar Rao *et al.* 1982), enumeration of the population at a given time may not necessarily indicate the potential of these strains to form nodules and/or to fix N_2. Nodule number can however, be used as a criterion to predict an inoculation response. If the crop is not nodulated, or poorly nodulated, then an enumeration of the background population can help us to understand the cause of nodulation failure, i.e. whether it is caused by a lack of groundnut rhizobia, or by adverse soil conditions.

Acetylene reduction

Although the acetylene reduction assay is influenced by a wide range of factors in groundnut (Nambiar & Dart 1983), this 'on the spot' measurement of N_2 fixation helps us understand the comparative efficiency of the native population. Surveys in many farmers' fields in southern India using acetylene reduction as a tool have indicated poor N_2 fixing efficiency (Nambiar *et al.* 1982a), suggesting the possibility of obtaining an inoculation response in these fields. It should be realized, however, that factors other than nitrogen, such as pests, diseases and nutrient deficiencies, can also result in poor plant growth which will in turn result in low acetylene reduction rates.

Response to fertilizer nitrogen

The positive response of a legume crop to fertilizer nitrogen indicates that the nitrogen demand of the crop is not being completely met by N_2 fixation and, therefore, symbiotic N_2 fixation could be limiting. Mineral nitrogen fertilization has improved groundnut yields in many countries (Shimshi *et al.* 1967; Schiffmann & Alper 1968; Ratner *et al.* 1979; Mazzani 1980; Hadad *et al.* 1982). Response to N fertilization as an indication of the N demand of the crop and possibilities for obtaining inoculation response in such fields has also been suggested by Schiffmann (1961) and Burton (1976). Schiffmann (1961) compared response to *Rhizobium* inoculation and fertilizer N at two sites in Israel. Both nitrogen fertilization (180 kg N/ha) and *Rhizobium* inoculation increased yields, although *Rhizobium* inoculation gave better yields, but in some soils other factors, such as soil pH, mineral toxicities or nutrient deficiencies, could influence symbiotic N_2 fixation without directly affecting plant growth. Under these conditions one may obtain a response to nitrogen fertilization, but not to *Rhizobium* inoculation. Moreover, fertilizer nitrogen could influence the symbiotic N_2 fixing system, and in many instances can decrease the existing N_2 fixing efficiency (Reddy & Tanner 1980; Nambiar 1985).

Nitrogen deficiency symptoms

Attempts have been made in the past to determine the N deficiency/demand of a groundnut crop by quantifying the N concentration in the leaves and other tissues (Reid & Cox 1973). Acute N deficiency symptoms are expressed by yellowing of the younger leaves as well as the older leaves. This invariably indicates the need to improve the symbiotic N_2 fixing system, possibly by *Rhizobium* inoculation. But groundnut crops in many farmers' fields do not express such N deficiency symptoms. This has lead to the general belief that such crops do not need *Rhizobium* inoculation. However, nitrogen fertilization of a groundnut cultivar, Robut 33-1, in fields at ICRISAT center, Patancheru, resulted in higher pod and haulm yield. At zero nitrogen this cultivar had normal foliage colour and did not exhibit any N deficiency symptoms. Moreover, nitrogen fertilization did not significantly influence the N concentration in the plant parts (author, unpublished). This clearly indicates that the nitrogen demand of the crop is not necessarily expressed as deficiency symptoms.

Hence it is rather difficult to assess the need to inoculate by any one of the above methods alone and field inoculation trials are mandatory for this purpose.

Response to inoculation

Newly cropped areas

There are several reports indicating that in fields not previously cropped, inoculation with efficient *Rhizobium* strains has increased yields. Inoculation of groundnut cv Florunner in virgin sandy soils in the USA improved the seed size and protein content, and increased yields by 93% (Burton 1976). Similar results have been reported in other countries (Seeger 1961; Schiffmann & Alper 1968; Pettit *et al.* 1975; Reddy & Tanner 1980). In Alabama, however, in fields where no groundnut was previously cultivated, granular *Rhizobium* application (commercial inoculum, 10^6 cells/seed) or fertilizer nitrogen application did not significantly increase the yields of cv Florunner in 12 experiments (Hitbold *et al.* 1983). These authors concluded that "while groundnut was not a host legume for these rhizobia during the years prior to these experiments, the rhizobia apparently persisted on alternate legume hosts in the cowpea miscellany in numbers adequate for effective inoculation of groundnuts".

