Linking Biological Nitrogen Fixation Research in Asia

International Crops Research Institute for the Semi-Arid Tropics

Abstract

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Reports from Bangladesh, Nepal, Thailand, and India on the on-farm use of rhizobial inoculants are presented. Other topics covered include the status of soybean *Bradyrhizobium* research in India, influence of cropping system and other factors on population of cowpea rhizobia, improvement of biological nitrogen fixation (BNF) in groundnut by host-plant selection, expectations of research administrators and breeders from BNF research, intra-varietal variability in nodulation in chickpea and pigeonpea, the role of legumes in cropping systems, and iron chlorosis in groundnut. Details are given of experiments on rhizobial inoculants and on host-plant selection for high BNF. Working Group work plans are outlined.

Résumé

La recherche en Asie sur la fixation biologique de l'azote: rapport d'une réunion du Groupe de travail Asie sur la fixation biologique de l'azote chez les légumineuses, 6–8 déc 1993, Centre ICRISAT pour l'Asie, Inde. **Cet ouvrage présente des rapports, en provenance du Bangladesh, du Népal, de la Thaïlande et de l'Inde, sur l'utilisation en milieu réel des inoculants rhizobiaux. D'autres sujets qui sont abordés: statut de la recherche sur *Bradyrhizobium* du soja en Inde, influence du système de culture et d'autres facteurs sur la population des rhizobia du niébé, amélioration de la fixation biologique de l'azote chez l'arachide par la sélection de la plante-hôte, résultats attendus par les administrateurs de recherche et sélectionneurs sur la recherche sur la fixation biologique de l'azote, variabilité intra-variétale de la nodulation chez le pois chiche et le pois d'Angole, rôle des légumineuses dans les systèmes de culture et enfin, chlorose ferrique chez l'arachide. Sont également inclus des détails des expériences sur les inoculants rhizobiaux et sur la sélection des plantes hôtes pour une fixation biologique de l'azote élevée. Des projets de recherche futurs du Groupe de travail sont présentés brièvement.**

Linking Biological Nitrogen Fixation Research in Asia: report of a meeting of the Asia Working Group on Biological Nitrogen Fixation in Legumes

6-8 Dec 1993
ICRISAT Asia Center, India

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1994

Preface

This publication was prepared from the proceedings of a meeting held at ICRISAT Asia Center (LAC), Patancheru, India, 6-8 Dec 1993. The meeting was organized to initiate activities of the Asia Working Group on Biological Nitrogen Fixation in Legumes (AWGBNFL). Research on BNF in legumes has been criticized as having promised much but delivered little. However, research in this area is quite relevant, particularly in developing countries, in view of the high financial and environmental costs of producing and using fertilizer nitrogen and the difficulties involved in transporting it to farmers' fields. It was considered that the working group concept, which allows pooling of resources of interested researchers is a sound approach to address BNF research needs in Asia and to achieve the objectives of the AWGBNFL (see Appendix).

The meeting was divided into sessions dealing with the following topics: on-farm experience of scientists from different countries in the application of BNF technology, research areas with potential for on-farm application, identification of best-bet BNF technology for validation on farmer's fields, identification of constraints to adoption of existing BNF technology, and Work Plans. The highlights of the meeting were shared through the informal newsletter of the group, 'AWGBNFL Notes'. Thus, the meeting provided an opportunity for a joint evaluation of BNF technologies that are available or are likely to be available in the near future.

We consider that not all the bacteria nodulating chickpea, groundnut, and pigeonpea, the legumes of major interest to the participants in this meeting, are slow growers and therefore belong to the genus Bradyrhizobium. For this and other considerations we have used Rhizobium, Bradyrhizobium and root-nodule bacteria (RNB) interchangeably.

The Organizing Committee of the meeting, appreciates the contributions of all their ICRISAT colleagues. They acknowledge the active participation of J K Ladha of the International Rice Research Institute, Philippines, in leading the group discussion and giving a direction to the formulation of Work Plans, and of K V B R Tilak of the Indian Agricultural Research Institute, New Delhi, India, and A L Khurana of CCS Haryana Agricultural University, Hisar, India, for providing guidance in finalizing the Work Plans.

And they thank the authors for their contributions.

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Opening Session

Introductory Remarks and Overview of the Meeting

O P Rupela[1]

On behalf of the ICRISAT management, and the Organizing Committee of the Working Group, I welcome you to this first meeting of the Asia Working Group on Biological Nitrogen Fixation in Legumes (AWGBNFL). This meeting has been organized as an activity of the Cereals and Legumes Asia Network (CLAN).

Working groups serve as important vehicles to address the specialized research needs of the participating countries within CLAN. The idea of a Working Group (WG) on biological nitrogen fixation (BNF) was conceived in the late 1980s, at a time when funding for BNF research was on the decline the world over. The support for this WG came from CLAN members and ICRISAT in 1992 after the likely benefits from research on the host-plant aspect of BNF became apparent. We will learn more about ICRISAT's experience as the meeting unfolds.

The economic recession that is affecting most countries of the world has made funds for agricultural research difficult to obtain. In such an environment, every dollar that we seek for BNF research has to be justified. Research administrators and funding agencies have a pragmatic approach, and they like to see a good return on every dollar that is invested. The onus of justifying the funding lies on us, the researchers. Hence, we need to review the strengths and weaknesses, and the costs and benefits of BNF research. As will be clear to all of you, this WG will largely confine itself to aspects of BNF research that are suitable for on-farm testing and adoption by farmers for sustainable production systems. I strongly believe that through BNF research we can support the research efforts aimed at achieving sustainable increases in yields in different cropping systems.

BNF researchers face the criticism that there is no readily apparent evidence of their research benefiting farmers. In this meeting, we will learn more about the expectations of research administrators and breeders, and these must be considered seriously before we plan for future work. The presentation of papers and group discussions will provide us an opportunity to discuss these expectations. We must also prepare Work Plans on specific research topics that are likely to yield a measurable output in 3-5 years. The research topics should also be of relevance to those who could not participate in this meeting. We propose to circulate such research topics in reasonable detail to those who are interested in BNF research through our informal newsletter 'AWGBNFL Notes'.

We hope that researchers will share their research responsibilities and work on the topics suggested by this group. The linkages created by the WG (as given in Appendix 1), will facilitate the sharing of germplasm (of both host plant and root-nodule bacteria), techniques, and other research outputs. We look forward to a useful interaction among participants on all relevant aspects of BNF in legumes and hope that our shared vision will generate appropriate recommendations for Working Group activities.

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Working Groups and Biological Nitrogen Fixation in Legumes

C L L Gowda[1] and A Ramakrishna[2]

Introduction

The purpose of this meeting is to discuss the modalities for the formation of an Asia Working Group on Biological Nitrogen Fixation in Legumes (AWGBNFL), and prepare a work plan for research collaboration. This paper provides a brief background to the concept of a working group (WG) and its operation.

Agricultural research in developing countries is facing an acute paucity of funds, and research administrators and scientists are being required to cut costs and maximize the cost efficiency of research. Laboratories and/or institutions are unable to take up comprehensive studies due to the scarcity of funds, facilities, and expertise. Therefore, it is not surprising that scientists are joining hands to find solutions to important regional problems. The concept of working groups is not new; scientists around the world have long been pooling their resources and sharing the results of their studies, either formally or informally. For instance, in India, the Coordinated Research Programs brought together scientists from universities and research institutions to review the research done and plan future activities. Many other countries have similar collaborative ventures. In the international arena, collaborative agricultural research networks are becoming increasingly popular as a means of using funds, facilities, and staff more efficiently and effectively.

Advantages of Working Groups

Working groups, also called subnetworks, working parties, or consortia, bring together committed scientists with a common interest in addressing high-priority regional problems. This approach aims at sharing resources, facilities, and staff to conduct research and exchange results. International working groups bring together expertise from developed and developing countries, international research centers, and specialized research laboratories and institutions, to work together on a common platform as equal partners.

There are many advantages in forming working groups:

- WGs identify and address problems that are important to a region; hence, they are a need-based activity.
- The WG approach allows scientists to initiate a series of discrete research topics as and when priority problems are identified; these topics can be wound up once solutions are found.

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- The small size of a WG makes it cost efficient and easy to operate.
- WGs use existing facilities and staff, avoid duplication of effort, and save time and resources.
- WGs in a network (such as CLAN) can help support those activities that are in common with other WGs, such as training.

Organization and Structure of Working Groups

The membership of a WG may include scientists from national programs, international and regional institutions, and advanced research laboratories (Fig. 1). Each WG nominates a Technical Coordinator (TC), normally an expert on the subject, to liaise, coordinate, and harmonize research. The TC is usually supported by a network or institution that provides the necessary administrative and logistical support.

CLAN supports a few working groups formed by scientists in Asia. The proposed AWGBNFL will also become part of CLAN.

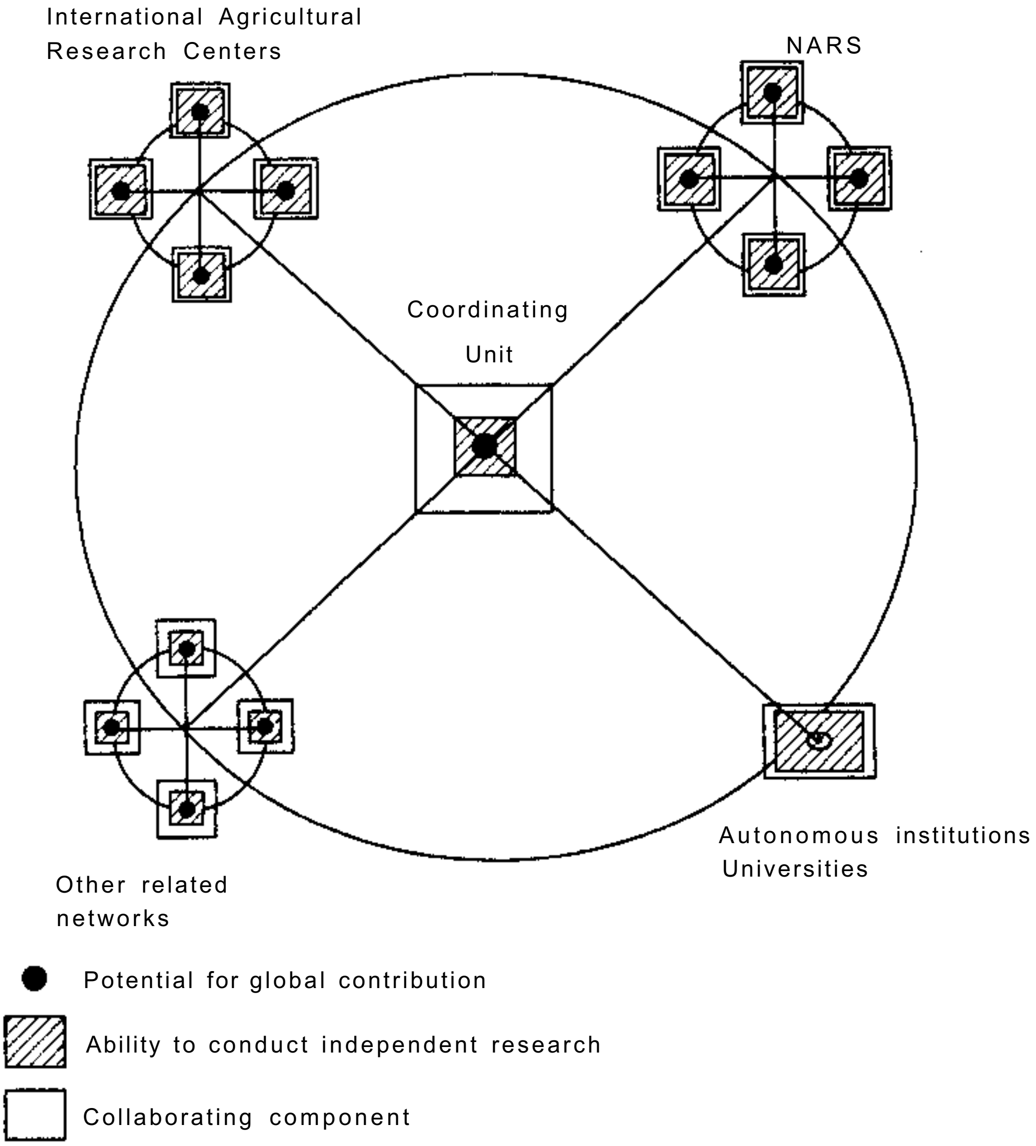

Figure 1. Structure of a Working Group.

Cereals and Legumes Asia Network

The Cereals and Legumes Asia Network (CLAN) was established in April 1992 to serve as a research and technology exchange network for Asia involving sorghum, millets, chickpea, pigeonpea, and groundnut. It was formed by merging the erstwhile Asian Grain Legumes Network (AGLN) and the Asian component of the Cooperative Cereals Research Network (CCRN).

CLAN consists of scientists and administrators from Asian countries who are willing to commit resources to undertake collaborative research, participate in network activities, and share results and technology. Its membership includes staff from more than 15 Asian countries, regional and international institutions in Asia and elsewhere, and ICRISAT scientists.

The overall objective of CLAN is to support, coordinate, and facilitate technology exchange involving CLAN's priority crops and their resource management among Asian scientists. The ultimate objective is to improve the well-being of Asian farmers by improving the production and productivity of crops in a sustainable manner.

