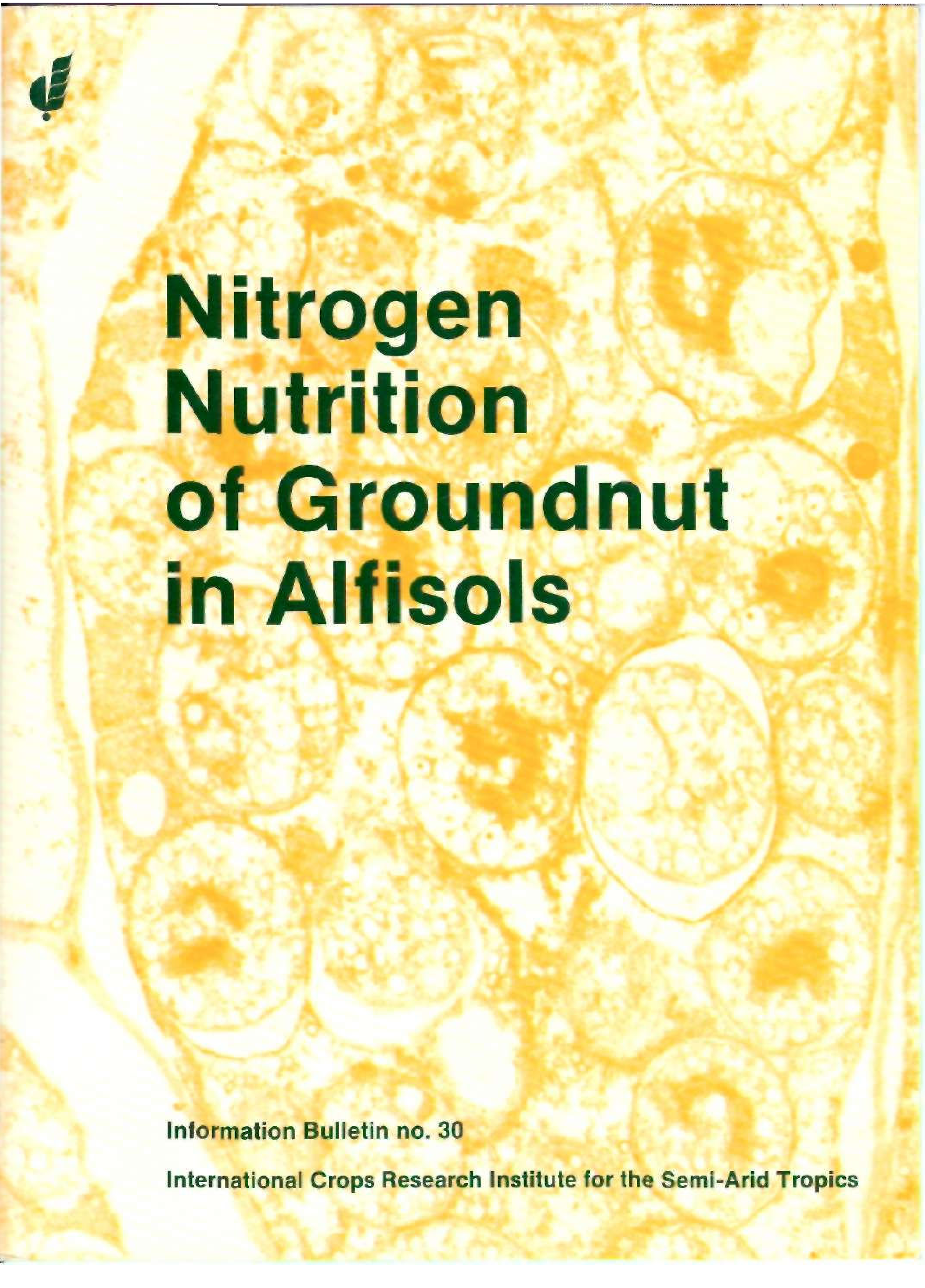

Nitrogen Nutrition of Groundnut in Alfisols

Information Bulletin no. 30

International Crops Research Institute for the Semi-Arid Tropics

Abstract

Citation: Nambiar, P.T.C. 1990. **Nitrogen nutrition of groundnut in Alfisols.** Information Bulletin no. 30. Patancheru, A. P. 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

This bulletin covers aspects of demand and acquisition of nitrogen by groundnut (*Arachis hypogaea*). Results of experiments conducted in Alfisols at ICRISAT Center to improve groundnut productivity with *Bradyrhizobiutn* inoculation and fertilizer-N, together with those relating to host cultivar specificity, and some possible problems in applying this information to farmers' fields are discussed. Although it is possible to increase N_2 fixation through plant breeding, it is suggested that breeding for increased N_2 fixation is not a practical proposition.

Résumé

Référence : Nambiar, P.T.C. 1990. **Nutrition azotée de l'arachide sur les Alfisols.** Bulletin d'information n° 30. Patancheru, A.P. 502 324, Inde : International Crops Research Institute for the Semi-Arid Tropics.

Ce bulletin traite des aspects de besoins et d'acquisitions azotés de l'arachide *(Arachis hypogaea).* Les résultats des expériences conduites sur les Alfisols au Centre ICRISAT pour améliorer la productivité arachidière avec l'inoculation de *Bradyrhizobium* ainsi que sur la spécificité des cultivars hôtes sont examinés. Certains problèmes éventuels dans l'application de cette information aux champs paysans sont aussi discutés. Bien qu'il soit possible d'accroître la fixation N_2 par la sélection, il est suggéré que la sélection pour l'accroissement de la fixation N_2 n'est pas pratique.

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Nitrogen Nutrition of Groundnut in Alfisols

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Cover: Electron micrograph of part of groundnut root nodule cell showing several rhizobial (bacteroid) cells.

Contents

Introduction

Legumes, because of their high protein content, require large amounts of nitrogen to produce good yields. Groundnut (*Arachis hypogaea* L.), being a legume, is capable of obtaining its nitrogen requirements from both symbiotic nitrogen fixation by root nodules, and soil nitrogen. The source for the reduction of nitrogen (nitrogen fixation) is gaseous nitrogen (N_2), while soil nitrogen is absorbed mainly as nitrate (NO_3) nitrogen.

Groundnut genotypes need approximately 1 kg of assimilated nitrogen to produce around 36 kg biomass, in contrast to cereals such as sorghum, that can produce as much as 120 kg biomass kg^{-1} assimilated nitrogen (Nambiar et al. 1986). This large amount of nitrogen is supplied to the groundnut plant mainly by its root nodules. Available nitrogen in soils is at its highest level soon after fertilizer application, and it decreases thereafter, depending on such factors as plant uptake, leaching, mineralization, and nitrification. In contrast, symbiotic N_2 fixation is a part of the plant's metabolism and, if well established, the nodules supply the plant with a regulated and continuous supply of nitrogen, depending on its the growth stage. Rhizobia that nodulate groundnut have recently been reclassified as *Bradyrhizobium* (Jordan 1984).

The purpose of this bulletin is to examine the use of both nitrogen utilization pathways to maximize crop productivity of groundnut. Generalized answers to some common questions on nitrogen nutrition of groundnut are given at the beginning of each of the four major sections, as a means of summarizing the topics that will be covered in detail in the sections. Most of the results discussed come from experiments conducted in Alfisols at ICRISAT Center, Patancheru. Currently, groundnut cultivation in Vertisols posses different problems including lime-induced iron (Fe) chlorosis which may limit N_2 fixation. Attempts to study nitrogen fixation in Vertisols in detail should be made after problems related to Fe chlorosis have been solved, and hence nitrogen nutrition in Vertisols will not be discussed.