Previously cropped areas

Most of the fields currently under groundnut cultivation in many countries have been previously cropped with either groundnut or other hosts, such as cowpea. Under these conditions inoculation must meet the challenge of providing superior strains in a manner that will result in the inoculated strain forming a large proportion of the total nodules. In soils containing other established native *Rhizobium* populations, the introduced *Rhizobium* should have the capacity to compete with the native population in nodule formation. There are no laboratory methods to test this competitive ability. Competitive strains can be selected only by field trials, which limit the number of strains that can be tested. Little is known of the factors controlling competitiveness, but host cultivar, soil microflora, soil type and other environmental factors, and the nature of the competing strains may influence the success of an inoculum strain in nodule formation (Alexander 1982). Probably because of these factors, *Rhizobium* inoculation has produced variable effects in fields where groundnut had been grown earlier.

Rhizobium inoculation did not increase pod yields either in Raleigh (J. C. Wynne, NCSU, personal communication) or in Georgia (Walker *et al.* 1976) in the USA. At Ludhiana, India, Arora *et al.* (1970) observed that seed protein content, but not the pod yield, was increased by inoculation. Subba Rao (1976) observed that *Rhizobium* inoculation resulted in decreased yields in national trials conducted at several locations. Van der Merwe *et al.* (1974) conducted 11 seed inoculation trials over three seasons in different locations in South Africa, where groundnuts had been cropped intensively. They obtained increased seed yield only in one trial, conducted at Buffelsport, so they suggested that "seed inoculation may be superfluous under the existing agricultural practices". In Sudan, inoculation of two groundnut cultivars with four *Rhizobium* strains did not result in increased yield (Hadad *et al.* 1982). Nagaraja Rao (1974) reported a yield increase with cv TMV 2 when inoculated with very high levels (10^7 cells/seed applied at sowing and then again 10 days after sowing) of strain 402-B, during the 1969 rainy season. They tested five strains and only strain, 402-B, outyielded the uninoculated control. However, yield levels in this trial were very low (998 kg/ha). Commenting on inoculation experiments conducted by various authors, Hegde (1982) noticed that "in India the necessity to inoculate groundnut has neither been shown conclusively nor investigated thoroughly".

In Queensland, no response to *Rhizobium* inoculation (strain CB 756, applied as liquid, granular or slurry seed coating) was observed on land where groundnuts were grown earlier, although response to inoculation was observed on 'new land' (Diatloff & Langford 1975). These authors concluded that inoculation of groundnuts was unlikely to be adopted in groundnut-growing districts in Queensland.

Rhizobium *strain NC 92 as a potential inoculant in traditional producing areas*

Many experiments have been conducted at ICRISAT to identify strains that could be used as inoculants, mainly in fields where groundnuts had been previously cropped. During the 1977 and 1978 rainy seasons 15 host cultivar X *Rhizobium* strain combinations were tested. There were no significant increases in the yields of any cultivar over the uninoculated control. However, during the 1978/79 post-rainy season the yield of cv Robut 33-1 was increased by 1000 kg/ha when inoculated with the strain NC 92 (Nambiar & Dart 1980). Subsequently, many rainy and post-rainy season trials have shown similar results, although the yield advantages varied (Nambiar *et al.* 1982a; Nambiar *et al.* 1984a; Table 1). A pooled analysis of variance done over eight experiments showed a significant ($P < 0.01$) increase in yield of cv Robut 33-1 inoculated with strain NC 92. The average increase in pod yield over the uninoculated control was 16% (Table 1). Many of these experiments were conducted in fields where the uninoculated plants had 200-600 nodules/plant and exhibited considerable

Table 1 Response of groundnut cv. Robut 33-1 to inoculation with *Rhizobium* strain NC 92 over several seasons at ICRISAT Center

Season	Pod yield (kg/ha)		
	Uninoculated	Inoculated	S.E. mean
Post-rainy (1978-79)	3500	4500	± 290
Rainy (1979)	870	1160	± 24
Post-rainy (1979/80)	4280	4400	± 104
Rainy (1980)	1350	1640	± 77
Post-rainy (1980/81)	3210	3300	± 79
Rainy (1981)	2350	2760	± 188
Rainy (1981)	1100	1160	± 35
Rainy (1981)	1530	2150	± 177
Mean	2274	2634	± 56

Source: Nambiar *et al.* (1984a)