The specific objectives of CLAN are to:

- strengthen linkages and enhance exchange of germplasm, breeding material, technical information, and technology options among members;
- facilitate collaborative research among members to address and solve high-priority production constraints giving attention to poverty and equity issues as per the needs and priorities of member countries;
- assist in improving the research and extension capability of member countries through human resource development;
- enhance coordination of regional research on sorghum, millets, chickpea, pigeonpea, and groundnut; and
- contribute to the development of stable and sustainable production systems through a responsive research capability in member countries.

Conclusion

In addition to encouraging research collaboration among scientists, WGs help strengthen national programs' capabilities to improve basic and strategic research, and provide answers that can be quickly channelled to farmers for enhanced impact. The critical mass of scientists in a WG can address and solve a problem at a much faster pace, considerably reducing the 'research lag'. Although the task of setting up WGs has been initiated and coordinated by international centers, we propose to gradually transfer the research and coordination responsibilities to national programs, depending on the availability of staff expertise and facilities. Therefore, it is essential to identify laboratories and institutions that can take the lead role in research and coordination of the WG in the future.

Chairperson's Address

K K Lee[1]

It is a great pleasure to extend my warm welcome to the participants of this meeting who are engaged in research on biological nitrogen fixation (BNF) in legumes in Asia.

As you all know, BNF is one of the most important biological processes which contributes to crop production by generating combined N from inert atmospheric N_2 gas. However, its importance was not fully appreciated in the past, and farmers relied mainly on chemical fertilizers. Since the 1970s, however, the rising cost of fertilizers, and concern for the environment have forced farmers to look for alternative agricultural methods that are more economical and environmentally friendly. One such method was to exploit BNF by including legumes in crop rotations or intercropping systems, and by using them as green manure. Legumes have been used to introduce N to soils and other crops and sometimes to avoid pests and diseases. In addition, N_2 fixed through BNF is less likely to be lost than inorganic fertilizer N. Therefore, BNF can be an important factor in sustainable agriculture.

Though BNF is beneficial to crop production, the technologies generated from BNF research have not been widely adopted by farmers. This, I guess, is one of the reasons why we are here today. By sharing our ideas, information, and experiences, we will have an insight into the current status of BNF research, which will help us identify topics for future on-farm research.

I understand that this Working Group has formulated five broad objectives, around which the discussions at this meeting will be centered.

The first objective is to validate best-bet BNF technology on farmers' fields and use the experience thus gained to update it. This objective has particular relevance to rhizobial inoculation technology. The technology has been used by farmers in many countries where inoculants are commercially available, but the results have often been unsatisfactory. One of the problems with inoculation is that it is difficult for farmers to determine the range of conditions under which it works best. I hope that the participants of this meeting will share their experiences regarding inoculation when they present their country reports.

The second objective is to characterize factors which impede BNF and to try to find solutions. The BNF process is influenced by such environmental factors as soil nutrients, soil pH, soil moisture, soil and atmospheric temperatures, light intensity, soil biota, etc. I understand that among these factors, soil nutrients, particularly mineral N, are of great relevance to this meeting. Combined N has a

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considerable influence on BNF in legumes. Its effect on N_2 fixation in vitro is always inhibitory. However, as all of you know, in the case of in planta or in situ N_2 fixation, combined N can either stimulate or depress BNF depending on the level of mineral N in the soil I do not think that it is possible to pinpoint the exact level at which stimulation stops and inhibition begins; the response varies with plant species and genotypes, and soil and plant conditions. This presents us with a problem in the case of legume-nonlegume intercropping or rotation systems in which the fertilizer or residual N may affect BNF in the legume crop. Appropriate soil-management practices can solve this problem, but another solution is to develop legume crops or genotypes whose BNF is not affected by high levels of combined N. Although no special session has been devoted to this problem, I have been told that it will be discussed in the meeting.

Thirdly, the Working Group will examine which task requires more attention at this stage: host-plant selection, or rhizobial selection. It seems to me that greater emphasis has so far been placed on rhizobial selection than on host-plant selection. Microbiologists have tried hard to obtain superior rhizobial strains that have high N_2-fixing ability and are tolerant to high or low temperatures, nutrient toxicity, high or low pH, etc. These strains have not always performed well in the field, but in many cases, they have made a great contribution to increased BNF in legumes.

Efforts must also be made to select or breed plant genotypes that possess high N_2-fixing capacity. A great advantage of having such genotypes is that BNF technology can be packaged in the seed. As we all know, such an approach is ideal if the technology is to reach farmers quickly and effectively.

The fourth objective of this Working Group concerns quantification of N_2 fixed by legumes. This is important not only from the economic point of view of saving fertilizer N, but also to develop strategies to support sustainable agriculture. Since some of the participants have worked on different legume crops and genotypes in different agroecological zones, we look forward to hearing about their experiences in this matter.

Lastly, but most importantly, the Working Group must develop work plans with emphasis on demonstration of BNF technology. We may have to generate not only topics for research and demonstration trials, but also some ideas about the likely sources of funding. As Dr Rupela rightly said, strict justification will be required for every dollar that we seek for BNF research. We must convince the donors that our research is likely to produce results, and promise them that the research will be collaborative in its approach. Fortunately, this meeting is placing an emphasis on on-farm technology, and I hope that this will lead to measurable impact. All of us assembled here today are interested in developing a collaborative and coordinated approach to conducting future research. I hope that the group will be able to develop research proposals for submission to donors.

I have great pleasure in chairing the Opening Session and wish you all success in the deliberations.

Country Reports

Use of Rhizobial Inoculants: On-farm Experience in Bangladesh

Delowara Khanam[1], Hasan H Rahman[2], and A K Maqbul Hossain[3]

Introduction

Bangladesh produces a variety of pulses and other legumes with yields ranging from 600 to 750 kg ha^{-1} (Bangladesh Bureau of Statistics 1991). The possibility of increasing the yield of these legumes through the application of effective nitrogen-fixing bacteria has been suggested in various studies. Khanam et al. (in press, a) found that the grain yield of chickpea increased by 37 to 119% with rhizobial inoculation in field trials. The grain yield of lentil increased by 64% due to rhizobial inoculation alone and by 106% due to the application of phosphorus + potash + rhizobia, Khanam et al., (in press, b). Such responses in trials on research stations encouraged us to more confidently promote the use of rhizobial inoculants in farmers' fields.

BNF Work in Bangladesh

Rhizobial strains nodulating lathyrus (Lathyrus sativus), lentil (Lens culinaris), chickpea (Cicer arietinum), black gram (Vigna mungo), mung bean (Vigna radiata), cowpea (Vigna sinensis), pigeonpea (Cajanus cajan), groundnut (Arachis hypogaea), and soybean (Glycine max) are collected locally or procured from laboratories abroad. These strains are maintained in the laboratories of several research institutions in Bangladesh, such as the Bangladesh Agricultural Research Institute (BARI), Joydebpur; the Bangladesh Agricultural University (BAU), Mymensingh; and the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh.

At BARI, the strains are first evaluated in pot trials and the most efficient ones are further tested in field trials. Sometimes, exotic strains reported to be efficient in other countries are directly included in field evaluations. Field trials are conducted to:

- identify combinations of high-nodulating and high N_2-fixing legume cultivars and suitable rhizobia;
- identify strains that are compatible with chemical fertilizers; and
- select cultivars with high nodulation and high N_2-fixing ability, and high yield potential.

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Khanam, Delowara., Rahman, Hasan H., and Maqbul Hossain, A.K. 1994. Use of rhizobial inoculants: on-farm experience in Bangladesh. Pages 7-12 in Linking Biological Nitrogen Fixation Research in Asia: report of a meeting of the Asia Working Group on Biological Nitrogen Fixation in Legumes, 6-8 Dec, ICRISAT Asia Center, India. (Rupela, O.P., Kumar Rao, J.V.D.K., Wani, S.P., and Johansen, C, eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

In trials at research stations, significantly higher yields resulting from rhizobial inoculation have been observed. The yield increases ranged from 28 to 114% in lentil (Khanam et al, in press, b), 34 to 110% in chickpea (Khanam et al., in press, a), and 44 to 84% in groundnut (Rahman et al. 1992). Similar results have been reported from BAU (Hoque et al. 1982, Hoque et al. 1983) and BINA (Podder and Habibullah 1982, Solaiman et al. 1991).

In Bangladesh, rhizobial inoculants for on-farm trials are prepared using locally available peat. The peat is powdered to 250 µm and sterilized in plastic bags before use. The rhizobial strains are generally grown in small flasks on shakers, using yeast extract mannitol broth (Vincent 1970). After testing the broth for purity, it is injected into the presterilized peat in plastic bags. The rhizobial population in these inoculants was generally found to be around log$_{10}$ 8.0, or above, g^{-1} dry peat. In an earlier study, it was established that at least log$_{10}$ 8.0 rhizobia survived for 75 days in inoculants prepared in locally available sterilized peat, even when stored at a room temperature of 25±5°C (Khanam et al. 1984).

Mineral N and Native Rhizobial Population

Alluvial, flood-plain, and terrace soils are the major soil types in Bangladesh. Their pH ranges from 5.0 to 7.5, organic matter from 0.75 to 1.60%, and total N from 320 to 2040 mg kg^{-1} soil. We did not measure the mineral N level in different soils. We also did not measure the mineral N and native rhizobial population at the experimental sites where on-farm inoculation trials were conducted. However, the native rhizobial population nodulating five different legumes—lathyrus, lentil, chickpea, cowpea, and pigeonpea—was assessed using the most probable number (MPN) plant infection method (Vincent 1970), in an experiment conducted in a greenhouse during December 1992. Soil samples were collected from 16 locations in Bangladesh, brought to BARI, and stored at 4°C until processed. One composite soil sample from the top 15 cm of soil was collected from a farmer's field in each district. The MPN count was done by the serial dilution plant infection test at BARI and ICRISAT Asia Center (IAC). The cultivars used as host plants were lathyrus Jamalpur, lentil L5, chickpea Nabin, cowpea Hathazari Local, and a local variety of pigeonpea. Plants were grown in test tubes with Jensen's agar, except for chickpea at IAC where coarse sand was used. Host plants were grown in test tubes and inoculated with serial dilutions of a given soil sample. Each dilution was replicated thrice. Nodulation was recorded 45 days after inoculation, and the data were used to calculate the most probable number of rhizobia g^{-1} dry soil as per Somasegaran and Hoben (1985). Number of rhizobia, where the appropriate strains were present, ranged from log$_{10}$1.2 to log$_{10}$ 5.6 of lathyrus, log$_{10}$1.2 to log$_{10}$ 4.8 of lentil, log$_{10}$ 1.2 to log$_{10}$ 5.2 of chickpea, log$_{10}$ 1.4 to log$_{10}$ 5.2 of cowpea, and log$_{10}$1.2 to log$_{10}$ 4.7 gr^{1} dry soil of pigeonpea (Table 1). Of the total 16 samples, the rhizobial count was undetectably low in 1 sample of cowpea, 2 samples each of lathyrus and pigeonpea, 4 samples of lentil, and 7 samples of chickpea. Flooded conditions under rice cultivation may have reduced the

Table 1. Native rhizobial populations nodulating five different legumes in soil samples from 16 locations in Bangladesh, Dec 1992.

	Rhizobial count ($\log_{10}$ g^{-1} dry soil)				
Location	Lathyrus	Lentil	Chickpea	Cowpea	Pigeonpea
Bagmara[1]	2.3	1.2	UD[2]	3.2	2.4
Barishal	2.4	UD	UD	5.2	4.4
Bogura[1]	3.6	3.2	UD	1.6	UD
Chittagong	UD	UD	UD	4.6	1.2
Dinajpur[1]	2.6	1.6	1.2	2.0	2.4
Gazipur[1]	2.6	3.3	2.3	3.4	4.7
Ishurdi	3.0	2.4	1.6	2.7	UD
Jamalpur[1]	4.0	4.6	5.0	2.3	3.7
Jessore[1]	3.0	3.4	3.6	4.0	2.4
Khulna	4.0	2.6	2.2	3.6	2.6
Kushtia	5.6	4.8	5.2	1.4	4.6
Panchagar[1]	3.0	1.4	UD	UD	2.4
Patuakhali	1.2	UD	UD	5.0	3.8
Rajshahi[1]	4.2	3.6	1.3	4.0	4.4
Sylhet[1]	UD	UD	UD	1.6	2.4
Thakurgaon[1]	2.0	2.1	2.3	3.2	4.0

1. Counting of rhizobia was done at ICRISAT Asia Center using the following trap hosts: Cultivar K 850 (= ICC 5003) for chickpea, siratro *(Macroptylium atropurpureum)* for cowpea, and cultivar 1CPL 227 for pigeonpea.
2. UD = Undetectably low count.

population of different rhizobia (Rupela et al. 1987). If this is the case, the survival of different rhizobia in a large number of other soil samples needs to be investigated. In nodulation surveys on farmers' fields in Bangladesh, very poor to extremely good nodulation of chickpea, without rhizobial inoculation, has been observed (O P Rupela, ICRISAT, personal communication).

Response to Inoculation

About 40 rhizobial strains of different legumes have been evaluated in field experiments at BARI Inoculants of the most efficient strains were provided to the On-farm Research Division of BARI for evaluation on farmers' fields in different agroclimatic regions of the country during 1992/93. The experiments consisted of four treatments: farmers' practice (without fertilizers and rhizobia); nitrogen (N) + phosphorus (P) + potash (K); PK + rhizobia; and rhizobia. Where relevant, N was applied as urea at the rate of 30 kg ha^{-1}, P as triple superphosphate at 50 kg ha^{-1}, and K as muriate of potash at 50 kg ha^{-1}. In the treatments without rhizobia, the number of nodules $plant^{1}$ and nodule mass $plant^{1}$ were invariably low in all the trials. Fertilizers were applied at sowing in plant rows. The plot size of each treatment was 24 m^2, and the experimental sites were not characterized for

Table 2. Response to rhizobial inoculation in four legumes in on-farm trials[1], Bangladesh, 1992/1993.