Bradyrhizobium Inoculation

Is it necessary to inoculate groundnut to obtain high yields?

It may be necessary to inoculate groundnut when new fields are brought under cultivation, but not to inoculate traditionally cultivated fields. But, recent work at ICRISAT Center has shown that even in traditionally cultivated fields marginal and inconsistent increases in yield have been obtained when certain genotypes were inoculated with particular strains of *Bradyrhizobium.*

Is it possible to pre-determine whether high yields could be obtained with *Bradyrhizobium* inoculation?

It is rather difficult to predict. If the crop is showing N-deficiency symptoms inoculation with *Bradyrhizobium* might increase yield. But, even if there are no deficiency symptoms, N can still limit plant growth and yield.

What are the symptoms of N deficiency on groundnut?

Acute N deficiency is expressed as yellowing (chlorosis) of the older leaves. In severe cases, younger leaves may also become chlorotic. In calcareous soils, N deficiency is sometimes coupled with Fe deficiency, but if it occurs independently, Fe deficiency is expressed as interveinal chlorosis on younger leaves.

How can good quality inocula be produced?

Detailed information is available in publications by Thompson 1984, and Nambiar and Anjaiah 1985b.

Rhizobia Nodulating Groundnut

Groundnut is nodulated by the rhizobia that also nodulate many species of tropical leguminous plants, and are classified as the cowpea miscellany (Allen and Allen 1981). These rhizobia have recently been classified as *Bradyrhizobium* (Jordan 1984), and most cultivated soils of the tropics appear to have relatively large populations (> 10^2 g^{-1} dry soil) of them. Groundnut nodules are formed at the junctions of root axils where lateral roots emerge (Allen and Allen 1940; Nambiar et al. 1983b). During the early stages of seedling growth rhizobia colonize the rhizosphere, enter the junction of root axils, penetrate into deeper cell layers of the root, and infect a cell. Soon after intracellular infection, the bacteria multiply rapidly. Further development of the nodule occurs by repeated division of the infected host cells (for details see Chandler 1978) . However, rhizobia differ in their ability to Fix N_2, and the presence of nodules on the roots of a groundnut plant does not necessarily mean that sufficient N_2 is being fixed to maximize its growth (Weaver 1974; Nambiar et al. 1982a). It may therefore be necessary to introduce superior strains of *Bradyrhizobium,* to ensure adequate N_2 fixation for maximum growth and yield of the host plant.

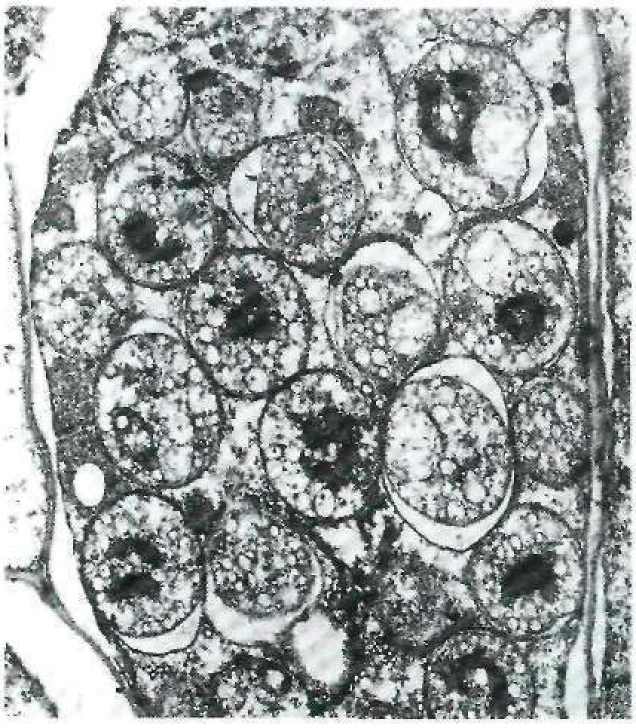
Rhizobium or *Bradyrhizobium* inoculation is a cheaper, and usually more effective, way of ensuring an adequate nitrogen supply to legumes than the application of fertilizer nitrogen. The development of an inoculant industry in many countries has largely been motivated by the desire to introduce legume species to new areas, mainly in temperate zones where more specific rhizobia are required (Burton 1982). *Rhizobium* or *Bradyrhizobium* inoculation of newly introduced crops has resulted in dramatic yield

Nodules formed by different strains of *Bradyrhizobium* differ in their N_2-fixing ability. The plant nodulated by an effective N_2-fixing strain (left) is green, but the plant nodulated by an ineffective strain (right) is chlorotic.

increases in several countries (Burton 1976). In USA, 80% of the total inoculants produced are for soybeans and alfalfa that are introduced crop species (Burton 1982). However, results of inoculation trials on many other legume crops have been neither consistent nor encouraging (Subba Rao 1976; Lopes 1977; Graham 1981; Hegde 1982; Hadad et al. 1982). Reviewing the prospects for inoculating groundnut, Lopes (1977) observed that "since advantages from seed inoculation of peanuts are not clearly established, the practice of inoculating this legume is not usual".

Assessing the Need to Inoculate

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



Electron micrograph of part of groundnut root nodule cell showing several rhizobial (bacteroid) cells (x 6950).

Photo: Electron Microscope Unit, 1CRISAT

Cropping History

Inoculation with efficient rhizobia in fields where no groundnut crop had previously been grown resulted in increased yields at several locations (Seeger 1961; Shimshi et al. 1967; Schifmann and Alper 1968; Chesney 1975; Pettit et al. 1975; Burton 1976; Burton 1982). The absence of those strains of *Bradyrhizobium* that nodulate groundnut could be a major constraint to crop growth and yield in some of these fields. Once established, an introduced *Bradyrhizobium* inoculant for groundnut does not have to compete with other *Bradyrhizobium* strains for nodule formation.

Bradyrhizobium Population

Low populations 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 (Hadad et al. 1982) but, as the *Bradyrhizobium* population varies during crop growth and over seasons (Kumar Rao et al. 1982), enumeration 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 to determine the cause of poor nodulation or it's failure, i.e., whether it is caused by a lack of groundnut rhizobia, or by adverse soil conditions.

Acetylene Reduction

The rate of reduction of acetylene gas to ethylene is considered to indicate the rate of N_2 fixation, and the technique of acetylene reduction (Burris 1975) is generally used to measure the rate of N_2 fixation. Although the acetylene reduction assay is influenced by a wide range of factors in groundnut (Nambiar and Dart 1983), this 'on the spot' measurement of N_2 fixation does help to understand the comparative efficiency of native rhizobial populations. Surveys in

Profusely nodulated groundnut roots from fields in USA. Nodulation is relatively less profuse in Indian fields.

Photo: J.C. Wynne, North Carolina State University, USA.

many farmers' fields in southern India using acetylene reduction assay have indicated poor N_2-fixing efficiency (Nambiar et al. 1982a), suggesting the possibility of obtaining a response to inoculation in these fields. It should be realized, however, that factors other than the supply of nitrogen, such as pests, diseases, and other nutrient deficiencies can also result in poor plant growth, and reduced rates of acetylene reduction.