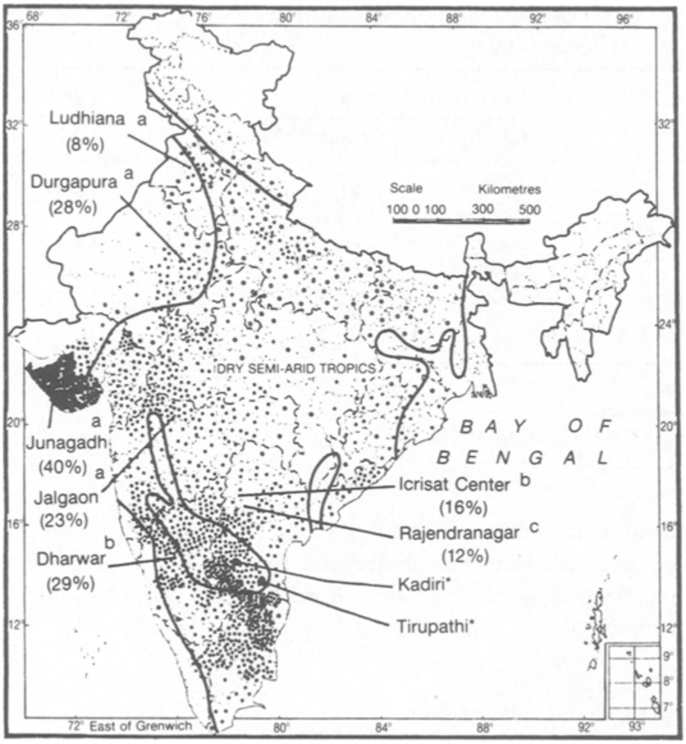

Fig. 1 Locations where increased yields of cv Robut 33-1 have been obtained with inoculum NC 92. Percentage yield increase given in brackets. (a) Mean of two season trials; (b, c) mean of seven season trials at ICRISAT Center and one season trial in other locations. Tirupathi and Kadiri, locations where no increased yields with inoculation were obtained. Dots indicate intensity of groundnut growing area. (*Source*: AICORPO 1983)

nitrogenase activity (60-80 μmol C_2H_4/plant/h) 60 days after planting. Similar observations on increased yield after inoculation were recorded at Dharwar, Karnataka State (Nambiar *et al.* 1984a), and at several other locations in India (Fig. 1). However, significant yield increases were observed at only four out of eight other locations in a trial conducted by the All India Co-ordinated Research Project on Oil Seeds (AICORPO 1983).

In Gujarat state in India, Joshi & Kulkarni (1983; 1984) also obtained increased yields from cv Robut 33-1 when it was inoculated with strain NC 92 (Table 2). Inoculation with strain NC 92 almost doubled the yield of cv Robut 33-1 in these fields in the first year. Another interesting aspect of these results is that yields of cv Robut 33-1 in the uninoculated plots were less than those of cv J 11, which is grown across locations as a check cultivar in yield trials. This is good evidence to show that the performance of varieties can be misjudged if they are not appropriately inoculated. During the 1982 experiment the percentage increase in yield was less than that observed during the 1981 experiment.

There are a few other reports on the effect of strain NC 92 on groundnut yield. In farmers' fields at Gulbarga in Karnataka state and at North Arcot in Tamil Nadu (in India) inoculation with strain NC 92 increased yields of cvs Robut 33-1 and JL 24 respectively (R. T. Hardiman, ICRISAT and G. Satyakumar, farmer, Pudupalayam; N.A. Dist., personal communications). Inoculation with strain NC 92 increased yields of cultivar 28-206 in Cameroon – a 26% yield increase was observed for an 28-206/NC 92 combination over the uninoculated control, which was nodulated by indigenous rhizobia. This cultivar is grown on 80% of the area under

Table 2 Yield response of two groundnut cultivars to inoculation with three *Rhizobium* strains at Junagadh, Gujarat, India

Treatment	1981 rainy season		1982 rainy season	
	J 11	Robut 33-1	J 11	Robut 33-1
Uninoculated	800*	590	1490	1600
Inoculated				
NC 92	900	1260	1800	1800
5a/70	800	1160	1820	1770
NGR 234	1000	760	1580	1760
SE	±50		±27	

*Yield in kg pod/ha.
Cultivars, J11, Robut 33-1.
Rhizobium strains, NC 92, 5a/70, NGR 234.
Data from Joshi & Kulkarni (1984).

groundnut cultivation in Cameroon (Anon 1983). This strain also produced higher yields when inoculated with cvs Hong-hua, E-hua and Robut 33-1 at Hubei Province in China (Zhang-Xue-Jiang, Wu Sheng Tang and Jiang Rong Wen, Oil Crops Research Institute, Hubei Province, China, personal communications).

Initial evaluation of the inoculant strain

Even though some other cultivar/strain combinations were found to be effective in some experiments, the inoculation responses have not been consistent (Nambiar *et al.* 1984a). Burton (1976) and Date (1976) recommended that *Rhizobium* strains should first be screened under controlled environmental conditions for effectiveness in N_2 fixation. Figure 2 depicts a regression analysis of the relationship between N_2 fixation and nodulation for two *Rhizobium* strains (NC 92 and 5a/70) and two groundnut cultivars (TMV 2 and Robut 33-1), grown in the glasshouse (Nambiar *et al.* 1983). At a given nodule number or nodule weight strain 5a/70 was found to be superior to strain NC 92 (Fig. 2), but NC 92 proved to be a better field inoculant than 5a/70 in fields containing natural populations of effective *Rhizobium* strains (Nambiar *et al.* 1984a). The N_2-fixing efficiency of 19 *Rhizobium* strains as observed in a glasshouse experiment and the effect of these inoculant strains on the pod yield of the same cultivar (Robut 33-1) in the field is shown in Fig. 3. The field soil contained a native *Rhizobium* population of 10^2-10^4 cells/g. No significant correlation (r = 0.19, df = 17) was obtained when N_2 fixing efficiency and pod yield were compared. The above results agree with the observation that "prudent use of the glasshouse and growth chamber can aid in eliminating poor or totally ineffective strains, but cannot substitute for field testing" (Burton 1976).