Location	Crop	Treatment	Nodule number plant^{-1}	Nodule mass (mg plant^{-1})	Grain yield (t ha^{-1})	Increase in yield over control (%)
Jessore	Chickpea	Farmers'practice	12	52	0.82	
		NPK	17	75	1.40	71
		PK + rhizobia	31	166	1.43	74
		Rhizobia	27	115	1.20	46
Nachole	Chickpea	Farmers' practice	9	22	0.74	
		NPK	11	32	1.20	62
		PK + rhizobia	28	103	1.24	68
		Rhizobia	21	83	1.02	38
Chuadanga	Chickpea	Farmers' practice	5	13	0.76	
		NPK	8	20	1.29	70
		PK + rhizobia	26	95	1.34	67
		Rhizobia	16	61	1.05	38
Jessore	Lentil	Farmers' practice	3	3	0.75	
		NPK	5	4	1.25	67
		PK + rhizobia	16	10	1.31	75
		Rhizobia	14	8	1.00	33
Faridpur	Lentil	Fanners' practice	4	6	0.69	
		NPK	6	5	1.04	51
		PK + rhizobia	21	12	1.10	59
		Rhizobia	18	9	0.90	30
Meherpur	Lentil	Farmers' practice	3	4	0.74	
		NPK	5	6	1.20	62
		PK + rhizobia	15	19	1.27	72
		Rhizobia	12	12	1.08	46
Faridpur	Lathyrus	Farmers' practice	6	6	0.95	
		NPK	6	8	1.62	71
		PK + rhizobia	23	26	1.75	84
		Rhizobia	20	22	1.25	32
Pabna	Lathyrus	Farmers' practice	6	6	0,82	
		NPK	6	7	1.40	71
		PK + rhizobia	20	22	1.52	85
		Rhizobia	17	18	1.10	34
Kishoregonj (inl990)	Groundnut	Farmers' practice	62	67	1.60	
		NPK	84	80	2.72	70
		PK + rhizobia	120	140	2.79	74
		Rhizobia	109	106	2.15	34

[1] One farmer was selected for the trial at each location.

chemical and biological properties. After inoculation with effective rhizobial strains, the number of nodules plant^{1} was generally 2 to 4 times higher than in the noninoculated treatments (Table 2). The number and mass of nodules of lentil, chickpea, and groundnut also increased with rhizobial inoculation in research station trials (Khanam et al. in press, a; Rahman et al. 1992). The highest nodule number, nodule mass, and grain yield due to rhizobial inoculation was obtained in the presence of P and K. There was a 30 to 46% increase in yield due to rhizobial inoculation compared to farmers' practice (control). However, the yield of different legumes with rhizobial application alone was generally lower than that of the PK + rhizobia treatment (Table 2). Farmers were interested in using rhizobial inoculum for different legumes after they had observed its yield benefits.

Constraints to Using Rhizobial Inoculants

Bangladesh produces legumes on 0.5 m ha on which no chemical fertilizers and rhizobial inocula are used (Bangladesh Bureau of Statistics 1991). About 2000 t of inocula would be required annually for leguminous crops grown on this area. The present annual production of inoculants in Bangladesh is estimated to be about 1.5 t. Data from research stations (Khanam et al. in press a, b; Rahman et al. 1992) and farmers' fields suggest that there is considerable scope to increase yield by means of rhizobial inoculation. However, the following constraints limit the use of inoculants by farmers:

- There is a great need for trained manpower and facilities to produce the required amount of effective inoculum and maintain its quality.
- Until recently there were no commercial producers of inoculants.
- There is no established system for training extension workers and farmers in the use of rhizobial inoculants.

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Use of Rhizobial Inoculants: On-farm Experience in Nepal

Shanti Bhattarai[1]

Introduction

About 90% of Nepal's population depends on agriculture for its livelihood. Of the 3.13 million ha of cultivated area, legumes occupy 268 000 ha. Soybean, cowpea, black gram, beans, and peas are the main leguminous crops in the hill region, while lentil, chickpea, and pigeonpea are the important crops of the Terai region (the plains). Lentil and chickpea are grown as a relay crop with rice with few inputs during winter (Oct-Mar) in the Terai. Though there is scope to improve legume yields by Rhizobium inoculation, the on-farm studies on this aspect are limited.

Native Rhizobial Population

The presence of adequate numbers of appropriate rhizobia in the soil is one of the prerequisites for optimum nodulation in legumes. The Rhizobium population is usually estimated by the serial dilution/plant infection method. Assessing the nodulation status of legumes by careful uprooting may be an alternative method despite of the fact that legume nodulation can be influenced by biotic and abiotic factors. The Rhizobium population, as assessed by the nodulation status of soybean in research farms and farmers' fields (Table 1) and of chickpea in farmers' fields (Table 2) was generally low. Similarly, nodulation in groundnut in some farmers' fields in Sarlahi, Nawalparasi, and Chitwan districts of Nepal was also reported to be low, with nodule number plant^{1} ranging from 30 to 80 (J V D K Kumar Rao and G V Ranga Rao, ICRISAT Asia Center, personal communication 1991). These observations indicate that there is scope to improve nodulation and nitrogen fixation in legumes through rhizobial inoculation.

Response to Rhizobium Inoculation

Effective Rhizobium and Bradyrhizobium strains have been identified for lentil, chickpea, groundnut, and soybean based on laboratory and greenhouse tests (data not presented) (Bhattarai 1992). Bhattarai and Shrestha (1989) reported that

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Table 1. Soybean nodulation[1] at different research farms and farmers' fields surveyed in Nepal, rainy season 1988/89.

Location	Number of nodules $plant^{-1}$ Effective	Ineffective	Total	Remarks
Bhairahawa				
Agricultural farm	3	8	11	Most of the nodules
Farmer 1	3	9	12	were not effective
Farmer 2	7	20	27	
Farmer 3	4	17	21	
Nepalgunj				
Agricultural station	1	2	3	The number of nodules
Farmer 1	1	2	3	$plant^{-1}$ was less than on
Farmer 2	1	2	3	other farms
Farmer 3	1	1	2	
Rampur				
Agricultural center	42	13	55	The number of nodules
Farmer 1	10	5	15	$plant^{-1}$ was higher in
Farmer 2	16	33	49	plants inoculated with
Farmer 3	12	39	51	Rhizobium
Farmer 4	24	13	37	
Parwanipur				
Agricultural center	17	26	43	Though the number of
Farmer 1	8	13	21	nodules was lower,
Farmer 2	22	6	28	most of them were
Farmer 3	4	8	12	effective
Tarahara				
Agricultural center				Both the number and
Inoculated	75	39	114	effectiveness of the
Noninoculated	18	10	28	nodules were higher in
Farmer 1	9	5	14	plants inoculated with
Farmer 2	9	14	23	Rhizobium
Farmer 3	3	11	14	

[1] Average of 4 plants $field^{-1}$.

Source NARC (1990).

inoculation with effective *Rhizobium* strains increased yields of soybean (15-62%), groundnut (16-34%), lentil (13-25%), black gram (49%), and broad bean (67%) over the noninoculated controls on research stations (Table 3). *Rhizobium* inoculation was reported to increase groundnut pod yields in farmers' fields at Babarganj (11%), Laukat (33%), and Piparpati (9%) during the 1991 rainy season (Sharma and Koirala 1993).

Table 2. Chickpea nodulation and rhizobial population in farmers' fields in Nepal, 1987/88.

Location	Soil pH	MPN[1] count of chickpea rhizobia g^{-1} dry soil	Nodulation rating[2]
Dang	7.6	2.15×10^5	4
Rupendahi	6.1	9.08×10^4	3
Nawalparasi	7.1	Less than 10	1
Bara	5.1	4.334×10^2	2
Bagmati	6.1	2.01×10^4	3
Chitwan	5.7	10.96×10^5	3

1. MPN = Most probable number.
2. Nodulation rating was scored on a 1-5 scale, in which 1 = minimum nodulation, 5 = maximum nodulation.

Source: O.P. Rupela and S. Bhattarai, personal communication.

Table 3. Effect of *Rhizobium* inoculation on different legumes grown on research farms in Nepal.

Crop	Year	Location	Yield ($kg\ ha^{-1}$) Noninoculated control	Inoculated	Increase in yield due to *Rhizobium* inoculation (%)
Soybean	1972	Khumaltar	3388	3962	17
	1976	Kakani	600	690	15
	1976	Lumle	1133	1822	62
	1987	Khumaltar	4340	5040	16
	1987	Khumaltar	1166	1726	48
Lentil	1976	Parwanipur	594	731	12
	1976	Khumaltar	2125	2725	25
Black gram	1987	Khumaltar	275	410	49
Groundnut	1978	Sarlahi	1097	1997	33
	1979	Nepalgunj	789	920	16
	1983	Trisuli	1034	1383	33
Broad bean	1986	Khumaltar	375	628	67

Source: Bhattarai and Shrestha (1989).

Constraints to Adoption of Rhizobium Technology by Farmers

- There is a demand for rhizobial inoculants in Nepal. However, there is no commercial production of inoculants in the country.
- A central *Rhizobium* culture-collection facility is needed to maintain and supply tested cultures to farmers and commercial enterprises.
- Lack of training in the use of biofertilizers for legumes other than chickpea and lentil to the personnel of the Department of Agriculture which is responsible for transfer of technology, particularly in major grain legume-growing districts of the country.

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Use of Rhizobial Inoculants: On-farm Experience in Thailand

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Introduction

Soybean, groundnut, and mung bean are the most important legumes in Thailand. While soybean and groundnut are produced mainly for domestic consumption, mung bean is exported as well. During the last decade, the average yields of the three crops have been increasing. Realizing the potential of rhizobial inoculants to increase crop yields at a lower cost than that of nitrogenous fertilizers, the Government of Thailand is promoting research on legume BNF technology and its extension to resource-poor farmers.

Organizations Involved in Legume BNF Technology Research and Extension

In Thailand, research in legume BNF technology is the main responsibility of the Soil Microbiology Group of the Division of Soil Science, Department of Agriculture (DOA), Ministry of Agriculture and Co-operatives (MOAC). Research is also carried out by Khon Kaen, Kasetsart, and Chiang-mai Universities.

The DOA is also responsible for the production of rhizobial inoculants. The Department of Agricultural Extension (DOAE) administers the distribution and promotion of the inoculants through training, on-farm trials, field days, and cooperative agreements with the private sector.

Research on Legume BNF in Thailand

Research activities on BNF in important legumes can be broadly grouped under:

- Rhizobium strain selection for released cultivars
- Inoculation trials under field conditions
- Methods and rates of inoculation

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- Ecology of Rhizobium
- Measuring the amount of N_2 fixed using the ^{15}N isotope dilution technique under field conditions
- Residual N benefit to succeeding crops.

Soybean. In studies on the ecology of soybean Rhizobium, it was found that the rhizobial population varied from season to season. Hot and dry conditions during the summer months decreased the population drastically. The population also varied with location (Table 1) and cropping history (Table 2). It was low in fields in which soybean had not been grown previously and high where it had. This was particularly so in northeastern Thailand, where soybean has recently been introduced.

Consistent responses to Rhizobium inoculation (up to 87% increase in yield) have been observed under field conditions, especially in northeastern Thailand. Rhizobial inoculation was found to have increased seed yield in soybean equivalent to what might have been achieved with the application of N fertilizer at the rate of 75-150 kg N ha^{1} (Vasuvat 1976). Liming is also important to achieve higher seed yields. The effects of different stickers for seed inoculation have also been studied. Some locally available stickers such as syrup, tapioca starch, rice starch, vegetable oil, etc., are recommended for seed inoculation. Soil inoculation, using either water or soil as spreading agents, seems to be superior to seed inoculation (Toomsan 1987). The presently recommended rate of inoculation is 200 g peat inoculant 10 kg^{*1} seed. However, this rate can be reduced by half without a significant decrease in yield.

Table 1. Population ($\log_{10}$ MPN) of soybean and groundnut rhizobia in soils of different provinces in northeastern Thailand.

Province	Number of samples	Population			
		Soybean		Groundnut	
		Range	Mean	Range	Mean
Chaiyaphoom	2	1.28-6.31	3.82	6.30-6.36	6.33
Loei	14	<0.32-6.34	3.28	<0.32-6.71	3.43
Khon Kaen	20	<0.28-7.35	3.41	<0.61-7.35	4.66
Mahasarakarm	5	<0.28-0.98	0.49	<0.28-2.98	1.16
Roi-Et	10	0.60-4.63	2.31	0.60-4.25	2.29
Ubol	8	<0.24-6.94	2.43	<0.24-7.26	3.55
Kalasin	9	<0.28-1.99	0.81	0.28-4.28	1.44
Sakolnakorn	10	<0.27-4.99	3.39	2.61-6.98	3.97
Mukdahan	9	<0.26-3.61	1.17	<0.28-5.65	2.89
Surin	9	<0.26-4.64	2.03	1.28-6.01	4.12
Srisaket	6	0.64-4.64	1.89	2.04-6.00	4.27

Source Toomsan (1990).

Table 2. Effect of crop history on population ($\log_{10}$ MPN) of soybean and groundnut rhizobia in soil samples taken from different provinces in northeastern Thailand.