Response to Fertilizer Nitrogen

The positive response of a legume crop to fertilizer-N indicates that the N demand of the crop is not being fully met by N_2 fixation and, therefore, symbiotic N_2 fixation could be limiting. The application of mineral-N fertilizers has improved groundnut yields in some trials (Shimshi et al. 1967; Schiffmann and Alper 1968; Ratner et al. 1979; Mazzani 1980; Hadad et al. 1982). Response to fertilizer-N as an indication of the nitrogen demand of the crop, and the possibility of obtaining a response to inoculation in such fields has also been suggested by Schiffmann (1961) and Burton (1976). Schiffmann (1961) compared responses to *Bradyrhizobium* inoculation and fertilizer-N at two sites in Israel. The application of fertilizer-N (180 kg N ha^{-1}) and *Bradyrhizobium* inoculation both increased yields, although *Bradyrhizobium* inoculation gave better yields than fertilizer application. In some soils other factors, such as soil pH, mineral toxicities, or nutrient deficiencies, can influence symbiotic N_2 fixation without directly affecting plant growth. Under these conditions a response to fertilizer-N, but not to *Bradyrhizobium* inoculation may be obtained. Moreover, fertilizer-N can influence the symbiotic N_2-fixing system, and in many instances decreases the existing N_2-fixing efficiency (Reddy and Tanner 1980; Nambiar 1985a).

N-Deficiency Symptoms

Attempts have been made to determine the N deficiency/demand of a groundnut crop by quantifying the N concentration in the leaves and other tissues (Reid and Cox 1973). Acute N-deficiency symptoms are expressed as yellowing of both the younger and the older leaves. This invariably indicates the need to improve the symbiotic N_2-fixing system, possibly by *Bradyrhizobium* inoculation. But groundnut crops in many farmers' fields do not express N-deficiency symptoms, and this has led to the general belief that such crops do not need *Bradyrhizobium* inoculation. The application of fertilizer-N to groundnut cv Robut 33-1 (Kadiri 3) in fields at ICRISAT Center resulted in increased pod and haulm yield. When grown without fertilizer-N, this cultivar had normal-colored foliage and did not exhibit any N-deficiency symptoms. Moreover, the application of fertilizer-N did not significantly influence the N concentration in the plant parts (Nambiar et al. 1986). This clearly indicates that the N demand of the crop is not necessarily expressed as deficiency symptoms.

It is rather difficult to assess the need to inoculate by any one of the above methods alone, therefore, field inoculation trials are essential for this purpose.

Response to Inoculation

Newly Cropped Areas

There are several reports indicating that, in fields not previously cropped with groundnut, inoculation with efficient *Bradyrhizobium* strains has increased groundnut yields. Inoculation of groundnut cv Florunner, in virgin sandy soils in USA improved seed size and protein content, and increased yields by 93% (Burton 1976). Similar results have been reported in other countries (Seeger 1961; Schiffmann 1961; Schiffmann and Alper 1968; Pettit et al. 1975; Reddy and Tanner 1980). In Alabama, USA, however, in fields where groundnut had not previously been cultivated, the application of granular *Bradyrhizobium* (commercial inoculum, 10^6 cells $seed^{-1}$) or fertilizer-N did not significantly increase Florunner yields in 12experiments (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 fields currently under groundnut cultivation in many countries have been previously cropped with either groundnut, or such *Bradyrhizobium* hosts 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 established native *Bradyrhizobium* populations, the introduced *Bradyrhizobium* should have the capacity to compete with the native population in nodule formation. Laboratory methods to test this competitive ability are not available. Competitive strains can be selected only by field trials, and this limits 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 all influence the success of an inoculum strain in nodule formation (Alexander 1982; Nambiar et al. 1987a). Probably because of these factors, *Bradyrhizobium* inoculation has produced variable effects in fields where groundnut has previously been grown.

In USA *Bradyrhizobium* inoculation did not increase groundnut pod yields in either Raleigh, North Carolina (J.C. Wynne, North Carolina State University, Raleigh, USA, personal communication) or Georgia (Walker et al. 1976). In Ludhiana, India, Arora et al. (1970) observed that inoculation increased seed protein content, but not pod yield. Subba Rao (1976) noted that *Bradyrhizobium* inoculation resulted in decreased yields in Indian national trials at several locations. Van der Merwe et al. (1974) conducted 11 seed-inoculation trials over three seasons at different locations in South Africa where groundnut had previously been intensively cropped. They obtained increased seed yield only in one trial, at Buffelsport, and suggested that "seed inoculation may be superfluous under the existing agricultural practices". In Sudan, inoculation of two groundnut cultivars with four *Bradyrhizobium* strains did not result in increased yield (Hadad et al. 1982). 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, Australia, no response to *Bradyrhizobium* inoculation (strain CB 756, applied as liquid, granular, or slurry seed coating) was observed on land where groundnut was grown earlier, although response to inoculation was observed on "new land" (Diatloff and Langford 1975). These authors concluded that inoculation of groundnut was unlikely to be adopted in groundnut-growing districts in Queensland.

Bradyrhizobium Strain NC 92 and Other Strains as Potential Inoculants in Traditional Groundnut-producing Areas

Many experiments to identify *Bradyrhizobium* strains that could be used as inoculants have been conducted, mainly in fields where groundnuts had previously been cropped at ICRISAT Center. This led to the identification of an inoculum strain NC 92 (Nambiar et al. 1984a) that was later released by the All India Co-ordinated Project on Oilseeds (AICORPO) to farmers in India (Nambiar 1985a). However, in 13 out of 15 trials conducted after 1983, inoculation with *Bradyrhizobium* strain NC 92 did not increase the yields of the groundnut cultivars tested. The reasons for this discrepancy are not clear.

There are a few other reports on the effect of strain NC 92 on groundnut yield. In farmers' fields at Gulbarga, Karnataka and at North Arcot, Tamil Nadu, India inoculation with strain NC 92 increased yields of Robut 33-1 by 22% and of JL 24 by 18%. Inoculation with strain NC 92 increased yields of 28-206 in Cameroon, and Hong-hua, E-hua, and Robut 33-1 produced higher yields when inoculated with this strain in Hubei Province, Peoples Republic of China (Nambiar 1985a). However in trials conducted in other countries i.e., Botswana, Brazil, Burkina Faso, Indonesia, Malawi, and Sudan, inoculation with NC 92 did not increase yields (Nambiar, unpublished). The results obtained with NC 92 are interesting, because there are no other examples reported in the literature where inoculation with a *Bradyrhizobium* strain increased yields across locations, over many seasons, and in soils with large native rhizobial populations capable of nodulating the host plant.

Inoculation with two other strains, TAL 176 and NC 43.3, did not increase the pod yield of Robut 33-1 (Nambiar et al. 1984a; Nambiar 1985a). The reason for the failure of inoculated plants to produce higher yield can be explained by the poor competitiveness of TAL 176. However, strain NC 43.3 fixed more N_2 than strain NC 92 in pot culture, and formed more or less the same percentage of nodules as NC 92 in field soil containing native rhizobia. Why then did inoculation with NC 43.3 not increase the pod yield of Robut 33-1? This is yet to be understood (Nambiar 1985a), but a possible explanation is that the effect of NC 92 on groundnut yield may not be entirely due to its symbiotic nitrogen-fixing ability. Mutants of NC 92, that form nodules, but do not fix nitrogen are available (Wilson et al. 1987). These could be used to test whether the effect of strain NC 92 is due to any attribute other than N_3 fixation.