In another experiment conducted in the glasshouse at ICRISAT Center, it was observed that *Rhizobium* strain NC 43.3, when nodulated on cv Robut 33-1 fixed more N_2 than strains NC 92 or TAL 176. These strains fixed 205, 161, and 152 mg N/plant (s.e. ±4.3) respectively, during a growth period of 42 days (author, unpublished). Experiments conducted to assess the competitive ability of these strains showed that (a) in pot-grown plants (cv Robut 33-1), where other *Rhizobium* strains were absent, strain NC 43.3 was a better competitor than strain NC 92 when mixed in equal proportions, but when inoculated as single strain in fields containing native rhizobia (10^2-10^4 cells/g dry soil), both strains formed

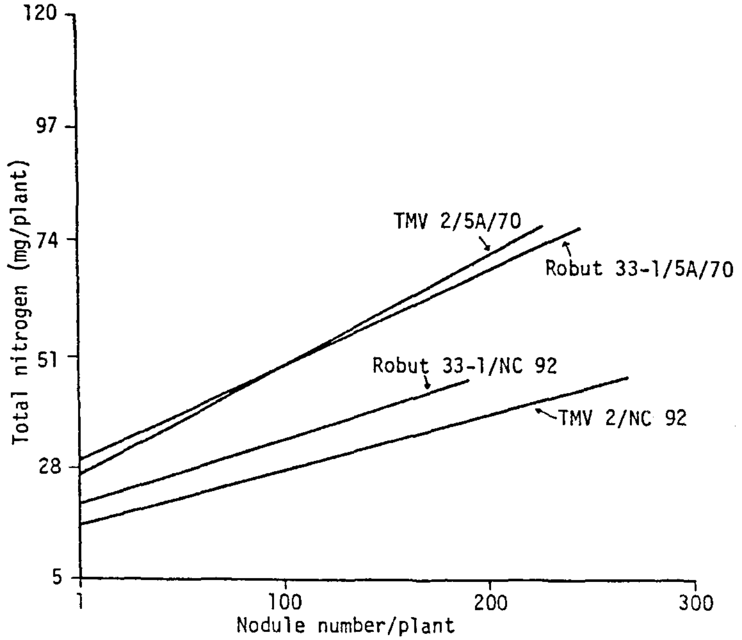

Fig. 2 Regression analyses showing relationship between plant nitrogen content and nodule number for two *Rhizobium* strains NC 92 and 5a/70 and two groundnut cultivars (TMV 2 and Robut 33-1). Plants were grown in a glasshouse under nitrogen free conditions. For a given nodule number strain 5a/70 fixed more nitrogen. However, field trials indicated that strain NC 92 is a better inoculant (for statistical details see Nambiar *et al.* 1983).

more or less the same number of nodules, (b) strain TAL 176 was a poor competitor in two strain mixtures (with NC 92 or NC 43.3) and in field soil containing native rhizobia (Table 3). These data emphasize that efficiency of *Rhizobium* strains in fixing N_2 and their competitive ability are different characters, and glasshouse experiments are not adequate to assess the competitiveness of an inoculant strain in the field.

Table 3 Nodulation by *Rhizobium* strains in the glasshouse and in the field

	Percentage nodules* formed by strain		
	NC 92	TAL 176	NC 43.3
In pot culture			
NC 92 + TAL 176	93	2	–
NC 92 + NC 43.3[1]	48	–	77
TAL 176 + NC 43.3	–	3	97
In the field as single strain	20	1	17

*Percentage of nodules formed by three *Rhizobium* inoculant strains (NC 92, TAL 176 and NC 43.3) in two strain combinations (10^6 cells each strain/seed) in pot-culture in the glasshouse and as single strains (10^6 cells/seed) in the field containing 10^2-10^4 native rhizobia/g dry soil, ICRISAT rainy season 1984, cv. Robut 33-1.

1. Values greater than 100% due to double occupancy by NC 92 and NC 43.3

Data from P.T.C. Nambiar, V. Anjaiah & B. Srinivasa Rao (unpublished).