Crop history	Number of samples	Population			
		Soybean		Groundnut	
		Range	Mean	Range	Mean
Standing legumes					
- cowpea group	6	<0.26-1.28	0.67	2.27-6.30	4.91
- soybean	19	1.60-7.35	5.55	2.62-7.35	5.34
Standing nonlegumes					
- without soybean history	7	<0.26-0.97	0.48	1.28-4.65	2.72
- with soybean history	9	<0.28-4.92	2.66	<0.28-6.98	3.56
Bare soil					
- without soybean history	9	<0.24-1.27	0.61	<0.24-6.01	2.99
- with soybean history	38	<0.28-6.95	2.28	<0.28-7.26	2.54

Source: Toomsan (1990).

The amount of nitrogen fixed, as determined by the ^{15}N isotope dilution technique, ranged from 32 to 161 kg N ha*^{1} depending on the *Rhizobium* strain, the soybean cultivar, the location, and the management practices adopted (Kucey et al. 1988a, b). In studies on the effect of residual N benefit from soybean, the seed yield of maize was found to be lower when it was grown after soybean than when it was grown after N_2-fixing groundnuts (Table 3).

Table 3. Seed and total dry matter yields (kg ha^{-1}) of maize grown after different legumes and maize on a Yasothorn soil (Yt soil series, Paleustult), Khon Kaen University Farm, 1992/93.

Crop grown before maize	Seed	Total dry matter
Soybean cv SJ 4	2215	4634
Multipurpose cowpea	2475	5765
Stakeless yard long bean	1940	4169
Nod^{-1} groundnut	2096	5127
Groundnut cv KK 60-1	4050	9490
Groundnut cv KK 60-3	4543	10180
Maize cv Suwan 2	1530	3357
LSD (.05)	768	1593
CV (%)	19	18

Groundnut The ecology of groundnut Rhizobium (cowpea group) varies from season to season. Waterlogging does not drastically reduce the rhizobial population as much as hot and dry conditions during the summer. The groundnut Rhizobium population also varies with location (Table 1) and cropping history (Table 2). It was found that the population is high in fields with standing legumes.

Rhizobium strains have been selected for the groundnut cultivars presently recommended in Thailand. Under greenhouse conditions, Rhizobium inoculation was found to give good results, but under field conditions it rarely resulted in significant increases in pod yield (Toomsan 1987). Inoculation may increase nodule number, nodule dry mass, and nitrogenase activity but it rarely increases stover dry mass and pod yield (Toomsan 1990).

Studies of inoculation methods indicate that soil inoculation may be better than seed inoculation. The present recommended rate for groundnut Rhizobium is 200 g peat inoculant 12 kg^{-1} of seed.

The amount of nitrogen fixed by groundnut, as determined by the ^{15}N isotope dilution technique, was found to range between 100 and 150 kg N ha^{-1} depending on the cultivar. It was found that groundnut fixed more N_2 than the amount removed in pod yield. Groundnut can help increase soil N provided the stover is returned to the soil. This can help increase the yield of succeeding crops (Table 3).

Mung bean. Compared with soybean and groundnut, there have been only a few BNF studies in mung bean. Selection of strains suitable for mung bean has been made (Wadisirisuk et al. 1988). However, the response to Rhizobium inoculation under field conditions has been inconsistent. Selection of mung bean lines for high N_2-fixing ability has also been carried out (Siripin 1992).

Green-manuring Legumes

In recent years, the focus of research on BNF in green-manuring legumes in Thailand has shifted from sunn hemp (Crotalaria juncea) to species of Sesbania and Aeschynomene. Most of the attention has been given to exotic species, especially Sesbania rostrata, which performed best at sites with adequate moisture. A basal dose of 72 kg P ha^{-1} as rock phosphate, and inoculation with effective strains of Azorhizobium caulinodans was found to result in rapid growth and high herbage yield. The growth of S. rostrata can be severely affected by drought.

Recently, an attempt was made by the Khon Kaen University's BNF research group to study the feasibility of utilizing native nodulating leguminous weeds **such as Aeschynomene americana, Crotalaria striata, and Mimosa pudica as green** manure. The results are very promising.

Production of Rhizobium Inoculant

The Department of Agriculture has the capacity to produce 200 t of Rhizobium inoculant per year. However, it produced only 134 t in 1989 and 126 t in 1990

Table 4. Rhizobium inoculant production by the Department of Agriculture (DOA), and distribution through the Department of Agricultural Extension (DOAE), the private sector (PS), and Marketing of Farmer Organization (MFO), 1977-90.

Year	Inoculant produced (t)	Number of bags of inoculant distributed		
		DOAE	PS	MFO
1977	5.00	6 950	-[1]	9 865
1978	10.59	17 430	-	40 323
1979	7.42	22 296	-	6548
1980	4.92	16 761	-	11429
1981	7.48	26 649	-	10 164
1982	6.58	20 877	8 584	3 763
1983	14.36	34 557	30 079	1 104
1984	36.16	112 073	56 885	-
1985	48.77	157 323	75 264	-
1986	78.00	285 796	88 115	-
1987	81.63	248 595	150 378	-
1988	140.70	593 941	90 237	-
1989	134.27	557 527	68 996	-
1990	126.35	557 772	30 578	-

1. Trace or nil.

Source: Chanaseni and Kongngoen (1992).

(Table 4). Private companies produced only 20 t per year. The Rhizobium inoculants produced by the Department of Agriculture were distributed mainly through the DOAE (Table 4). They are sold along with seed to farmers at the rate of 10 baht (US $ 0.40) bag^{-1} of 200 g of peat inoculant. The farmers have to buy 1 bag of inoculant per 10 kg of seed purchased. Such efforts have led to an increase in the percentage of crops inoculated with rhizobia (Table 5). Between 1977 and 1990, there has been an approximately 35-fold increase in yearly legume inoculant use.

Table 5. Monitoring and evaluation report (MER) on the use of rhizobial inoculant, Thailand, 1986/87-1989/90.

	Crop area inoculated with rhizobia (%)			
Crop	1986/87	1987/88	1988/89	1989/90
Soybean	30.3	44.8	52.4	50.9
Groundnut	9.8	12.3	17.4	22.0

Source: Chanaseni and Kongngoen (1992).

Constraints to Adoption of BNF Technology

The adoption of legume BNF technology by farmers is hampered by the following factors:

Shortage of Rhizobium inoculant. Although inoculant production has increased tremendously in the last 10 years in Thailand, it still does not meet the demand. Moreover, inoculants are not available off the shelf as readily as chemical fertilizers and pesticides. The need to keep the inoculants in cold storage also limits their distribution and use.

Lack of a simple technology of inoculation. Farmers tend to prefer a technology that is simple and less labor-intensive to one that is relatively sophisticated. Though soil inoculation is superior to seed inoculation, it is not likely to be accepted by farmers because it requires more labor to open the furrow, mix and spread the inoculant, sow the seed, and close the furrow again.

Inconsistent field responses to rhizobial inoculation. This is particularly true in the case of legumes like groundnut and mung bean that nodulate with cowpea-type Rhizobium. The lack of a clear response to inoculation has also been noticed in a field with a long history of soybean cropping. This is especially so in northern Thailand, where soybean has been grown for a long time.

Lack of an immediate cash income and price incentive. Though green-manure legumes are known to increase soil fertility, they are not readily accepted by resource-poor farmers because they do not generate an immediate cash income. Furthermore, a green-manure crop like S. rostrata requires a cash input in terms of phosphorus fertilizer, and sometimes, Rhizobium inoculation. There are also considerable difficulties in cutting and plowing under the green-manure residue.

The price of the produce is also a criterion in the adoption of BNF technology. If the price is high, the technology is likely to be accepted readily. In the case of most economic legumes, e.g., soybean, groundnut, and mung bean, the price is of utmost importance. For instance, many soybean growers in Nong-Kai province have recently shifted to banana. This is not because of lack of knowhow. Most of them do know that legumes nodulate and that inoculation enhances nodulation as well as yield. The main reason for the change is that the farmers find that soybean is not profitable enough.

The authors feel that the adoption of BNF technology both at the national and global levels can be achieved only if farmers change from other crops to legumes. Therefore, governments should set up national legume production plans and guarantee good prices to farmers.

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Application and Adoption of Rhizobial Inoculation in Chickpea: On-farm Experience in Madhya Pradesh, India

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Introduction

Chickpea (Cicer arietinum) is an important pulse crop in Madhya Pradesh state, India, where it occupied an area of 2.48 million ha in 1990/91. Annual production was 1.93 million t in 1990/91 but productivity was only 779 kg ha^{-1}. The state containes about 33% of the total area under chickpea and produces about 37% of the total production in the country.

Studies conducted in different parts of the country during the past two decades under the All India Coordinated Pulses Improvement Project (AICPIP) of the Indian Council of Agricultural Research (ICAR) have shown that inoculation with efficient strains of Rhizobium increased yield of grain legumes (Rewari 1985). A significant improvement in yield and biological nitrogen fixation due to Rhizobium inoculation has been reported in chickpea (Khurana and Dudeja 1981, Raut and Ghonsikar 1982, Namdeo et al. 1989). Efficient N_2-fixing Rhizobium strains specific to chickpea, e.g., F 75, IC 76, and H 45, have been identified by AICPIP. However, there is inadequate data on the impact of their use on chickpea grain yield in farmers' fields. Therefore, a survey was conducted to demonstrate the usefulness of Rhizobium inoculants on chickpea in farmers' fields and to identify areas in the Vindhyan plateau of Madhya Pradesh that require such technology.

Physiography, Soil, and Climate

Madhya Pradesh lies between latitudes 17-26° and longitudes 74-84° and has a mean annual rainfall of 1143 mm. The Vindhyan plateau is one of the six physiographic regions of the state, which includes Bhopal, Sehore, Raisen, Vidisha, Sagar, Damoh, and Guna districts, and has mainly medium black soils.

In 38 of the 45 districts of Madhya Pradesh, the available N status of the soil (measured by the alkaline permanganate method which includes mineralizable N) was reported to be low (Figure 1). In general, the status of available P was low to medium, and that of available K medium to high (Gupta et al. 1986). Chickpea Rhizobium population in soils of the Vindhyan plateau in the plow layer (top 15 cm) generally ranged from 10^2 to 10^3 g^{-1} soil. This indicates that the soil of this

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Figure 1. Available N (mineral and mineralizable N) in the different districts of Madhya Pradesh.

Source: Gupta et al. 1986.

region is poor in chemical fertility as well as biofertility. This suggests that use of Rhizobium inoculation technology would yield good results in chickpea in this area.

Survey of Nodulation in Chickpea

The nodulation survey of chickpea was carried out from 1988/89 to 1992/93 in 79 villages in Sehore, Raisen, and Vidisha districts. In all, 135 fields were surveyed. Ten 45 to 60-day-old plants were selected randomly from each location, carefully uprooted with a pickaxe, and observed for nodule number plant^{1}.

Of the 135 fields surveyed, 106 showed poor nodulation (79%), 26 moderate (19.3%), and only 3 had good nodulation (2.2%) (Table 1). Several researchers have reported poor nodulation of grain legumes by native rhizobia.

Table 1. Survey of nodulation status[1] in chickpea in farmers' fields in villages of the Vindhyan plateau, Madhya Pradesh, India, 1988/89-1992/93.

		Number of fields with		
Year	Number of fields surveyed	Poor nodulation	Moderate nodulation	Good nodulation
1988/89	12 (12)[2]	8	4	
1989/90	15 (15)	13	2	
1990/91	44 (19)	38	6	
1991/92	35 (16)	26	9	
1992/93	29 (17)	21	5	3
Total	135	106	26	3

1. Nodulation status: Poor = 0-10 nodules plant^{-1}; moderate = 11-20 nodules plant^{-1}; good = 21-30 nodules plant^{-1}.
2. Figures in parentheses are number of villages represented.

Performance of Chickpea Rhizobium Inoculant in Farmers' Fields

Demonstration trials were conducted from 1989/90 to 1992/93 in farmers' fields that had shown poor nodulation in the preceding years. Sixteen trials, each covering an area of 2000 m^{2}, were laid out with two simple treatments: noninoculated control and Rhizobium inoculation with strain IC 76. These nonreplicated trials were conducted with cultivar JG 315 or JG 74 under rainfed conditions with a basal fertilizer dosage of 40 kg P_2O_5 ha^{-1} as single superphosphate.

Data on grain yield (Table 2) revealed that the inoculation treatment gave 18-24% higher yield than the noninoculated control. This response may have been due to the low chickpea rhizobial population, poor nodulation, the low available

Table 2. Performance of chickpea Rhizobium inoculant of strain IC 76 in farmers' field trials, 1989/90 to 1992/93[1].

		Grain yield (kg ha^{-1})		Increase in yield over
Year/trial no.	Village	Control	Inoculated	control (%)
				90
1989/90				
1	Khajuri	605	730	21
2	Khajuri	640	795	24
3	Phanda	625	765	22
4	Thoona	655	780	19
1990/91				
1	Jakakhedi	635	765	21
2	Jharkheda	600	740	23
3	Khajuri-Bangla	650	775	19
4	Pachama	615	750	22
1991/92				
1	Rafiq-ganj	625	760	22
2	Gurbhela	655	775	18
3	Naplakhedi	630	755	20
4	Sonda	610	745	22
1992/93				
1	Semlikhurd	655	780	19
2	Hasnabad	630	770	22
3	Phanda	665	800	20
4	Pachama	650	765	18

1. Chickpea cultivar JG 315 was used everywhere except in 1992/93 when JG 74 was used. Size of each treatment plot at each location was 1000 m^2.