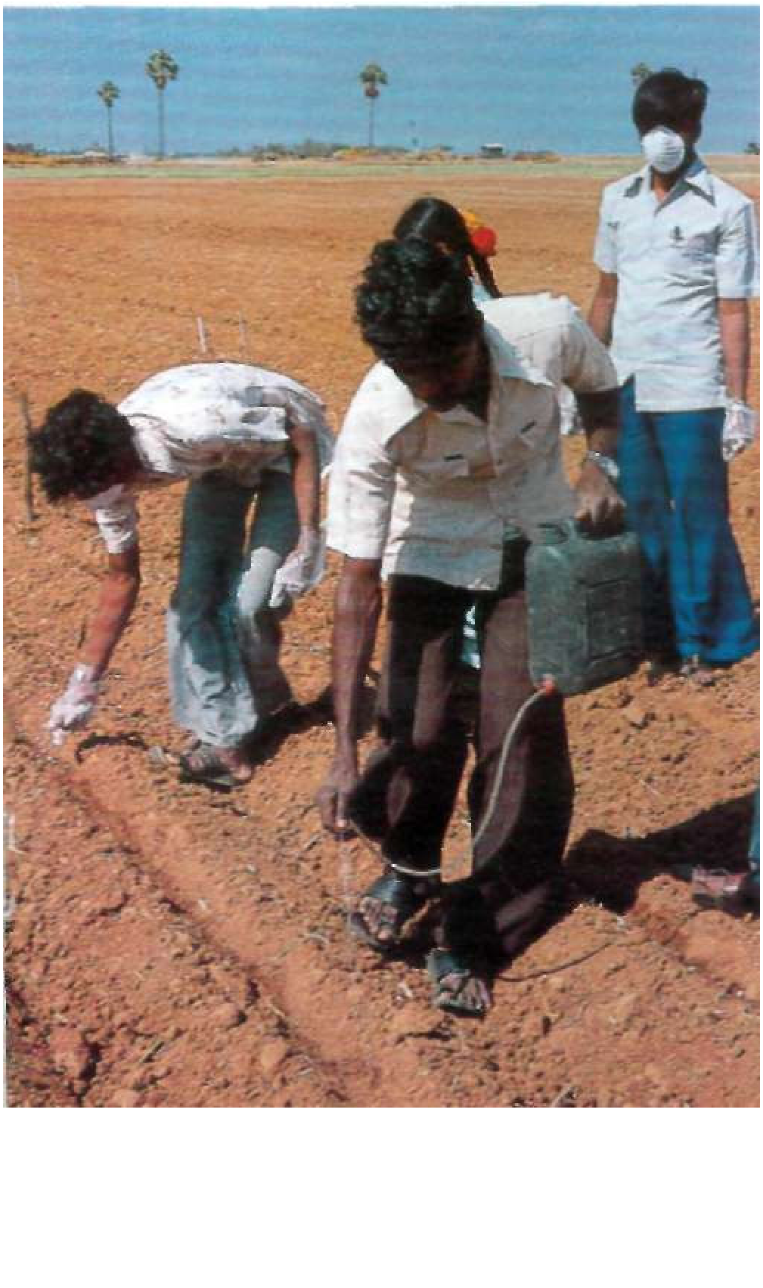
Under natural aerobic soil conditions most of the iron in the soil exists in an insoluble ferric form, that is not available to the plant (Neilands 1981). Strains NC 92 and NC 43.3 secrete siderophores (Fe-chelating compounds) into their culture media, but NC 92 secretes more siderophore than NC 43.3 (Nambiar and Sivaramakrishnan 1987). It is possible that the siderophore produced by NC 92 could help in the Fe nutrition of the crop, and that the effect of NC 92 on groundnut yield may be partially due to its siderophore-producing ability (Nambiar and Sivaramakrishnan 1987).

Other *Bradyrhizobium* strains, e.g., IGR 13, IGR 40, IGR 14, and IGR 6 have increased groundnut yields at few locations in India (ICAR 1987a).

Bradyrhizobium Inoculation Methods

Direct application of *Bradyrhizobium* to seed is the most common form of legume inoculation. However,

The best method of inoculation is to mix the *Bradyrhizobium* inoculum with water to form a suspension that is applied in the furrow before the seed is sown.



Applying liquid inoculum to the furrow below seeds with a bullock-drawn seeder; this method has proved more effective at ICRISAT Center than conventional methods of inoculating groundnut seeds with *Rhizobium* strains. Inset shows inoculum flowing into the seeder.

groundnut seeds are fragile, and are often coated with fungicides, so other methods of inoculation have been suggested (Bonnier 1960; Burton 1976). When NC 92-coated seeds were treated with fungicides the success of the strain in nodule formation was considerably reduced. Schiffmann and Alper (1968) reported large yield increases when groundnuts were inoculated by applying a slurry of peat-based inoculum in the seed furrow. This method of application has also given good results at ICRISAT Center (Nambiar et al. 1982a, 1984b). A bullock-drawn seeder, commonly used by farmers in India, has been modified for simultaneous *Bradyrhizobium* application in the seed furrow (Nambiar, 1985b).

Inoculum Application Rate and Persistence

Groundnut grown under greenhouse conditions needs large numbers of rhizobia (10^6-10^8 cells seed^{-1}) for maximum nodulation and N_2 fixation (Nambiar et al. 1983d). With a background *Bradyrhizobium* population of 10^2-10^4 cells g^{-1} soil higher rates of inoculum may be required for field inoculants. Experiments conducted in fields at 1CRISAT Center suggest that a minimum of 10^6 cells seed^{-1} is required by Robut 33-1 (Nambiar et al. 1984b; Nambiar et al. 1987a).

The percentage of nodules formed by the inoculated strain (NC 92) increases with subsequent inoculations (Nambiar et al. 1984b). Using a high inoculum rate (10^6-10^8 cells seed^{-1}) for the initial inoculation could help in early establishment of the inoculant strain, and if so, inoculation would be economical. However, this needs further testing under different soil conditions.

***Bradyrhizobium* inoculants (left) may be contaminated with other microorganisms that severely reduce the number of bradyrhizobia they contain. A good quality inoculant (right) should contain around 10^{10} bacteria g^{-1} carrier material.**

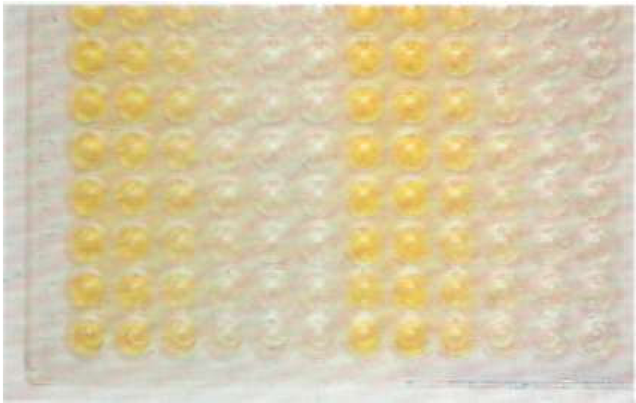
Problems in Technology Application

Data suggest that *Bradyrhizobium* inoculation can increase yields of certain groundnut cultivars in India in fields where the crop is currently cultivated. Based on 3 years of testing at many Indian locations during the 1981-83 rainy seasons, AICORPO recommended *Bradyrhizobium* strain NC 92 as an inoculant for cultivars Robut 33-1 and JL 24 that have been released in India (AICORPO 1983). However, the nonavailability of quality inoculum could be one of the major constraints to its use in developing countries (Thompson 1982; 1984). Quality control of inoculants 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 enzyme-linked immunosorbent assay (ELISA) technique could be used to estimate the number of rhizobia in peat inoculum (Nambiar and Anjaiah 1985a; 1985b; Nambiar 1986; Reddy et al. 1987). This test has an advantage over other methods because it can be selectively used to enumerate a specific strain, while other tests count the population of all rhizobia present. Large numbers of samples can be handled using ELISA and the results known within 2 days. The alternative plant-infection method requires incubation periods of 3 to 4 weeks.