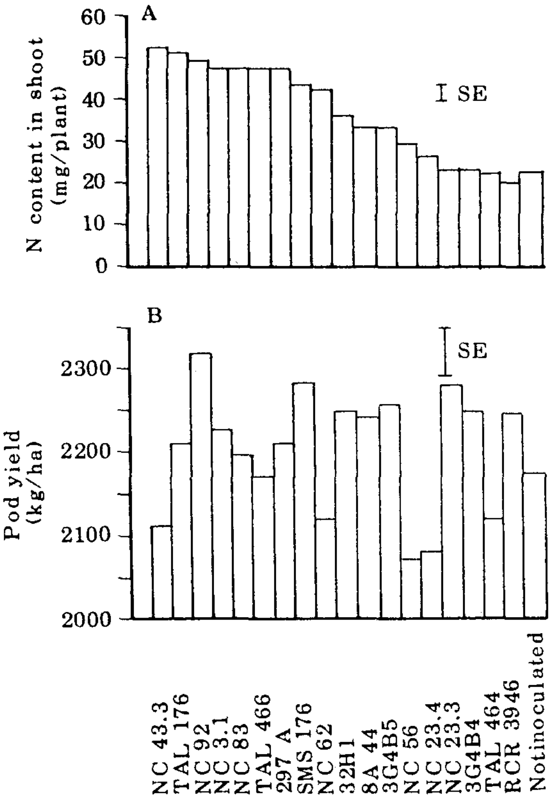

Fig. 3 Comparative evaluation of *Rhizobium* strains for N_2 fixing effectiveness (N content in shoot) in the glasshouse (A) and their effect on pod yield in the field during the 1982 rainy season, ICRISAT Center (B). Cultivar tested – Robut 33-1. (*Source*: ICRISAT 1983).

Siderophore production by strain NC 92

Inoculation with strains TAL 176 and NC 43.3 did not increase the pod yield of cv Robut 33-1 (Nambiar *et al.* 1984a; ICRISAT 1983). The reason for the failure of strain TAL 176 inoculated plants to produce higher yield can be explained because of the poor competitiveness of this strain. However, strain NC 43.3 fixed more nitrogen than strain NC 92 in pot-culture and formed more or less the same percentage of nodules as that formed by strain NC 92 in field soil containing native rhizobia. Why inoculation with NC 43.3 did not increase the pod yield of cv Robut 33-1 is yet to be understood.

One possible explanation is that the effect of strain NC 92 on groundnut yield may not be entirely due to its symbiotic nitrogen-fixing ability. Strain NC 92 secretes a siderophore, an iron chelating compound, into the culture medium, whereas no siderophore was detected in the culture medium of NC 43.3 (P.T.C. Nambiar, in preparation). Under natural aerobic soil conditions most of the iron exists as insoluble ferric form, which is not available to the plant (Neilands 1981). Hence it is possible that the siderophore produced by strain NC 92 could help in the iron nutrition of the crop and the effect of strain NC 92 on groundnut yield, may be due at least partly, to its siderophore-producing ability (P. T. C. Nambiar, in preparation).

Cultivar X strain specificity

Based on the earlier observations, Nambiar *et al.* (1983) suggested that genetic compatibility in nodulation could exist between cv Robut 33-1 and *Rhizobium* strain NC 92. However, it was observed that following field inoculation, NC 92 form more or less the same percentage of nodules on cvs J 11 and Robut 33-1 (Table 4). Why then does inoculation with NC 92 increase the yield of Robut 33-1, but not usually that of cv J 11? The findings suggest that cv Robut 33-1 has a high yield potential and thus has greater demand for nitrogen than cv J 11. Results of a nitrogen fertilizer trial with these two cultivars during the 1982 rainy season at ICRISAT support this hypothesis. Pod yields of cv Robut 33-1, but not of J 11 were increased by fertilizing with mineral nitrogen (Table 5). However, inoculation with NC 92 and 5a/70 increased yield of cv J 11 during the 1982 rainy season at Junagadh (Table 2). Soil and other environmental factors could be responsible for these observed differences. Alternatively it is also possible that a host genotype X *Rhizobium* interaction could also occur after nodule formation, and the Robut 33-1/NC 92 combination fixes more N_2 than the J 11/NC 92 combination.

Table 4 Cultivar differences in yield response to *Rhizobium* inoculation and the percentage of nodules formed by the inoculum strain NC 92

Treatment	Cultivar			
	Robut 33-1		J 11	
	Yield*	NC 92 nodules (%)	Yield	NC 92 nodules (%)
Uninoculated	2350	1	1950	4
Inoculated				
NC 92	2760	30	1870	30
Mixture (5a/70 + NC 92 + IC 6006	2710	14	1600	6
S.E. mean	±188	±2.6	±188	±2.6

*Yield in kg pod/ha.
Cultivars, J 11, Robut 33-1.
Rhizobium strains, NC 92, 5a/70, IC6006.
Data from Nambiar *et al.* (1984a, b)

Table 5 Effect of nitrogen fertilization on pod yield of two groundnut cultivars during the 1982 rainy season at ICRISAT Center

Cultivar	Fertilizer N applied (kg/ha)	
	0	200
J 11	1650*	1650
Robut 33-1	2000	2330
S.E. Mean	±41	

*Yield in kg pod/ha.
Data from P.T.C. Nambiar (unpublished).