N and P status of the soils, and the use of a more efficient rhizobial strain. Similar results have been reported by Chandra and Ali (1986). Generally, small gains from inoculation have been reported in areas having a rhizobial population of more than 10^3 g^{-1} soil (Rupela and Saxena 1987).

Adoption of Rhizobial Inoculation Technology

Biofertilizers are at present being manufactured in India by about 60 units operated by central and state governments, agricultural universities, and private companies. In Madhya Pradesh, Nafed, Indore; Madhya Pradesh Agro Industries, Bhopal; Oilfed, Dhar; and the Regional Biofertilizers Development Center, Jabalpur, are the major producers of rhizobial inoculants. The chickpea area receiving inoculation in the state increased from 2.4% in 1987/88 to 11.0% in 1990/91

(Table 3). This indicates a steady increase in the acceptance of the technology by farmers. Considering the large area under chickpea and the limited use of Rhizobium inoculation technology, there is a tremendous scope for the development and adoption of this technology in Madhya Pradesh.

Table 3. Adoption of rhizobial inoculation technology in Madhya Pradesh.

Year	Area under chickpea ('000 ha)	Total number of inoculant packets supplied by leading procedures[1]	Chickpea area receiving inoculationt (%)
1987/88	2236	167 975	2.40
1988/89	2234	135 150	1.94
1989/90	2130	489 080	7.35
1990/91	2482	853 114	11.00

1. One inoculant packet can coat seed sufficient for one acre (= 0.405 ha) area.

Source: Directorate of Agriculture, Government of Madhya Pradesh, Bhopal (MP), unpublished.

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Field Response of Groundnut to Bradyrhizobium Inoculation

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Introduction

Groundnut is a major oilseed crop of India where it accounts for 45% of the area under oilseed crops, and 55% of the oilseed production. Poor nitrogen fixation is one of the major reasons for the low yield of groundnut (Kulkarni and Joshi 1988). Groundnut is nodulated by strains of Bradyrhizobium which are abundant in Indian soils (Nambiar 1988, Joshi et al. 1989). But these strains differ greatly in their N_2-fixing ability and competitiveness to form nodules, resulting in varying responses to rhizobial inoculation under field conditions (Nambiar 1988, Joshi et al. 1989). Biological nitrogen fixation in groundnut is affected by various agroecological factors including native strains of Bradyrhizobium, temperature, soil moisture, soil fertility, pesticides, and agronomic practices under farm conditions. The effects of all of these factors on BNF of groundnut in real farm situations, and the problems encountered in the implementation of BNF technology are discussed in this paper.

Nodulation Status of Farmers' Fields

Nodulation surveys of farmers' fields in India indicated that groundnut crops grown in the red soils of the Rayalaseema region in the state of Andhra Pradesh had poorer nodulation than those grown in the medium-black soils of the Dharwad and Raichur districts of the state of Karnataka, and Parbhani and Latur districts of the state of Maharashtra. Poor nodulation was observed in the state of West Bengal (P K Joshi, personal communication) and in the coastal plain of the Saurashtra region, while the medium-black calcareous soils of Junagadh, Rajkot, and Amreli districts of Gujarat state supported adequate nodulation (Kulkarni and Joshi 1988). Similarly, poor nodulation of groundnut was observed in 52 farmers' fields out of 96 surveyed in southern India (Nambiar 1988). In areas where there is poor nodulation of groundnut, there is a possibility of getting a good response to inoculation with Bradyrhizobium.

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Crop Response to Inoculation

In virgin or cultivated fields inoculating groundnut with the effective Bradyrhizobiutn strain NC 92 increased pod yield in several field trials (Nambiar 1985, Joshi et al. 1989). Based on its performance in various trials conducted at different locations (Table 1), this strain was recommended for inoculating groundnut cultivars Kadiri 3 and JL 24 during the rainy season (AICORPO 1983). On-farm trials conducted in nontraditional groundnut-growing areas in West Bengal and in the traditional groundnut-growing areas of Karnataka showed a 6.6% to 36.8% increase in pod yield due to inoculation with rhizobial strains IGR 6 and IGR 40 (Table 2). In such areas, the crop response to Bradyrhizobium inoculation varied from no response (Nambiar 1985, Subba Rao 1976) to significant increases in pod yield (Nambiar 1985, Joshi et al. 1989).

Table 1. Increase in pod yield of groundnut (cv Kadiri 3) inoculated with Bradyrhizobium strain NC 92.

Location	State	Increase in pod yield over noninoculated control (%)
Ludhiana[1]	Punjab	8
Durgapura[1]	Rajasthan	28
Junagadh[1]	Gujarat	40
Jalgaon[1]	Maharashtra	23
Dharwad[2]	Karnataka	29
ICRISAT[3]	Andhra Pradesh	16
Rajendranagar[2]	Andhra Pradesh	12

1. Mean of two trials.
2. One-season trial.
3. Mean of seven seasons' trials.

Source: AICORPO 1983.

Constraints Affecting Nodulation and Nitrogen Fixation

Native population of Bradyrhizobiutn. Bradyrhizobiwn population in groundnut-growing areas in India ranged from 10^2 to 10^6 cells g^{-1} dry soil (Nambiar 1988, Nambiar et al 1988, Joshi et al. 1989). However, paddy fallows contained zero to low populations of Bradyrhizobiutn (Nambiar et al. 1988). Many of the native strains of Bradyrhizobium are not effective in nodulation. Development and introduction of effective and competitive strains should thus improve BNF in groundnut.

Temperature. Nodulation and nitrogen fixation in groundnut is better in the rainy season than in the postrainy/summer seasons. In the rainy season, the

Table 2. Effect of Bradyrhizobium inoculation on groundnut yield[1] (kg ha^{-1}) in farmers' fields in West Bengal (rainy season, 1988) and in Karnataka (postrainy season, 1990), India.

	Strains		
Location	IGR6	IGR40	Control
West Bengal			
24-parganas (I)	1923 (16.4)[2]	1888 (14.2)	1653
24-parganas (II)	1594 (14.2)	1588 (13.7)	1396
Hooghly	1650 (11.9)	1600 (8.5)	1475
Nadia	1775 (14.7)	1695 (9.5)	1548
Karnataka			
Hagari Bommanahalli	730 (9.9)	821 (23.5)	665
Bellary (I)	400 (6.7)	400 (6.7)	375
Bellary (II)	1045 (6.6)	1120 (14.3)	980
Hospet	756 (36.8)	606 (23.7)	548
Harrapanahalli	896 (25.7)	880 (23.4)	713

1. Nonreplicated demonstration plots on farmers' fields.
2. Figures in parentheses are percentage increase in pod yield over control.

smaller variations in diurnal temperature (25-35°C) contribute to high nodulation on the hypocotyl and roots of groundnut, whereas the wide variations in diurnal temperature in summer (28-45°C) and in winter (9-37°C) lead to low nodulation and nitrogen fixation (NRCG 1985). This indicates the need to develop strains of *Bra*dyrhizobium and host cultivars that are tolerant to both high and low temperatures. The adverse effect of low temperature on nodulation in groundnut in the postrainy season can be partly overcome by the use of polythene mulching (Joshi et al 1992).

Moisture. In India, rainfed groundnut often suffers from drought stress during different stages of crop growth, this adversely affects nodulation and BNF. Drought stress during the vegetative or flowering or pod-filling stages inevitably retards nodulation, leaf area, dry-matter production, and N uptake (Kulkarni et al. 1988). Drought stress is often coupled with increased temperature. Therefore, there is a need to identify strains of *Bradyrhizobium* and host cultivars that can perform well under drought stress and high temperature in terms of nodulation, nitrogen fixation, and pod yield.

Soil fertility. The physical and chemical properties of different soils affect nodulation and BNF in groundnut (Nambiar 1988). High levels of soil nitrogen adversely affect nitrogen fixation (Joshi and Kulkarni 1984). Poor nitrogen fixation in the medium-black soils of Jalgaon in Maharashtra and Junagadh in Gujarat has been attributed to low available phosphorus, zinc, and iron content and high pH and calcium carbonate (Kulkarni and Joshi 1988). Soil acidity resulted in manganese and aluminium toxicities which affected nitrogen fixation (Nambiar 1988). For higher nitrogen fixation in groundnut, optimum soil fertility must be ensured.

Effect of fungicides. Seed treatment with fungicides adversely affected nodulation, nitrogen fixation, and survival of *Bradyrhizobium* (Nambiar 1985). To overcome such constraints, such alternative methods of inoculation as liquid inoculation (Nambiar 1985) and use of inoculants mixed with compost (NRCG 1983) have been suggested.

Agronomic Practices

Intercropping. Intercropping cereals and legumes is a common practice followed by Indian farmers. Tall-growing cereal intercrops like pearl millet and maize reduced nodulation, nitrogen fixation, and pod yield of groundnut, mainly due to reduced photosynthesis because of shading (Nambiar et al. 1983).

Plant population. Farmers in India sow groundnut at a higher seed rate than is recommended. This results in a high plant population which affects nitrogen fixation. The optimum plant population for high nodulation and nitrogen fixation is 222 000 for Virginia and 160 000 plants ha^{*1} for Spanish bunch varieties (NRCG 1986).

Depth of sowing. Sometimes groundnut is sown deep in the soil to exploit moisture at deeper layers. This results in the development of an elongated hypocotyl, poor rooting, and poor nodulation and nitrogen fixation, especially in Spanish bunch varieties (Nambiar 1988).

Sowing on broadbed-and-furrow system. Higher nodulation was observed in groundnut sown in a broadbed-and-furrow system than in a flatbed system at two locations in Maharashtra (Pawar et al. 1993).

Problems in Application of Technology

Although effective strains of Bradyrhizobium have been identified, the non-availability of inoculants of good quality to farmers is one of the major constraints in developing countries (Nambiar 1988). Another major problem lies in convincing farmers about the effect of inoculation on yield. The lack of visible differences between inoculated and noninoculated plots and the wide fluctuations in pod yield in noninoculated plots make the task more difficult. An awareness needs to be created among farmers, through various extension agencies, of the benefits of BNF technology.

Adoption of BNF technology. Data on the production of inoculants of some effective strains of Bradyrhizobium in India is given in Table 3. This gives an estimation of the adoption of available BNF technology by farmers. However, data on the production of inoculants of other effective strains of Bradyrhizobium is not available. Biological nitrogen fixation technology is poorly adopted by farmers because of the reasons stated above.

Table 3. Production of Bradyrhizobium inoculants (strains IGR 6[1] and IGR 40) for groundnut in India.

Agency	Year	Production	Supplied to
Bharatiya Agro Industries Foundation, Pune	1990	85 000 packets	Government of Maharashtra
Micro BAC India, West Bengal		200 liters[2]	Farmers of West Bengal and Orissa
Madhya Pradesh Agro Industries Corporation Ltd., Bhopal	1988	6 000 packets	Farmers of Madhya Pradesh
	1989	55 400 packets	
	1990	28 900 packets	
	1991	35 500 packets	
Eastern Enterprises, Calcutta	1991	2-3 t	Government of West Bengal
Maharashtra Agro Industries Development Corporation Ltd., Bombay	1991/92	155 600 packets	Government of Maharashtra and farmers
National Federation of Agricultural Cooperatives, Indore		100 000 packets	Farmers of Maharashtra, Karnataka, and Tamil Nadu

1. Information was provided by the listed agencies. Information on actual use of inoculants by farmers was not available.
2. Production of IGR 6.

Future Research

In view of the problems encountered in implementing BNF technology in groundnut, the following aspects of the problem need the attention of researchers:

- Development of more efficient and competitive strains of Bradyrhizobium tolerant to biotic and abiotic stresses.
- Identification of host cultivars that are tolerant to abiotic stresses such as temperature, drought, etc.
- Identification and development of appropriate cropping patterns and agronomic practices including the correction of nutrient deficiencies observed in groundnut fields.
- Ensuring the quality of inoculants produced by different agencies at different stages like transportation and storage.
- Detailed studies on the persistence of inoculant strains in the field.

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On-farm Experience in the Use of Rhizobial Inoculants on Pigeonpea in India

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Introduction

Pigeonpea (Cajanus cajan) is an important grain legume crop of the Indian subcontinent. The area under pigeonpea in India has increased from 2.73 m ha in 1979/80 to 3.72 m ha in 1991/92, an increase of 36% (DPR 1993). In the state of Haryana it increased from 31 000 ha during 1966/67 to about 55 000 ha in 1991/92. Haryana is situated in northwestern India between latitudes 27°10' N and 30°55' N. It has a subtropical, semi-arid, and monsoonal climate. The maximum temperature reaches 49°C during May-June and the minimum temperature falls to 3°C in January. The average annual rainfall is less than 300 mm in the southwestern region of the state and more than 1000 mm in the northeastern region. The rainfall is highly erratic and over 70% of it occurs between July and September.

Since pigeonpea is grown mostly on marginal soils in India, Rhizobium inoculation could be one way of improving yields. Rewari et al. (1981) report that Rhizobium inoculation increased pigeonpea grain yield under field conditions. However, responses to inoculation were not consistent, probably because various biotic and abiotic factors influence the symbiosis between the Rhizobium and the host plant under field conditions. The major abiotic factors that influence the interaction are mineral N, available P, temperature, and moisture. The biotic factors include the native rhizobial population and the competitiveness of the inoculant strain. This paper summarizes the results of studies on pigeonpea response to Rhizobium inoculation in farmers' fields, at different locations in India, the likely reasons for the inconsistent responses to inoculation, and the constraints to the adoption of this technology.