A second important problem in applying inoculum technology to groundnut production is to convince farmers of the effect of inoculation on yield. Fluctuations in yields of noninoculated plots from year to year, even under similar growing conditions, are often larger than yield differences between inoculated and noninoculated plots. During the rainy season, in noninoculated fields at ICRISAT Center, yields ranged from 0.9 to 2.4 t ha^{-1}. Yield response to inoculation cannot always be ensured. Moreover, there were no visible differences in plant growth between inoculated and noninoculated plots.

To summarize, *Bradyrhizobium* inoculation is important in newly cleared fields where native rhizobia are in low numbers or ineffective. In traditionally cultivated soils, marginal, but inconsistent increases in pod yields might be obtained by inoculation with such strains as NC 92. The inoculation potential under these conditions is better judged by a consistent demand for inoculant by farmers.

To test the quality of eight packets of *Bradyrhizobium* inoculant it is necessary to use 168 siratro (*Macroptylium atropurpureum*) plants (above). A single ELISA plate (below) can be used to make the same assessment.



N_2 Fixation, Residual N Effects, and Applications of Nitrogen Fertilizer to Maximize Groundnut Yields

How much N_2 does a groundnut crop fix during a growing season? Is any N left behind for a subsequent crop?

The amount of N_2 fixed depends on yield levels and available soil N. A large proportion (60-80%) of the N content in a groundnut crop is normally derived from N_2 fixation. If almost all the pods and haulms are harvested, and the foliage is not lost due to leaf fall or predation, then it is unlikely that significant quantities of N will be left behind for the next crop.

Does N_2 fixation require more energy than fertilizer-N utilization? If so, can a groundnut crop be grown substituting fertilizer-N for symbiotic N_2 fixation?

The conclusion that N_2 fixation requires more energy than fertilizer-N utilization is based on calculations derived from data collected in pot experiments. This perhaps does not apply to field situations. Moreover, one needs to apply very large amounts of N to produce a good yield, e.g., to produce a pod yield of 3.5 t ha^{-1} it is necessary to apply around 300 kg N ha^{-1}. This is not economical.

Can we supplement N_2 fixation with fertilizer-N application?

Application of fertilizer-N reduces N_2 fixation. Hence it may not be feasible to supplement N_2 fixation by fertilizer-N application.

Is it necessary to apply a starter dose of fertilizer-N, to provide N to the plant before N_2 fixation is initiated?

Only in a few situations would a starter (basal) dose of fertilizer-N increase crop yield. There are many recommendations to farmers that suggest starter N application. Although the levels recommended are low (15-25 kg N ha^{-1}) and may not be costly to a farmer, on a country-wide basis the wastage of fertilizer-N could be substantial. It is preferable to use fertilizer-N on other crops that are better able to use it.

Measurements of N_2 Fixation

As described in the Introduction (page 4) groundnut genotypes need considerable qualities of assimilated N to produce biomass equivalent to that produced by cereals (Fig. 1). There are three different methods of measuring N_2 fixation by legumes. These are: (1) differential N uptake using a non-N_2 fixing line as a control to determine the soil-N uptake by the legume (Ham 1978); (2) ^{15}N-isotope dilution using labeled fertilizer-N (Witty 1983); and (3) natural abundance of ^{15}N (Shearer and Kohl 1986).

These measurements are related to crop yield levels. Large variations in yields also reflect the amount of the N_2 fixed by a crop. Using the differential N uptake method, Nambiar et al. (1986) estimated that when pod yields are around 3.5 t ha^{-1}, the crop fixes approximately 190 kg N ha^{-1}. Under these conditions much of

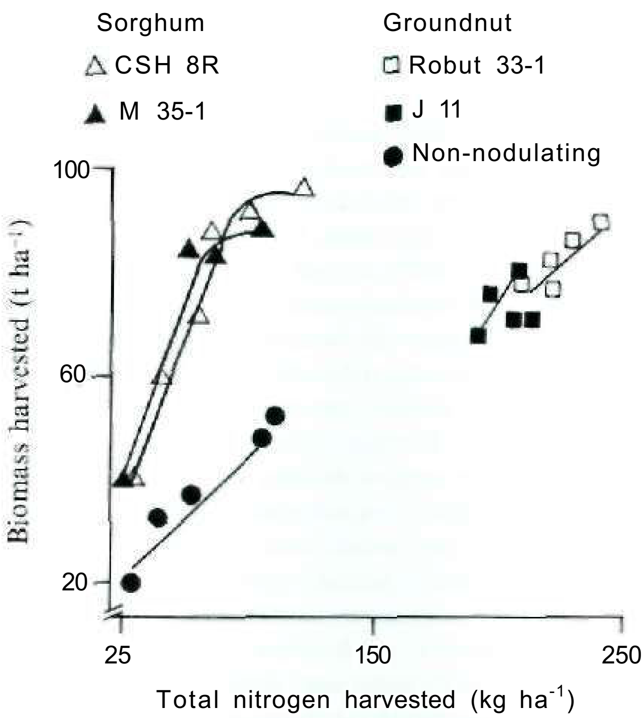


Figure 1. Regression analyses of total biomass production as a function of total N assimilated by sorghum genotypes CSH 8R and M 35-1, and groundnut genotypes Robut 33-1, J 11, and a non-nodulating genotype, ICRISAT Center, postrainy season 1983/84.

(Source:.Nambiarctal. 1986. Field Crops Research 15: 165-179 (Courtesy Elsevier Science Publishers B.V.)

the N (60-30%) is derived from N_2 fixation (Giller et al. 1987; Yoneyama et al. 1990). At higher yield levels the amount of N_2 fixed could be even greater.

Residual Effect of N_2 Fixed by Groundnut

In a crop rotation experiment, yields of pearl millet (*Pennisetum glaucum*) and maize (*Zea mays*) were higher when grown after groundnut than those of the same crops grown after cowpea (*Vigna unguiculata*) or sorghum (*Sorghum bicolor*) (Giri and De 1979; Jones 1974). At ICRISAT Center, irrigated pearl millet grown in the postrainy season yielded 45% more grain when it followed rainy-season groundnut than when maize was the preceding rainy-season crop (Nambiar et al. 1982c). However, if either groundnut or maize were grown in the postrainy season no residual effect was observed on pearl millet grown in the following rainy season (Nambiar et al. 1982c). Although other factors could be involved, it is possible that the observed residual effect of groundnut was due to leaf fall as a result of foliar diseases in the rainy season, whereas leaf fall due to foliar diseases was minimal during the postrainy season; indicating that if all the plant parts of groundnuts are removed during the harvest, then very little N will be left behind by groundnut for a subsequent crop.

Application of Fertilizer-N to Substitute for or Supplement N_2 Fixation

Williams (1979) suggested that at very high yield levels, the N requirement of nodulated groundnut cannot be met from symbiotic N_2 fixation alone. To examine the possibilities of supplying N to the plant by fertilizer-N application as a substitute for. or supplement to N_2 fixation, it is important to understand the pattern of N_2 fixation during the plant's ontogenic changes and some of the major environmental factors that affect this pattern.

Effect of Fertilizer-N on Noduiation and N_2 Fixation

In general, soil nitrogen. applied or residual, reduces noduiation and N_2 fixation in legumes. Hence, if higher yields are to be obtained, application of fertilizer-N, should not only compensate for the loss of N from reduced N_2 fixation, but should also result in higher N metabolism. Although N_2 fixation in groundnut is reduced by fertilizer-N application, the reduction is not so marked as that observed in some other legumes, e.g., cowpea. The application of 200 kg N ha^{-1} decreased N_2 fixation by cowpea by 58% while N_2 fixation by groundnut decreased only by 26% (Fig. 2). Groundnut is also considered to be a poor utilizer of fertilizer-N (Nambiar et al. 1986).