Table 6 Response of three groundnut cultivars to *Rhizobium* NC 92 inoculation in the 1982 rainy season at ICRISAT Center

Treatment	Cultivars		
	JL 24	ICGS 27	Robut 33-1
Uninoculated	1490*	1580	1650
NC 92	1580	1750	1750
S.E. mean for comparing two treatment levels within a cultivar = ±35			

*Yield in kg pod/ha.
Data from ICRISAT (1983).

Recently it was observed that strain NC 92 could also increase the yields of cv JL 24 (Joshi & Kulkarni 1983) and cv ICGS 27 (Table 6), ICGS 11, and ICGS 12 (author, unpublished). Commenting on the impact of biological N_2 fixation (BNF) technology, App & Eaglesham (1982) pointed out that the failure to obtain larger yields of grain legumes with inoculants or N fertilizers may be caused, partially, by a yield barrier; once the yield barriers can be broken, greater inputs of N and other nutrients may be necessary to exploit this enhanced yield potential. Hence varieties with inherent low yield potential may not respond to *Rhizobium* inoculation. This could be one of the causes for the failure of strain NC 92 to produce any significant effects on the yields of cvs J 11, Kadiri 71-1, and Argentine at ICRISAT Center, Patancheru (Nambiar *et al.* 1984a).

It is interesting to note that strain NC 92 originates from South America, where *Arachis* also originated. The nodules were collected from a groundnut cultivar, *Arachis hypogaea* cv Overo Colorado Blanco, from the dark alluvial loam soils in Bolivia by Professor W. C. Gregory of North Carolina State University, (NCSU, Raleigh, USA). The strain was isolated at NCSU and was supplied to ICRISAT as a part of NCSU/ICRISAT collaborative project on biological N_2 fixation.

By further field screening it may be possible to identify other strains for effective use as inoculants. Based on the data discussed above, and on experiences in farmers' fields, groundnut growing fields could be classified into three categories as shown in Table 7. The ICRISAT fields where we obtained most of the results are Type 3 fields where the native *Rhizobium* population is effective. Root nodules of uninoculated plants collected from these fields

Table 7 Classification of fields suitable for growing groundnuts and criteria for selecting *Rhizobium* inoculant

Field type	Cropping history of groundnut	Native *Rhizobium* Strain	
		Population	Selection
1	No	Nil to small	Effective strain
2	Yes	Small to large (mostly ineffective e.g., white nodules)	Effective and competitive strain
3	Yes	Large; apparently effective (red nodules, C_2H_4 reduction activity)	Effective, competitive, host cultivar-specific strain

reduced acetylene to 20-100 μmol ethylene/plant/h, depending on the cultivars, at around 60 days after sowing, which indicate high N_2 fixation capacity of native rhizobia (Nambiar *et al.* 1982a). Both host cultivar and *Rhizobium* strains should be selected under Type 3 situations, whereas a competitive and efficient strain should be suitable for fields of Types 1 and 2. This may explain the reason why strain 5a/70 performed better than strain NC 92 in Sri Lanka (Senaratne & Amarasekara 1984), but not in ICRISAT fields. Strains like 5a/70 and NC 43.3 could be recommended for fields of Type 1 whereas strain NC 92 could be recommended for Type 3 fields. It may not be practical to select strains for each of the field types. A strain selected under Type 3 conditions should also be able to perform well in fields of Types 1 and 2. Field surveys and acetylene reduction assays conducted in many farmers' fields in southern India indicate that these fields fall in category 2 (author, unpublished observations). Inoculation trials need to be conducted in farmers' fields to substantiate this hypothesis.

Method of *Rhizobium* inoculation

Direct application of *Rhizobium* to seed is the most common form of legume inoculation. However, groundnut seeds are fragile, and are often coated with fungicides, so other methods of inoculation have been suggested (Bonnier 1960; Burton 1976). Schiffmann & Alper (1968) reported large yield increases when peanuts were inoculated by applying a slurry of peat-based inoculum in the seed furrow. This method of *Rhizobium* application has given good results at ICRISAT Center also (Nambiar *et al.* 1982a; 1984b; Table 8). When NC 92 coated seeds were treated with fungicides, the percentage success of this strain in nodule formation was considerably reduced (Table 8). The Farm Power and Equipment Subprogram at ICRISAT has modified a bullock-drawn seeder, commonly used by farmers in India, for simultaneous *Rhizobium* application in the seed furrow.