Response to *Rhizobium* Inoculation in On-farm Trials

The response of pigeonpea to *Rhizobium* inoculation has been studied in farmers' fields in India since 1978/79 as part of the All India Coordinated Pulses Improvement Project (AICPIP). A positive response was observed in these trials, with increases in grain yield in the inoculated treatment over the noninoculated control ranging from 4% to 25% (Table 1). However, Kumar Rao et al. (1984) found

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Table 1. Response of pigeonpea to Rhizobium inoculation at different locations in India, 1978/79-1992/93[1].

			Grain yield (kg ha^{-1})		Increase over control
Year	Center	Locations	Noninoculated	Inoculated	(%)
1978/79	Hisar		1000	1200	20.0
1979/80	Hisar		1000	1100	10.0
1980/81	Hisar		1050	1150	9.5
1987/88	Bangalore		1030	1160	13.0
	Gulbarga		1295	1485	15.0
	Sehore	1	410	500	22.0
		2	485	570	18.0
		3	450	545	21.0
1990/91	Badnapur	1	402	466	16.0
		2	560	610	9.0
		3	574	660	15.0
		4	602	686	14.0
		5	345	392	14.0
	Sehore	1	625	765	22.0
		2	700	825	18.0
		3	675	805	19.0
		4	655	790	21.0
1991/92	Badnapur	1	618	684	11.2
		2	639	745	16.6
		3	612	765	25.0
		4	692	729	13.5
	Sehore	1	630	745	18.3
		2	655	780	19.1
		3	650	760	16.2
		4	610	735	20.5
1992/93	Badnapur	1	590	570	14.0
		2	470	490	4.3
		3	515	550	6.8
		4	490	530	8.2
	Gulbarga		850	930	9.0
	Sehore	1	710	830	16.9
		2	660	790	19.7
		3	675	860	18.5
		4	640	775	21.0

1. Plot size 0.2 ha.

Source: All India Coordinated Pulses Improvement Project (AICPIP), Consolidated Reports on Kharif Pulses (Microbiology).

no significant effect on pigeonpea yields in simple inoculation trials (with and without Rhizobium) in farmers' fields near Hyderabad, Andhra Pradesh.

Nodulation Status

By estimating the soil population of pigeonpea rhizobia by the most probable number (MPN) plant infection method, we get an indication of whether the native population is adequate for nodulating pigeonpea or not. The MPN counts of pigeonpea rhizobia in five farmers' fields near Hyderabad were generally found to be low (range $<10^2$ to 2.5×10^3 g^{-1} dry soil) (Kumar Rao et al. 1984). The other method of determining the presence or absence of rhizobia in farmers' fields is by looking for nodules on the roots of field-grown pigeonpea plants. Using the latter method, the nodulation status of pigeonpea in 635 farmers' fields in different parts of India was assessed. The nodulation was found to be poor at 75% of the locations, moderate at 20%, and good at 5% (Table 2). Similarly in the state of Haryana, out of the 417 farmers' fields surveyed, nodulation was found to be poor

Table 2. Nodulation status of noninoculated pigeonpea[1] in farmers' fields at various locations in India, 1980-92.

Center	Number of locations surveyed	Nodulation status[2]		
		Poor	Moderate	Good
Akola	38	21	15	2
Coimbatore	3	3	-[3]	-
Dholi	33	19	10	4
Gulbarga	20	15	5	-
Hisar	417	326	75	16
Pudukkottai	18	14	4	-
Sehore	80	56	17	7
Sardar Krishi Nagar	9	8	1	-
Varanasi	17	14	3	-
Total	635	476 (75%)[4]	130 (20%)	29 (5%)

1. Plants about 50 days old were carefully dug out, and the mean nodulation of 5 plants field^{-1} was calculated.
2. Poor = 0-5 nodules plant^{-1}.
   Moderate = 6-10 nodules plant^{-1}.
   Good = 11-20 nodules plant^{-1}.
3. No observation.
4. Values in parentheses represent percentage of total number of samples.

Source: AICPIP Consolidated Reports on Kharif Pulses (Microbiology).

at a majority of the locations (78%) (Table 3). Good nodulation was observed at only 4% of the locations. One interesting observation was that the locations (particularly sand dunes in Bhiwani district) where no nodules had been observed 10 years ago, showed good nodulation recently due to the maintenance of a good moisture status through sprinkler irrigation. Thus, the moisture status of the soil has a direct bearing on the rhizobial population which in turn affects nodulation.

Table 3. Nodulation status of noninoculated pigeonpea grown in farmers' fields at different locations in Haryana, India, 1980-92.

District	Number of locations surveyed	Nodulation status[1]		
		Poor	Moderate	Good
Ambala	10	10	-[2]	-
Bhiwani	45	34	11	-
Faridabad	42	34	7	1
Gurgaon	50	39	5	6
Hisar	22	20	2	-
Jind	16	14	2	-
Karnal	29	15	7	7
Kurukshetra	30	10	20	-
Mohindergarh	65	64	1	-
Rohtak	58	49	9	-
Sonepat	50	37	11	2
Total	417	326 (78%[3])	75 (18%)	16 (4%)

1. Poor = 0-5 nodules plant^{-1}.
   Moderate = 6-10 nodules plant^{-1}.
   Good = 11-20 nodules plant^{-1}.
2. No observation.
3. Values in parentheses represent percentage of total number of samples.

Source: AICPIP Consolidated Reports on Kharif Pulses (Microbiology).

Nitrogen and Phosphorus Status of Soils

Nodulation and nitrogenase activity in pigeonpea were found to have been depressed by soil nitrogen concentrations greater than 25 mg N kg^{-1} soil as NO_3, while the addition of P stimulated nodulation in an Alfisol and a Vertisol (references cited in Kumar Rao 1990). Grewal (1990) estimated available N and P in 470 farmers' fields in Haryana. The available N of surface soil (0-15 cm depth), analyzed by the alkaline permanganate method (Subbiah and Asija 1956), varied from 14 to 220 mg kg^{-1} soil, while available P [extracted by 0.5 N $NaHCO_3$ (Olsen et al. 1954)] ranged from 1.2 to 14.9 mg kg^{-1} soil. Singh et al. (1992) estimated the amount and distribution of different forms of N in the surface soil and soil profiles taken from the major soil groups in different agroclimatic regions of Haryana. In 20 samples (0-15 cm depth) obtained from various locations, the NH_4-N ranged from 25 to 68 mg kg^{-1} soil while the NO_3-N ranged from 8 to 31 mg kg^{-1} soil. Similarly, in soil samples taken from different soil groups (i.e., Typic Ustipsamments, Udic Ustochrepts, Typic Solorthids, Typic Haplustalfs, Fluventic Ustochrepts, Typic Dystrochrepts, and Typic Camborthids) and various soil depths (ranging from 0 to 226 cm), there was a wide variation in levels of NH_4-N and NO_3-N. In the top soil (0-23 cm depth), the ammonical N level ranged from 21 to 54 mg kg^{-1} soil and nitrate N ranged from 8 to 31 mg kg^{-1} soil.

On-farm studies have shown low pigeonpea nodulation at a majority of locations, thus promising a positive response to *Rhizobium* inoculation. One of the reasons for this appears to be the high levels of mineral N at least in soils in Haryana. Another reason is that as the crop is grown on marginal soils, various stresses such as those caused by drought and high temperature may be affecting the pigeonpea-Rhizobium symbiosis. Therefore, it is imperative to develop a stress-tolerant symbiotic system.

Constraints to Use of Rhizobium Inoculation Technology

Rhizobial inoculation of seed has not been widely adopted by farmers (except for crops like berseem and soybean) in spite of it being relatively inexpensive and potentially remunerative. One of the major constraints to popularizing this technology is the inconsistent response to it, and extension workers' inability to demonstrate visible differences between inoculated and noninoculated plants under field conditions. Indian farmers tend to adopt only those practices which show a visual and tangible response. If the farmers could be convinced that by using rhizobial inoculation they would need to spend less on nitrogen fertilizer for the succeeding crop, then this technology might be accepted. The nonavailability of quality inoculants within an accessible distance is another constraint to adoption. The poor quality of inoculants is yet another constraint to building up farmers' confidence in the technology. To overcome these constraints, monitoring and quality control should be ensured. Further, extension agencies must convince farmers that inoculation ensures the presence of efficient and suitable types of rhizobia.

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Soybean Bradyrhizobium Research in India

V R Balasundaram[1], K Annapurna[2], and K V B R Tilak[3]

Introduction

Among the various leguminous crops grown in India, soybean occupies a unique place with respect to rhizobial investigations. Since the improved soybean cultivars of North American origin were introduced in India in 1968, the area under the crop has increased to about 4.0 million ha in 1992/93. Remunerative prices for oilseeds, the policies of the Government of India, and support for the export of soybean meal have aided the spread of soybean cultivation. This paper deals with biological nitrogen fixation in soybean in general, and the response to inoculation with Bradyrhizobium in India.

Current Status

Newly introduced American soybean cultivars seemed to require inoculation with efficient strains of Bradyrhizobium japonicum as the inoculated plots in some cases had better nodulation and higher yields than the noninoculated plots (Table 1). Hence, an infrastructure for mass production of inoculants was developed with indigenous selection of strains under varying agroclimatic conditions, and

Table 1. Grain yield of soybean varieties inoculated with a composite culture of Rhizobium japonicum.

	Grain yield (kg ha^{-1})	
Variety	Noninoculated	Inoculated
Black Hawk	1870	1440
Gold Soy	2050	2810
Grant	2000	1810
Harrow Manchu	1020	950
Portage	400	1160
SE	±220.1	

Source: Kumar et al. 1976.

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Balasundaram, V.R., Annapurna, K., and Tilak, K.V.B.R. 1994. Soybean Bradyrhizobium research in India. Pages 42-46 in Linking Biological Nitrogen Fixation Research in Asia: report of a meeting of the Asia Working Group on Biological Nitrogen Fixation in Legumes, 6-8 Dec, ICRISAT Asia Center, India. (Rupela, O.P, Kumar Rao, J.V.D.K., Wani, S.P., and Johansen, G, eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

with different varieties. A case study conducted in two villages in Indore district of Madhya Pradesh—the major soybean growing state in India—showed that only 35% of the farmers used rhizobial inoculants (Table 2).

Table 2. A case study of constraints to increased soybean production in farmers' fields in Hingonia and Piplya villages in Indore district of Madhya Pradesh, India.

Description	Number of farms	Yield (kgha-i)
Farms using fertilizer	24 (60[1])	697
Farms using partial or no fertilizer	16 (40)	617
Farms using adequate seed rate	22 (55)	712
Farms not using adequate seed rate	18 (45)	689
Farms using Rhizobium culture	14 (35)	717
Farms not using Rhizobium culture	26 (65)	690

1. Figures in parentheses are percentages.

Source: D P Motiramani, JNKVV Jabalpur, personal communication 1975.

Field trials at different locations during 1968-85 revealed that mean soybean yield across locations increased by 3-49% due to rhizobial inoculation (Table 3).

Table 3. Mean yield of promising local soybean cultivars inoculated with Rhizobium on experimental farms at different locations in India during 1968-1985.

Year	Location[1]	Mean yield (kg ha^{-1}) Noninoculated	Mean yield (kg ha^{-1}) Inoculated	Increase in yield over noninoculated control (%)
1968	1,2	2383	2756	16
1969	1-6	2106	3080	46
1970	1-10	1605	2384	49
1971	1-12	1408	1773	26
1972	1-5,7-9,11,13	1410	1739	23
1973	1-5,7,8,10,11,15	1659	2104	27
1974	1-3,5,8,10-12,14	1181	1393	18
1975	1-3,5,8-12,14,15	1197	1535	28
1976	1-4,7,11,15-17	1353	1670	24
1977	1-3,7,11,15-17	1267	1352	7
1978	1	1813	1958	8
1979	1-3,11,15,17	1051	1076	3
1984	2,15,17	1422	1595	12
1985	1-3,5,11,15,17	1742	2235	28

1. 1 = Delhi, 2 = Pantnagar, 3 = Jabalpur, 4 = Kalyani, 5 = Amaravati, 6 = Katrain, 7 = Junagadh, 8 = Coimbatore, 9 = Ludhiana, 10 = Berhampur, 11 = Bangalore, 12 = Kanpur, 13 = Pura (UP), 14 = Pune, 15 = Parbhani, 16 = Jorhat, and 17 = Ranchi.

Source: AICRPS (1987).

However, at some locations, inoculation did not increase yield substantially. In another study, it was observed that the normal rate of inoculation of soybean plants (0.2 kg carrier-based inoculant 25 kg $seed^{-1}$) resulted in very poor nodulation and low yield (Table 4). The poor nodulation was mainly due to adverse soil conditions at sowing. However, when the rate of inoculation was raised to 2.5 kg inoculant 25 kg $seed^{-1}$, it resulted in increased nodulation and yield.

Table 4. Effect of inoculum rate on nodulation and yield (kg ha^{-1}) of soybean cultivar Bragg at IARI, New Delhi, rainy season 1972.

Treatment	Number of nodules $plant^{-1}$ at 5 weeks	Yield
Normal rate of inoculum (0.25 kg 25 kg^{-1} of seed)	1	847
Increased rate of inoculum (2.5 kg 25 kg^{-1} of seed)	37	1917
SE	NA[1]	±46.2

1. NA = Not available.

Source: Balasundaram (1975).