Ontogenic Changes in N_2 Fixation

N_2 fixation varies with the growth stages of the plant. Nodule formation is generally initiated when the seedlings are in the quad rifoliolate stage and can be greatly influenced by environmental factors, e.g., in the Alfisols at ICRISAT Center, nodules become visible 7 to 10 days after sowing during the rainy season (sown Jun-Jul), yet in the postrainy season (sown Nov-Dec), nodules are not visible until 15 to 18 days after sowing. This difference is probably due to the cooler weather at the start of the postrainy season. Nodule formation continues until the crop is almost mature, but nodules that are formed early start to

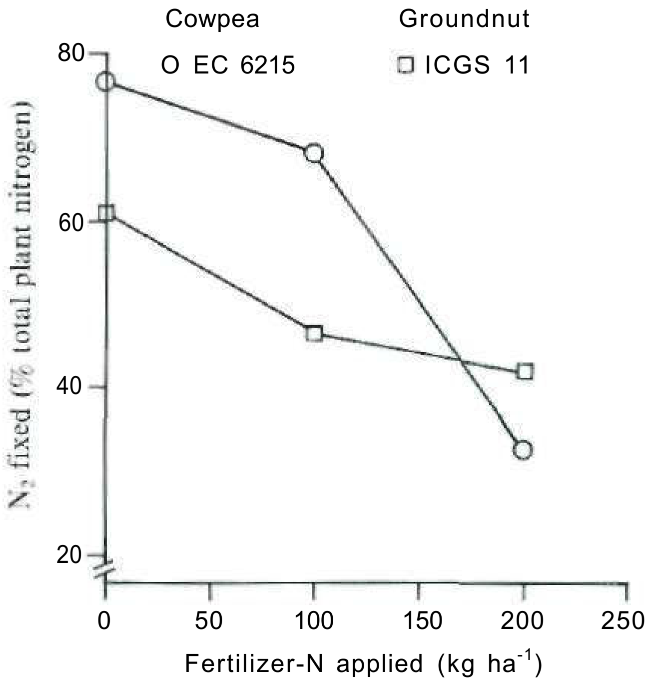


Figure 2. Effect of fertilizer-N application on N_2 fixation by a groundnut genotype (ICGS 11) and a cowpea genotype (EC 6215), ICRISAT Center, postrainy season 1985/86.

N_2 fixed (% nitrogen in the plant derived from atmospheric N_2) was estimated by the ^{15}N natural abundance method.

Source: Yonevama et al. 1990.

senesce after 50 to 60 days (Nambiar et al. 1987b). The factors responsible for this senescence of early-formed nodules, whilst many new nodules continue to develop, are not well understood (Nambiar et al. 1987b; Nambiar 1988).

As discussed earlier, the rate of N_2 fixation is usually measured by the acetylene reduction method. Whilst using this technique the following observations have been made.

Patterns of N_2 fixation differ according to the growing season. At ICRISAT Center, N_2 fixation started early during the rainy season, but was considerably delayed in the postrainy season crop sown in November (Nambiar, 1988). N_2 fixation peaked during the pod-filling stage, and declined at maturity (Nambiar et al. 1982b; Nambiar and Dart 1983). But recent evidence indicates that this is perhaps due to anomalies in the acetylene reduction assay, and that N_2-fixation continues during the pod filling stage (Dutta et al. 1988; Yoneyama et al. 1990).

Environmental Factors Affecting N_2 Fixation

N_2 fixation follows a diurnal pattern, with nitrogenase activity building up in the early morning, peaking after 8-10 hours, and decreasing during the night (Nambiar and Dart 1983). Other major factors that influence N_2 fixation are soil moisture, soil temperature, and light intensity. Groundnut experiments at ICRISAT Center on showed that; (a) excess or insufficient soil moisture reduced N_2 fixation, and (b) a 40% reduction in light intensity considerably reduced the rate of N_2 fixation. It was also observed that integrated nitrogenase activity during the growth period, was higher during the postrainy season than in the rainy season (Nambiar and Dart 1983).

It is important to consider these issues when deciding whether or not to supplement the nitrogen nutrition of groundnut by fertilizer-N application. Soil moisture and temperature should be considered before recommending fertilizer-N application since these factors also influence N_2 fixation. Even on N-deficient soils, responses by groundnut to fertilizer-N have been small and erratic (Acuna and Sanchez 1968; Chesney 1975; Gutstein 1978; Balasubramanian et al. 1980). It has been suggested that, since N_2 fixation is low during the early stages of plant growth, the application of a basal starter dose of fertilizer-N would help early plant growth and hence increase crop yield. But it is not clear to what extent root development and N assimilation by the plant occurs during this stage, and therefore whether the crop can utilize early applications of N. However, the Indian Council of Agricultural Research (ICAR 1987b) recommends farmers to apply 10-20 kg N ha^{-1}. In many trials conducted at ICRISAT Center, responses to basal application of 20 kg N ha^{-1} were not consistent. In two trials involving two and three genotypes there were no significant increases in pod yields; while in two other trials only one out of two genotypes responded to basal N application (P.T.C. Nambiar and B.S. Rao, unpublished). The application of small amounts of fertilizer-N, even if crop yields are not improved, may not cost the farmer very much, but in most developing countries fertilizer-N is subsidized, and hence this practice could result in a substantial national loss if such fertilizer applications do not increase crop yields.

The N_2 fixation rate increases 20-40 days after germination. When acetylene-reduction activity (ARA) is used as a criterion for the measurement of N_2 fixation, N_2 fixation declines during the pod-filling stage. Hence, it was suggested that application of fertilizer-N during pod filling may help to supplement the N requirement and boost crop yield. However, Williams (1979) and Dutta et al. (1988) reported increased N accumulation at the onset of reproductive growth in groundnut, which differs from the observation that ARA decreases during the same growth stage. Recent studies based on total N uptake and ^{15}N natural abundance indicated that decline in ARA during the pod-filling stage is not reflected in N_2 fixation rates as measured by the ^{15}N natural abundance method, and that there is considerable N_2 fixation during the pod-filling stage (Yoneyama et al. 1990). If this is true, application of fertilizer-N during the pod-filling stage may not be required, and may even reduce N_2 fixation. Two experiments conducted at ICRISAT Center on four genotypes did not indicate any positive yield advantage of applying fertilizer-N during the pod-filling stage (P.T.C. Nambiar and B.S. Rao, unpublished). However, a positive response to the application of N as a foliar spray during the pod-filling stage was reported in USA during 1980 (J.C. Wynne, North Carolina State University, Raleigh, USA, personal communication).

Application of N Fertilizer as a Substitute for N_2 Fixation

Since adequate N_2 fixation during crop growth is not always assured, N application could be considered as an alternative production technology, even though the economic cost is higher than *Bradyrhizobium* inoculation.

The overall plant energy cost involved in obtaining nitrogen through biological fixation includes the costs of nodule growth and maintenance, ammonia assimilation, and use of carbon skeletons for the transport of fixed N_2 (Neves and Hungria 1987). The energy required for nitrate (NO_3^-) N utilization by a given crop species depends on the site of nitrate reduction, energy required for synthesis and maintenance of the enzymes that have a high rate of turnover, pH regulation during N assimilation, etc. (Neves and Hungria 1987). Theoretical calculations on the energy requirement of N_2 fixation have led to the conclusion that at high levels of NO_3- N, assimilation through N_2 fixation requires more energy than NO_3- incorporation. Results from soybeans, cowpea, and white clover

Non-nodulating groundnut plants (center) yield less well than normal nodulating plants.