Table 8 Effect of fungicide dressing and method of *Rhizobium* inoculation on percentage nodules formed by strain NC 92*

Fungicide treatment†	Method of *Rhizobium* inoculation		
	Liquid	Seed	Uninoculated
Untreated	30 (27)	22 (20)	4 (2)
Captan	28 (23)	7 (4)	3 (1)
Thiram	25 (18)	6 (4)	7 (2)
Dithane	19 (10)	14 (9)	7 (3)
Bavistin	24 (16)	14 (8)	10 (3)
Mean	25 (19)	13 (9)	6 (2)

S.E. mean for comparing *Rhizobium* inoculation mean within a fungicide treatment is = ±5.5.

*Nodules typed by ELISA 60 days after sowing. Data analysed after arcsine transformation; original means are given in parentheses.
†The seeds were treated with the fungicides as recommended.
Data from Nambiar *et al.* (1984b).

Inoculum rate and persistence of inoculum

Groundnut grown under glasshouse conditions needs large numbers of rhizobia (10^6-10^8 cells/seed) for maximum nodulation and N_2 fixation (Nambiar *et al.* 1983). With a back-

Table 9 Persistence of inoculum strain NC 92 over two seasons (% nodules formed by NC 92)*

Season		Days after sowing	
1st	2nd	72	116
Uninoculated	Uninoculated	9 (5)†)	11 (8)†
Uninoculated	Inoculated	31 (27)	27 (25)
Inoculated	Uninoculated	28 (25)	42 (32)
Inoculated	Inoculated	39 (41)	75 (54)
SE		±2.5	±5.4

*Mean of four cultivars; nodules typed during the second season.

†Data analysed after arcsine transformation; original means are given in parentheses.

Data from Nambiar *et al.* (1984b).

ground *Rhizobium* population of 10^2-10^4 cells/g soil higher rates of inoculum may be required for field inoculants. Experiments conducted in ICRISAT fields suggest that a minimum of 10^6 cells/seed is required for cultivar Robut 33-1 (Nambiar *et al.* 1984b).

It has also been shown that the percentage of success of the inoculated strain (NC 92) increases with subsequent inoculation (Nambiar *et al.* 1984b; Table 9). Persistence of this strain is being examined under several cropping systems. Higher inoculum rate (10^6-10^8 cells/seed) at the initial inoculation could help in early establishment of the inoculant strain, and if so, then it would be economical. However, this needs to be further tested under different soil conditions.

Problem areas

Out of 11 locations tested by AICORPO, inoculation with strain NC 92 failed to produce higher yields of cultivar Robut 33-1 in two locations, namely Tirupathi and Kadiri (AICORPO 1983). Chemical analyses of soil collected from Tirupathi revealed a high manganese content (50 ppm; ICRISAT 1984). Manganese and aluminium could be toxic to symbiotic N_2 fixation even if they were not at a level to affect plant growth (Franco 1977). Soil acidity and alkalinity could also pose problems for symbiotic N_2 fixation. Crops in such fields may not respond to *Rhizobium* inoculation unless the inoculant strains have the capacity to overcome these adverse factors. These problem areas need research attention.

In the above suggestions, it is assumed that other nutrient requirements (other than nitrogen) of the crop are fullfiled. However, this is not usually the case in farmers' fields, and unless other nutrients are supplied, *Rhizobium* inoculation alone may not increase the crop yield.

Problems in technology application

These data suggest that *Rhizobium* inoculation can increase yields of certain groundnut cultivars in India in fields where the crop is currently cultivated. Based on three years of testing at many locations in India during the 1981-1983 rainy seasons, AICORPO has recommended *Rhizobium* strain NC 92 as an inoculant for cultivars Robut 33-1 and JL 24 (AICORPO 1983). At present cultivars Robut 33-1 and JL 24 are being tested for national release by AICORPO (AICORPO 1983). However, the availability of quality inoculum could be one of the major constraints in developing countries (Thompson 1982). Quality control of inoculant needs expertise and certain minimum facilities, if enumeration of rhizobia in the inoculant carrier has to be done by the plant infection technique (Vincent 1970). The ELISA (enzyme-linked immunosorbent assay) technique could be used to estimate the number of rhizobia in the peat inoculum (Nambiar & Anjaiah 1985). This test has an advantage over other methods in that it could be selectively used to enumerate a recommended strain, while other tests count the population of all rhizobia present. Although it is expensive to set up ELISA tests, they can be an extremely valuable tool for a quality control laboratory, since large numbers of samples can be handled daily and results can be known within two days. The alternative plant-infection method requires incubation periods of three or four weeks.