Soybean genotypes were screened for nodulation with native rhizobia at selected locations in India. At Sehore, Madhya Pradesh, the screening program revealed wide variation among genotypes for nodule number, nodule mass, and yield. Local soybean cultivars that nodulated well with native rhizobia were used in the breeding program. The crosses made between local and American cultivars showed promising adaptation and were also nodulated by native rhizobia.

Inoculation of soybean with rhizobia along with other beneficial microorganisms, particularly phosphate-solubilizing microorganisms and vesicular arbuscular mycorrhizae (VAM), showed varying results. Soybean inoculated with VAM cultures yielded significantly ($P < 0.05$) more than the noninoculated control (Table 5). Both rainfed and irrigated soybean showed a similar trend with respect

Table 5. Response of soybean to inoculation with vesicular arbuscular mycorrhizae (VAM), New Delhi, India, rainy season 1992.

Treatment	Mycorrhizal colonization in root (%)	Grain yield (kg ha^{-1})
Noninoculated control (no VAM and no phosphorus)	10.4	2180
Glomus fasciculatum (no phosphorus)	52.5	2850
Gigaspora gilmorei (no phosphorus)	56.3	3150
Glomus mosseae (no phosphorus)	54.2	3070
40 kg P_2O_5 (no VAM)	5.0	2290
SE	±1.88	±104.5

Source: K V B R Tilak, IARI, New Delhi, personal communication.

to inoculation response. Rhizobial inoculation of soybean also gave residual benefits to the subsequent wheat crop. Such benefits were also observed with the application of soil from the soybean field. The yield of wheat without application of nitrogen was 65% higher when it succeeded an inoculated crop of soybean than when it succeeded a noninoculated crop (Table 6). This increase in yield is equivalent to the response of wheat to the direct application of about 30 kg N ha^{-1}.

Table 6. Response of soybean to rhizobial inoculation or application of soil from an inoculated field and their effect on the subsequent wheat crop, Pantnagar, 1973.

Treatment	Grain yield of soybean (kg ha^{-1})	N applied to wheat (kg ha^{-1})	Yield of wheat (kg ha-^{1}) Grain	Straw
Control		0	1350	2910
(noninoculated)	950	120	3910	5620
Seed inoculated with		0	2240	3520
Nitragin® culture	1840	120	4270	6180
Soil from soybean		0	2350	3710
field, @ 2 t ha^{-1}	1650	120	4020	5900
SE	± 75.1		±202.3	±465.3

Source: Saxena and Tilak (1975).

Soils amended with manures, in general, recorded better nodulation, and consequently, higher nitrogen fixation than the soils amended by cakes of linseed and mustard. Application of the cakes beyond 2.5 t ha^{-1} impaired nitrogen fixation because of poor nodulation. The reason for such an adverse effect on nodulation needs further investigation.

Peat is an ideal carrier for Rhizobium, but high quality peat is not available in India. The possibility of utilizing several indigenously available materials as carriers for legume inoculants remains to be explored. Presently, charcoal-amended carriers are being used in most parts of the country.

Objectives

The present objectives of our research on BNF in soybean are:

- Maintenance of efficient bradyrhizobial strains compatible with plant germplasm selection for high BNF and yield under adverse soil conditions.
- Use of nitrate-tolerant symbiotic lines of soybean in mixed cropping with hybrid maize, cotton, etc.
- Biocontrol of soilborne diseases by identifying and incorporating antibiotic-producing genes from other sources into bradyrhizobia.
- Studies on the effect of various plant growth regulators on BNF and yield.

Research Possibilities

As the area under soybean increases year after year, there exists scope for screening and identifying soybean genotypes which would nodulate well in the new areas. Mixed cropping of soybean with cereals or cotton is practical in many areas of the country Nitrate-tolerant symbiotic germplasm lines will be compatible for use as intercrops with the generally N-fertilized cereals. There is a need to identify soybean genotypes which can derive a maximum proportion of their plant N requirement through BNF.

These research aspects may need the expertise of researchers in such areas as physiology, biochemistry, molecular biology, and biotechnology. Such a holistic approach to improving BNF in soybean will help improve crop production and save fertilizer N.

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Population of Cowpea Rhizobia in Farmers' Fields in Southern Karnataka: Influence of Cropping System, Locations, and N-level

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Introduction

The important cropping systems in Karnataka state of India are cereal-legume rotation, cereal/legume intercropping, cereal/cereal, cereal-nonlegume, and occasionally legume-legume. Ninety percent of the grain legume cultivation in the state is in rainfed areas while the irrigated areas are mainly occupied by rice and sugarcane. In southern Karnataka the grain legumes commonly cultivated are groundnut (*Arachis hypogaea*), horse gram (*Macrotyloma uniflorum*), cowpea (*Vigna unguiculata*), mung bean (*Vigna radiata*), black gram (*Vigna mungo*), pigeonpea (*Cajanus cajan*), and lablab (*Lablab purpureus*). Ail these are nodulated by cowpea-group rhizobia.

The indigenous population of rhizobia has a great influence on the success of inoculation (Singleton and Tavares 1986, Rupela and Sudarshana 1990, Thies et al 1991). Many biotic and abiotic factors in the soil are known to influence the rhizobial population and nodulation of legumes. Therefore, in order to decide whether inoculation is required or not, knowledge of native rhizobial number and their effectiveness is essential. This study reports on the population of cowpea-group rhizobia and the nodulation status in red soils under different cropping systems in the southern districts of Karnataka.

Materials and Methods

The six locations where the studies were conducted were Bangalore, Mandya, Sirsi, Kadur, Mangalore, and Madenur, covering the plains, hilly areas, and coastal regions. The most commonly cultivated cereals were finger millet (Eleusine *coracana*), rice (*Oryza sativa*), sugarcane (*Saccharum officinarum*), maize (*Zea mays*), and sorghum (*Sorghum bicolor*), while the common grain legumes were groundnut, cowpea, pigeonpea, horse gram, lablab, mung bean, and black gram.

Soil cores of 7.5 cm diameter from the top 15-cm soil profile were collected randomly from four spots in each test field/site and mixed thoroughly. A subsample of each was used to estimate the rhizobial population. Such sampling was done four times a year at quarterly intervals, once before sowing, twice during crop

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growth, and once after harvest for 3 years from 1988 to 1990. The data in the tables give only the mean values for the 3 years.

The population of cowpea-group rhizobia in each soil sample was estimated by the serial dilution-plant infection-most probable number method (Brockwell 1980) using siratro (*Macroptylium atropurpureum*) or a relevant legume as trap hosts. Cultivars of different trap hosts other than siratro were horse gram BGM1, pigeonpea TTB7, lablab Hebbal Avare, chickpea Annigeri 1, and soybean KHSP1. Plants were grown in icecream cups containing 250g sand - vermiculite (1:1 by volume) and inoculated with a 1 mL aliquot of relevant dilution of a soil sample, four replications per dilution. Plants were harvested at 35 to 40 days after sowing and observed for the presence or absence of nodules. Rhizobial populations were expressed as $\log_{10}$ (MPN) g^{-1} dry mass of soil.

Surface-sterilized seeds of different legumes were grown in a greenhouse in pots containing a portion of the soil sample used for determining the rhizobial population. Nodulation was visually rated as 'very good' (VG), 'good' (G), 'moderate' (M), or 'poor' (P). Nodule number, mass, and color were also observed once from 20 to 40 DAS but are not reported here.

Soil mineral N available for plant growth was determined according to the procedures of Keeney and Nelson (1982). Total soil N was determined by micro-Kjeldahl digestion (Bremner and Mulvaney 1982). Available soil P was determined by the method of Olsen and Dean (1954).

Results and Discussion

Influence of cropping system on rhizobial population. All the test soils contained cowpea-group rhizobia (Table 1) irrespective of the cropping system and

Table 1. Most probable numbers (MPN $\log_{10}$ g^{-1} dry soil) of cowpea-group[1] rhizobia and nodulation status[2] (in parentheses) in fields under different cropping systems in southern Karnataka, India.

	Locations						Mean
Cropping system[3]	1	2	3	4	5	6	±SE
Cereal-legume rotation	5.85 (M)	4.71 (M)	5.53 (VG)	1.76 (M)	5.08 (M)	4.28 (M)	4.53 ± 0.600
Cereal-legume intercrop	5.26 (M)	3.49 (M)	3.76 (M)	1.49 (M)	5.26 (M)	3.76 (M)	3.84 ± 0.568
Sole legume	5.09 (G)	5.01 (M)	6.07 (VG)	2.02 (M)	5.53 (G)	5.08 (G)	4.80 ± 0.580
Sole rice or sugarcane	2.23 (G)	1.20 (M)	2.76 (M)	1.49 (P)	1.20 (M)	2.76 (M)	1.94 ± 0.302

1. Siratro was used as trap host.
2. Nodulation was rated as very good (VG), good (G), moderate (M), and poor (P).
3. Soil sampling was initiated after at least 2 years of these cropping systems.

their location but their MPN varied from 1.20 to 6.07 log$_{10}$ g^{-1} dry soil. The highest mean population of rhizobia was found in soils cropped to sole legumes, followed by cereal-legume rotation, cereal-legume intercropping, and soils cropped to cereals alone. It was interesting to note that at least 1.49 log$_{10}$ rhizobia g^{-1} dry soil were recorded in soils continuously cropped to rice and sugarcane for many years. In Mandya, a field continuously cropped to rice for more than 50 years contained 1.2 log$_{10}$ rhizobia g^{-1} dry soil and siratro nodulated well.

Survival of rhizobia in submerged paddy fields has also been reported by Weaver et al (1987). It is well known that cultivation of legumes increases rhizobial number. However, this study shows that rhizobia can survive for many years in cultivated soils even in the absence of legumes. Increase in rhizobial number did not always result in increased nodulation.

The rhizobial population was always <3.0 log$_{10}$ g^{-1} dry soil in the absence of a legume crop for 2 years (Table 2), suggesting that rhizobial multiplication on

Table 2. Most probable numbers (MPN, log$_{10}$ g^{-1} dry soil) of cowpea group rhizobia[1] and nodulation status[2] (in parentheses) in fields under different cropping systems in southern Karnataka, India.

	Locations						Mean
Cropping system[3]	1	2	3	4	5	6	±SE
Finger millet-legume	3.26 (M)	2.28 (M)	2.02 (P)	1.71 (P)	2.29 (G)	2.08 (M)	2.27 ± 0.216
Sunflower-legume	3.97 (M)	2.48 (M)	3.32 (P)	NA[3]	1.68 (M)	3.79 (M)	3.05 ± 0.391
Maize-legume	4.26 (G)	3.26 (G)	4.27 (G)	4.66 (G)	2.02 (M)	3.84 (G)	3.72 ± 0.391
Sugarcane-legume	4.20 (VG)	3.76 (VG)	NA	3.08 (VG)	NA	NA	3.68 ± 0.230
Rice-legume	5.20 (G)	4.20 (G)	3.71 (G)	2.48 (M)	5.09 (VG)	NA	4.14 ± 0.455
Finger millet (3 years)	1.68 (P)	0.81 (P)	2.90 (P)	NA (P)	2.02 (P)	1.41	1.76 ± 0.316
Sunflower (2 years)	2.02 (M)	1.68 (P)	1.19 (P)	2.28 (M)	2.26 (M)	1.23 (P)	1.78 ± 0.199
Maize (2 years)	2.28 (M)	3.08 (M)	2.10 (P)	3.10 (M)	1.19 (P)	1.49 (P)	2.21 ± 0.323
Sugarcane (many years)	5.00 (G)	4.20 (G)	3.76 (G)	1.23 (G)	1.23 (M)	1.00 (M)	2.74 ± 0.727
Rice (many years)	2.23 (G)	2.76 (G)	5.20 (VG)	2.10 (G)	1.49 (M)	2.10 (M)	2.65 ± 0.537

1. Siratro was used as trap host.
2. Nodulation was rated as very good (VG), good (G), moderate (M), and poor (P).
3. NA = Not available.

host roots was important for maintaining their high soil population. Nodulation of siratro was good to very good even after the soil was submerged for several months during rice cropping. The nodulation status of other legumes under the other four cropping systems was poor to moderate in several cases.

Rhizobia nodulating siratro, horse gram, pigeonpea, lablab, chickpea (Cicer arietinum), and soybean (Glycine max) were estimated separately in soils under the four cropping systems (Table 3). The mean population of siratro and horse gram rhizobia was the highest (5.12-5.18 $\log_{10}$) followed by pigeonpea, lablab, soybean, and chickpea rhizobia. It is well known that most of the cowpea-group rhizobia nodulate siratro. The present study, however, shows that horse gram can serve as an equally efficient trap host. It is a small-seeded legume that grows well in a seedling agar tube of 18 x 150 mm size. It seems that a large population of rhizobia nodulating siratro and horse gram failed to nodulate pigeonpea and lablab. Also, the rhizobia nodulating chickpea and soybean were in negligible numbers (Table 3). Nodulation of soybean in soils with no history of soybean cultivation has been reported (Nautiyal et al. 1988). The failure of some siratro rhizobia to nodulate pigeonpea has also been reported by Kumar Rao and Dart (1981). Thus within the cowpea group rhizobia, there exists considerable host specificity. It is, therefore, important to estimate rhizobial numbers of relevance to a legume using the same legume as the trap host.