(*Trifolium repens*) suggested that plants that fix their own N_2 may have a respiratory burden up to 13% greater than that of plants dependent on NCy, and it has been suggested that because of this legumes grow more slowly than other crop plants (Silsbury 1977; Ryle et al. 1979).

Arnon (1980) argued that because of the high energy requirements of N_2 fixation, plant breeders have not been able to raise the yield levels of legumes, despite considerable breeding efforts. Hence, experiments were undertaken at ICRISAT Center to study the effect of continuous fertilizer-N application on the growth and yield of groundnut. The results showed that even the application of high levels of fertilizer-N (200 kg N ha^{-1}, applied in six doses) did not significantly influence crop yield. This is perhaps because the theoretical calculations were based on pot experiments. It was observed that plants grown in the greenhouse and supplied with adequate amounts of fertilizer-N grew better than those nodulated by the most effective *Bradyrhizobium* strain. In contrast, the growth and total nitrogen uptake of a non-nodulating genotype in the field was always poorer than the growth of many nodulating genotypes, and the growth of non-nodulating genotypes even at a high rate of applied-N (400 kg N ha^{-1}) was not significantly superior to that of nodulating genotypes grown without applied N (Fig. 3). These results suggest that respiratory costs of N_2- fixation and NCy utilization could be different in plants grown in the field from those grown in pots in a greenhouse. Hence the hypothesis that N_2 fixation requires more energy than NCy uptake and utilization may not be valid under field conditions. Experiments at ICRISAT Center suggest that the high protein content in legumes is not a consequence of N_2 fixation, but that apparently legumes acquire symbiotic N_2 fixation because of their high N requirement to produce an equivalent biomass, and that because of this, legumes grow more slowly than other crop plants. Moreover, legumes differ in their fertilizer-N use efficiency (Nambiar et al. 1986; Nambiar et al. 1988; Yoneyama et al. 1990). Much of the NCy it absorbs is accumulated in the leaves of groundnut, probably due to low levels of leaf nitrate reductase activity, the enzyme that converts nitrate to nitrite. In contrast, sorghum leaves have

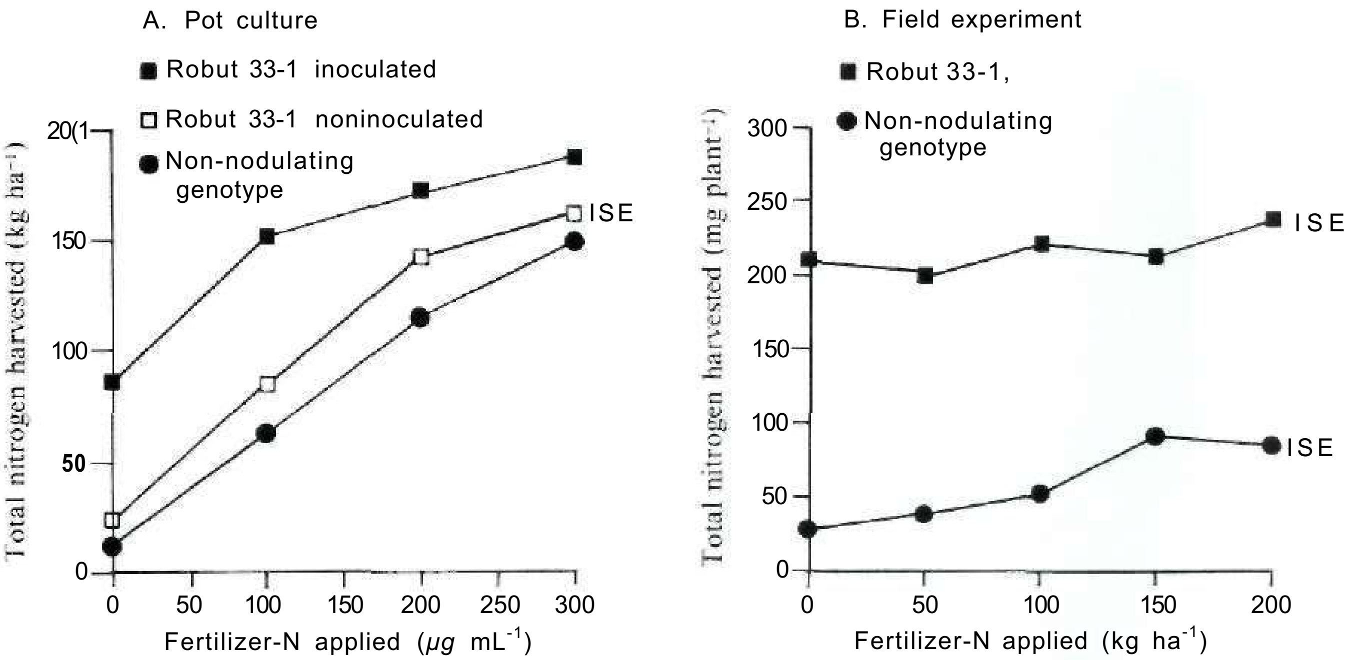


Figure 3. Effects of fertilizer-N application on total nitrogen uptake in pot culture (A) and field trial (B). In pot culture a nodulating genotype (Robut 33-1) was inoculated with one of the most effective strains of bradyrhizobia, or **grown without inoculation. A non-nodulating genotype was also grown. In the field the nodulating genotype was nodulated by the native rhizobia and the non-nodulating genotype was the control. The nodulated genotype responded very little to fertilizer-N in the field, but responded well in pot culture.**

Source: P.T.C. Nambiar and B.S. Rao, 1986 unpublished.

Non-nodulating groundnut plants (left) can not fix nitrogen, and therefore appear yellow (chlorotic) because they are nitrogen deficient. Nodulating groundnut plants (right) can fix nitrogen, they grow better and appear green. ICRISAT Center, rainy season, 1982.

Field trial to compare nitrogen accumulation by nodulating and non-nodulating groundnut genotypes and two sorghum cultivars. The groundnut plants in the center are nodulating types, obviously growing better than the non-nodulating ones (left), ICRISAT Center, postrainy season 1983/84.

Source: ICRISAT 1985, Annual Report 1984

higher nitrate reductase activity and lower nitrate contents, indicating faster conversion of NO_3^- (Nambiar et al. 1986). Groundnut can therefore be considered to utilize fertilizer-N less well than sorghum (Nambiar et al. 1986). It may not therefore be possible to obtain high biomass yields in such legumes as groundnut by substituting fertilizer-N application for N_2 fixation.

To summarize, with the exception of few circumstances, application of fertilizer-N at early growth stages (as a basal N application), or during all growth stages as a split application, or only during the pod-filling stage may not influence groundnut pod yield. This could be because groundnut is a poorer utilizer of fertilizer-N than cereal crops like sorghum (Nambiar et al. 1986; Nambiar et al. 1988).

Agronomic Practices and N_2 Fixation

How do other nutrient deficiencies affect N_2 fixation?

Deficiency of other nutrients could affect the process of N_2 fixation directly or indirectly through poor crop growth. It is thus important to supply the crop with adequate amounts of other essential nutrients.

Are there *Bradyrhizobium* strains that tolerate acid soils?

Results show that the selection of groundnut genotypes tolerant to acid soil conditions is more important than selection of *Bradyrhizobium* strains tolerant to acid conditions.