A second important problem in applying inoculum technology for groundnut production is to convince farmers of the effect of inoculation on yield. Fluctuations in yields of uninoculated plots from year to year, and even under similar growing conditions, are often larger than yield differences between inoculated and uninoculated plants grown in the same season. For example, yields of cv Robut 33-1 in uninoculated plots during the rainy seasons in ICRISAT fields ranged from 870-2350 kg/ha (Table 1). Also, response to inoculation in terms of yield cannot always be ensured. Moreover, there were no visible differences in the plant growth in inoculated *vs* uninoculated plots. However, *Rhizobium* inoculation can be a substantial component in groundnut production, and national government agencies and universities could play a key role in inoculum production, quality control and distribution.

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Summary

A successful *Rhizobium* inoculant strain needs to be more competitive than native soil rhizobia in forming nodules and to be effective in fixing nitrogen. Persistence of the strain from one season to the next will be an added advantage, since it eliminates the need to inoculate every season. Earlier studies indicated that groundnut, *Arachis hypogaea*, seldom responds to *Rhizobium* inoculation in soils already containing rhizobia which nodulate groundnut. However, the results of seven years of research at ICRISAT have opened up the prospect of inoculating groundnut in such soils. These experiments indicate that inoculating with sufficient numbers of an effective *Rhizobium* strain, NC 92, applied as a liquid slurry below the seed, increased yields of certain groundnut cultivars. Similar results were reported from other research centres in India. Inoculation with NC 92 for two consecutive seasons increased the proportion of nodules formed by this strain, from 25–32% in the first season to 41–54% in the second season, indicating that this strain can persist in the field for the following season. This paper also discusses results of experiments relating to host cultivar specificity and some possible problems in applying this information to farmers' fields. Strain NC 92 also produces a siderophore, an iron chelating compound, which may help in the iron nutrition of the plant.

Résumé

Réponse de l'arachide (Arachis hypogea *L.*) *à l'inculation de* Rhizobium *dans les champs. Problèmes et perspectives.*

Pour être couronnée de succès, une souche de *Rhizobium* inoculée doit supplanter les rhizobiums natifs du sol en ce qui concerne la formation de nodules et doit fixer l'azote efficacement. La persistance de la souche d'une saison à l'autre, rendant inutile la répétition saisonnière de l'inoculation, est un avantage supplémentaire. Des études antérieures ont montré que l'arachide (*Arachis hypogea*) ne répond que rarement à l'inoculation dans des sols contenant déjà des rhizobiums capables de noduler cette plante. Cependant, les résultats de sept années de recherches éffectuées à l'ICRISAT ont ouvert des possibilités quant à l'inoculation dans ce type de sols. Ces expériences montrent qu'une bouillie liquide d'une souche efficace de *Rhizobium* (NC 92), répandue en quantité suffisante sous les graines, augmente le rendement de certains cultivars d'arachide. Des résultats similaires ont été rapportés par d'autres centres de recherches en Inde. L'inoculation de NC 92 pendant deux saisons consécutives a augmenté la proportion des nodules formés par cette souche de 25 à 32% pour la première saison, et de 41 à 54% pour la seconde, ce qui indique que cette souche peut persister dans le sol d'une saison à l'autre. Dans cet article sont également discutés les résultats d'expériences concernant la spécificité de l'hôte et quelques problèmes concernant l'utilisation de ces informations par les agriculteurs. La souche NC 92 produit aussi un sidérophore, agent complexant du fer pouvant jouer un rôle dans le métabolisme de ce métal chez la plante.

Resumen

Respuesta del maní (Arachis hypogaea *L.*) *a la inoculación con* Rhizobium *en el campo. Problemas y perspectivas.*

La inoculación con *Rhizobium* para tener exito debe de realizarse con una cepa que sea competitivamente mejor que las cepas nativas del suelo en cuestión, en relación a la habilidad para formar nódulos y a la eficacia en la fijación de nitrógeno. La persistencia de la cepa de una temporada para otra es una característica deseable ya que elimina la necesidad de reinocular cada temporada. Estudios previos indicaban que el maní (*Arachis hypogaeae*) raras veces responde a la inoculación con *Rhizobium* en suelos que ya contienen *Rhizobium* específicos. Sin embargo los resultados de siete años de investigaciones en ICRISAT han abierto nuevas perspectivas para la inoculación del maní en dichos suelos. Esta experiencias indican que la inoculación, en cantidades suficientes de una cepa de *Rhizobium* eficaz (NC 92) aplicada en forma de liquido viscoso bajo la semilla incrementa el rendimiento de ciertos cultivars de maní. Resultados semejantes se han observado en otros centros de investigación en India.La inoculación de la cepa NC 92 durante dos años consecutivos incrementó la proporción de

nódulos formados desde 25–32% en la primera temporada hasta 41–54% en la segunda indicando pues la persistencia de dicha cepa en el suelo de una a otra temporada. Este trabajo también evalua los resultados de investigaciones relacionando la especificidad del inóculo con la posible problemática que conlleva la aplicación de esta informacion por el agricultor.