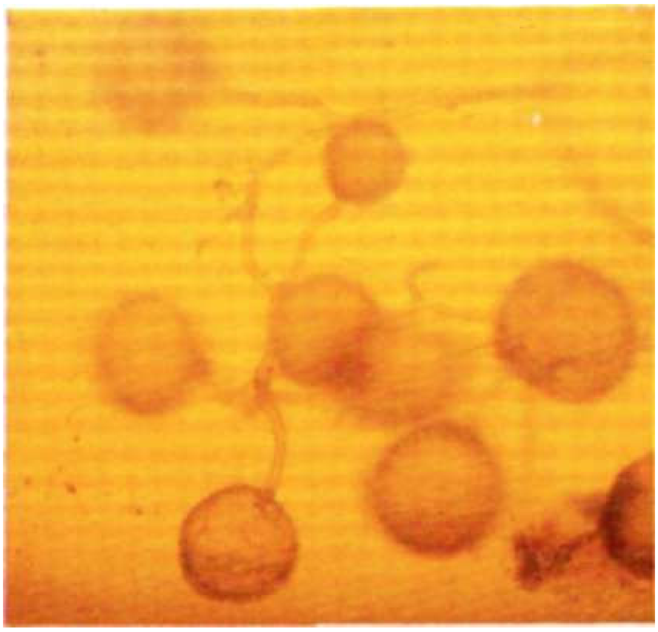
Do agronomic practices alter N_2 fixation?

Yes, depth of sowing is one of the important factors. Deeper seed placement results in a longer hypocotyl, poor rooting, poor nodulation, and decreased N_2 fixation. Intercropping groundnut with cereal crops such as maize or sorghum also reduces N_2 fixation.

Effects of Other Nutrients on N_2 Fixation

Effect of Phosphorus Nutrition and Vesicular-arbuscular Mycorrhiza

Limitations of other nutrients, e.g., phosphorus (P) will limit N_2 fixation either directly by affecting nodule initiation, nodule development, and N_2 fixation, or indirectly by affecting plant growth. Legumes form symbioses with both *Bradyrhizobium* and vesicular-arbuscular mycorrhiza (VAM) (Hayman, 1986). Mycorrhizae have been found beneficial to the plant mainly because they improve P uptake. In general, groundnut roots seem to be adequately colonized with VAM in many fields, but at low yield levels responses to inoculation with strains of VAM have been reported (ICRISAT 1987). Strains of *Bradyrhizobium* influence root colonization by species of VAM, and species of VAM influence root nodulation by strains of *Bradyrhizobium* (Nambiar and Anjaiah 1989a). Inoculation with strain NC 92 increased root colonization by native VAM in Alfisols at ICRISAT Center (Nambiar and Anjaiah 1989a).



The microscope photograph shows propagules and hyphae (x 120) of a VAM (*Glomus* sp.) abundant in the groundnut rhizosphere of Alfisols at ICRISAT Center. Inoculation with *Bradyrhizobium* increases the colonization by native VAM in the field.

Source: Nambiar and Anjaiah 1989a.

Other Nutrients

Molybdenum (Mo) and iron (Fe) are micronutrients implicated in N_2 fixation, because both these elements form a structural part of nitrogenase, the enzyme that reduces nitrogen. Soil microflora, including bradyrhizobia produce chelants called siderophores that may improve Fe uptake (Neilands 1981; Nambiar and Sivaramakrishnan 1987). *Bradyrhizobium* strains also influence the uptake of mineral nutrients by groundnut. The uptake of calcium (Ca), manganese (Mn), zinc (Zn), Fe, and sodium (Na) differed in groundnut plants when inoculated with different strains of rhizobia (Howell 1987). It has been reported that Fe deficiency specifically limits nodule development in groundnut grown in the calcareous soils of Thailand (O'Hara et al. 1988). Soil acidity along with Mn and aluminium (Al) toxicities can also restrict N_2 fixation in groundnut. Excess Mn was detrimental to plant growth per se rather than to nodulation, but nitrogenase activity was more affected by Al than plant growth (Nambiar and Anjaiah 1989b; Nambiar and Anjaiah unpublished). Under the conditions of the experiment, using a quartz-sand nutrient culture, the cause of Al toxicity was due more to a deficiency of P in the plant, because P precipitated in the presence of Al, than to Al toxicity per se. However, it appears to be more practical to select groundnut genotypes that are tolerant of acid soils than *Bradyrhizobium* strains for that environment.

Effect of Agronomic Factors on Nodulation and N_2 Fixation

Two of the major agronomic practices that influence nodulation and N_2 fixation are the intercropping of groundnut with cereals, and sowing depth. Intercropping a cereal with a legume crop is a common practice in developing countries. Competition for light by the cereal (e.g., millet, maize, or sorghum) component of the intercrop can reduce in N_2 fixation by groundnut (Nambiar et al. 1983c).

Many farmers sow groundnut 8-12 cm deep to utilize residual soil moisture for germination and seedling growth. Some conventional sowing devices also place the seed deeper than is necessary for good crop establishment, although deep sowing does protect the seed from birds and rodents. Under certain conditions deep sowing decreased nodulation, N_2 fixation, and pod and haulm yields (Nambiar and Srinivasa Rao 1987). Modifying these agronomic practices could lead to higher N_2 fixation, but under moisture-limiting conditions deeper sowing was found to result in better yields (Nageswara Rao et al. 1989).

Breeding for Increased N_2 Fixation

Can the inherent N_2-fixing potential of groundnut genotypes be improved by plant breeding?

Yes, this is theoretically possible. Thereare high and low N_2-fixing lines, and in theory there should be ways to improve N_2 fixation by plant breeding. However, this is very difficult in practice.

Many host genes influence nodulation and N_2 fixation (Nambiar and Dart 1980; Nigam et al. 1980; Nigam et al. 1982; Nigam et al. 1985; Wynne et al. 1983; Nambiar 1982; Nambiar et al. 1982b; Nambiar et al. 1983a; 1983b; Dutta and Reddy, 1988). Considerable variation in the ability to nodulate and fix N_2 exists in groundnut genotypes, and nodulation and N_2 fixation are also dependent on the interaction between the host cultivar and the nodulating *Bradyrhizobium* strain. These traits are heritable (Wynne et al. 1978; Wynne et al. 1980; Wynne et al. 1983; Arunachalam et al. 1984; Nigam et al 1985). However, these authors' conclusions were drawn from experiments using wide row spacings. Williams et al. (1989) noted that canopy-related effects were probably a confounding factor in the above experiments; ARA is dominated by leaf area and there is apparently relatively little genetic variation in nodulation and nitrogen fixation. On the contrary, it could also be argued that leaf-area development is proportional to protein synthesis, which under N-limiting conditions, is dependent on the rate of N_2 fixation. Hence, leaf- area development is dependent on N_2 fixation. In theory, it should be possible to estimate the ARA of genotypes after adjusting for leaf area effects (Williams et al. 1989). But, because of the practical difficulties in screening and selecting high N_2-fixing genotypes, it is rather difficult to envisage the success of a breeding program aimed at improving nodulation and N_2 fixation.

One of the successes of ICRISAT's groundnut breeding program has been the development of genotypes that can produce very high yields without fertilizer-N application, or *Bradyrhizobium* inoculation, and without deliberately attempting to increase N_2 fixation by plant breeding (1CRISAT 1985). This suggests that the above three methods are not very important to groundnut crop productivity, and that plant breeders have been inadvertently selecting high N_2-fixing lines. However, high yields are rarely realized in farmers' fields in India or other developing countries, and the factors contributing to the differences between experimental station and farmers' field yield levels are not understood. It is possible that good management of the research fields at ICRISAT Center over many seasons has resulted in optimum conditions for crop growth, and that these have a strong effect on nodulation and N_2 fixation.

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