Table 3. Most probable numbers (MPN $\log_{10}$ g^{-1} dry soil) of rhizobia and nodulation status[1] (in parentheses) of different legumes in four cropping systems in **Karnataka, India, assessed by using different trap hosts.**

	Cropping system				
Trap host	Cereal-legume rotation	Cereal-legume intercrop	Rice-legume intercrop	sole legume	Mean ± SE
Siratro	5.27 (G)	5.09 (G)	4.27 (VG)	6.07 (G)	5.18 ± 0.369
Horse gram	5.26 (VG)	4.19 (VG)	5.02 (VG)	6.00 (G)	5.12 ± 0.373
Pigeonpea	4.19 (M)	3.17 (M)	2.28 (M)	3.26 (M)	3.23 ± 0.390
Lablab	2.29 (P)	1.68 (G)	0.00 (P)	1.00 (P)	1.24 ± 0.491
Chickpea	0.0	1.02	0.00	NA[2]	0.34 ± 0.294
Soybean	1.02 (P)	0.44 (NIL)	0.00 (NIL)	2.10 (NIL)	0.89 ± 0.454

1. Nodulation was rated as very good (VG), good (G), moderate (M), and poor (P).
2. NA = Not available.

Influence of mineral N, P, and organic carbon on rhizobial population. Rhizobial number and nodulation of groundnut and pigeonpea were estimated at eight locations differing in mineral N and P levels and in organic carbon content (Table 4). The NH_4 + NO_3 N levels ranged from 147 to 51.7 mg kg^{-1} soil. The total N ranged from 0.03 to 0.29%. In general, rhizobial populations and N levels showed an inverse relationship. Groundnut showed better nodulation at lower N levels while the inverse applied for pigeonpea. Variations in rhizobial population among locations are well known, and the population at a given site is governed by the combined influence of various biotic and abiotic factors. Recently, a model was developed to predict the success of inoculation in diverse environments based on indices of the size of native rhizobial populations and availability of mineral N (Thies et al. 1991). However, the problems of poor nodulation and symbiotic nitrogen fixation remain to be solved. New strategies have to be evolved if BNF technology is to make worthwhile contributions to food production.

Table 4. Most probable numbers (MPN) of cowpea-group rhizobia[1] and nodulation status in fields with different fertility levels in Alfisols of Karnataka, India.

location	Mineral N2(mg kg^{-1} soil)	Total N(%)	Organic C(%)	Olsen P (mg kg^{-1} soil)	MPN log_{10} g^{-1} dry soil)	Nodulation rating[3]	
						Groundnut	Pigeonpea
Bangalore	26.9	0.04	0.5	15	4.71	M	P
Mandya	19.9	0.15	1.2	52	5.26	G	P
Shimoga	14.7	0.03	0.5	15	4.20	G	M
Sirsi	16.6	0.29	1.9	13	4.26	G	NA[4]
Hiriyur	40.4	0.14	1.7	18	2.76	M	G
Madenur	33.5	0.08	1.9	43	2.23	M	G
Mangalore	37.0	0.20	1.6	50	3.49	G	NA
Kadur	51.7	0.23	1.7	33	1.49	M	G

1. Siratro was used as trap host.
2. Measured in 2N KCl extract of soil samples using the method of Keeney and Nelson (1982).
3. Nodulation was rated as very good (VG), good (G), moderate (M), and poor (P).
4. NA = Not available.

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Improvement of Nitrogen Fixation in Groundnut by Host-plant Selection

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Introduction

Legumes can derive much of their nitrogen needs through biological nitrogen fixation (BNF). In symbiotic plants BNF plays a vital role in N accumulation, leaf-area development, and seed yield. Under field conditions, a number of factors influence BNF, including the host cultivar, the Rhizobium strain, soil water availability, and mineral nutrition (Nambiar 1990). Efforts to enhance BNF in groundnut have been made through Rhizobium strain improvement, cultivar selection, and improved agronomic management (Nambiar 1990). Inoculation with efficient strains of Rhizobium led to improved yields in some trials but the results have not been consistent (Kulkarni and Joshi 1988, Nambiar 1990).

It has often been argued that native soil rhizobial populations are inadequate. However, in many soils in dry areas, we have consistently observed adequate native rhizobia of the cowpea group (that also nodulate groundnut) at the time of groundnut sowing (Venkateswarlu 1992). The other presumption that native rhizobia are ineffective in BNF also does not seem convincing in groundnut. For instance, the specific nitrogenase activity (SNA) of groundnut nodules formed by native rhizobia on 60-day-old plants was at least 67% higher than that of cowpea (*Vigna unguiculata*), green gram (*Vigna radiata*), black gram (*Vigna mungo*), horse gram (*Macrotyloma uniflorum*), and cluster bean (*Cyamopsis tetragonoloba*) 35-46 days old grown on a shallow Alfisol at the Central Research Institute for Dryland Agriculture (CRIDA) research farm, Hyderabad (Table 1). This site contained >10^3 rhizobia g^{-1} dry soil at sowing (Venkateswarlu 1992). Similarly, the leghaemoglobin content was 40% higher than the mean of all the other legumes and was comparable to inoculated groundnut under analogous conditions.

These results suggest that the native rhizobial populations or their effectiveness per se may not be the primary limiting factors for optimum BNF in groundnut. An important constraint in realizing the optimum BNF in rainfed groundnut is the occurrence of droughts during the growing season that affect plant growth and nitrogen fixation. Therefore, in order to optimize N_2 fixation in rainy-season groundnut, greater emphasis is required on host-plant selection and agronomic management.

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Venkateswarlu, B. and Katyal, J.C 1994. Improvement of nitrogen fixation in groundnut by host-plant selection. Pages 53-60 in Linking Biological Nitrogen Fixation Research in Asia: report of a meeting of the Asia Working Group on Biological Nitrogen Fixation in Legumes, 6-8 Dec, 1CRISAT Asia Center, India. (Rupela, O.P., Kumar Rao, J.V.D.K., Wani, S.R, and Johansen, C, eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Table 1. Leghaemoglobin content and specific nitrogenase activity (SNA) of different cowpea-group legumes grown in an Alfisol and nodulated by native rhizobia, CRIDA, Hyderabad, India.

Crop	Leghaemoglobin content[1] (mg g^{-1} dry mass of nodules) ± SE	SNA[1] (u moles C_2H_4 g^{-1} dry mass of nodules) ± SE
Groundnut (60)[2]	3.8 + 0.42	77 ±6.5
Cowpea (35)	2.9 ±0.21	43 ± 5.2
Green gram (35)	2.4 ± 0.31	39 ± 4.9
Black gram (35)	2.6 ±0.36	46 ±7.1
Horse gram (46)	3.1 ±0.38	43 ±4.8
Cluster bean (40)	2.6 ± 0.41	35 ±5.3

1. Mean of six samples.
2. Figures in parentheses are the days after sowing when the samples were analyzed.

Genotypic Variability for Nodulation and N_2 Fixation

Wide genotypic variability exists for nodulation and N_2 fixation among cultivars of both botanical types of groundnut, Spanish and Virginia (Wynne et al. 1980, Nambiar et al. 1982). In general, virginia-type cultivars exhibit higher nodulation and acetylene-reduction activity (ARA) than the Valencia and Spanish types (Wynne et al. 1980). NC Ac 2821, a Virginia cultivar, ranked consistently high during a three-season evaluation at ICRISAT Asia Center involving 42 germplasm lines (Nambiar et al. 1982). However, in a field study involving 12 cultivars, Venkateswarlu et al (1991) found that the superior nodulation of Virginia cultivars becomes significant only 60 days after sowing (DAS) when hypocotyl nodulation begins. Until that time, the nodule biomasses were comparable in both the types. Kulkarni et al. (1988) indicated that the differences in the nodulation pattern of the botanical types are primarily related to their growth habit. However, the differences in nodulation and N_2-fixation rates were not reflected in the pod yields, but they related well with total dry matter and N accumulation at harvest (Nambiar et al. 1982).

Breeding for High N_2 Fixation

A few attempts have been made to breed for high N_2 fixation in groundnut at ICRISAT Asia Center in India and at the North Carolina State University, USA. Early reports on gene action for traits associated with N_2 fixation revealed a predominantly nonadditive genetic variance for nodule number, nodule mass, SNA, and total N (Wynne et al. 1980). However, later studies by Wynne et al. (1983) indicated that these were due to both additive and nonadditive genetic effects. In a diallel cross involving NC Ac 2821 and six parent lines selected for high or low N_2-fixing ability, a good combining ability was observed mainly due to the high additive genetic variance contributed by this germplasm line to the hybrid progeny (Nigam et al. 1985). These authors also reported that due to the greater importance of specific combining ability variance for nitrogenase activity selection for high N_2 fixation may not be effective in the early generations. Limited attempts at ICRISAT Asia Center (P T C Nambiar, personal communication) to establish strain-specific symbiosis with nonnodulating plants did not succeed. These efforts are, however, worth pursuing in order to establish a specific Rhizobium strain - host cultivar combination for high BNF and to overcome competition from native rhizobia.

Despite these reports, a clear breeding strategy for high N_2 fixation has not emerged due to the complex nature of some of the BNF traits used as selection criteria and the interaction among the host, the microsymbiont, and the environment. A critical examination of these reports led Nambiar (1990) to spell out the limitations of breeding for high N_2 fixation. He argued that separate efforts for breeding for high BNF may not be required as breeders have been unwittingly selecting for high nodulation when they select for higher yield. However, this may not hold true for farmers' fields. Moreover, observations on other crops such as chickpea suggest that high yields can often be obtained even with poor nodulation, with a considerable uptake of soil N. Therefore, improvement of BNF through host-plant selection is worth pursuing with native rhizobia as a first step, because of the complexities involved in working with specific host-strain combinations.

Nitrogen Fixation and Dry Matter Accumulation in Rainfed Groundnut

Although groundnut can derive most of its N requirement from BNF, a positive correlation does not always exist between N_2 fixation and seed yield. Research on soybean showed that N assimilation was more sensitive to drought than was C assimilation (Sinclair et al. 1987). This may also be true for other grain legumes. Specific leaf N (amount of leaf N/unit leaf area) regulates the pace of leaf area development and biomass accumulation in many legumes including groundnut. Hence, N availability regulates the rate of dry matter production under rainfed conditions. The higher sensitivity of nitrogenase activity to drought than net photosynthetic rate and stomatal conductance is well known in several legumes including groundnut (Venkateswarlu et al. 1989). Therefore, nitrogen fixation and its partitioning hold the key for dry matter accumulation and its partitioning in rainfed groundnut.

Remobilization and Partitioning of Nitrogen in Relation to the Nodulation Pattern

Cregan and van Berkum (1984) reported genetic differences for remobilization and partitioning of nitrogen in several crops and suggested that these traits can be used for genetic improvement for high grain yield and seed protein. We conducted a comprehensive study on these traits in groundnut cultivars and related the data with nodule biomass and ARA at different growth stages. Twelve cultivars from three botanical groups were included in this study which was conducted on a shallow Alfisol in Hyderabad (mean annual rainfall 750 mm) under rainfed conditions. Nodule biomass, ARA, and N accumulation were recorded periodically during the crop ontogeny. The nitrogen harvest index (NHI) and the pod yield harvest index (HI) were derived from the harvest data through individual estimates of dry matter and N content in roots, stem, leaves (including fallen leaves), pod walls, and seeds. Nitrogen remobilization efficiency (NRE) was calculated as the ratio of the difference between maximum vegetative N and final vegetative N to maximum vegetative N (Cregan and van Berkum 1984). The NRE values were calculated for each plant component separately and expressed as percentages. The correlation coefficients (r) between different parameters of nodulation and N metabolism and yields are presented in Tables 2 and 3. The highest correlation was found between nodule dry mass and ARA at 58 DAS and NHI.

Table 2. Correlation coefficients (r) for nodule dry mass, ARA, and N uptake with seed yield and NHI, CRIDA, Hyderabad, India[1].

Parameter	Seed yield	NHI[2]
Nodule dry mass		
at 35 DAS[3]	0.63*	0.64*
at 48 DAS	0.64*	0.68*
at 58 DAS	0.69*	0.76*
at 85 DAS	0.54*	0.53*
at harvest	0.48	0.51
ARA[4]:		
at 35 DAS	0.48	0.52
at 58 DAS	0.71*	0.81**
at 85 DAS	0.56	0.62*
N uptake:		
at 35 DAS	0.59	0.57
at 48 DAS	0.61*	0.66*
at 58 DAS	0.78*	0.83**
at 85 DAS	0.58	0.62*
at harvest	0.47	0.49

* Significant at $P = 0.05$.

** Significant at $P = 0.01$.

1. Data are based on means of two years from 12 cultivars.
2. NHI = Nitrogen harvest index.
3. DAS = Days after sowing.
4. ARA - Acetylene reduction activity.

Source: Venkateswarlu 1993.

Table 3. Correlation coefficients (r) between seed yield and different parameters related with N accumulation and partitioning, CRIDA, Hyderabad, India.

	Factors[1]					
Year	YX_1	YX_2	YX_3	X_1X_2	X_1X_3	X_2X_3
1989	0.52	0.95**	0.97**	0.42	0.39	0.99**
1990	0.43	0.70**	0.71*	0.40	0.36	0.82**
1991	0.05	0.73*	0.53	-0.40	-0.68**	0.82**
Mean	0.39	0.80**	0.83**	0.28	0.16	0.75*

* Significant at $P = 0.05$.

** Significant at $P = 0.01$.

1. Y = Seed yield (g nv^{2}), X_1 = total nitrogen uptake (g nr^{2}), X_2 = Nitrogen harvest index (%), and X_3 = Plant nitrogen use efficiency (g seed g^{-1} N).

Source: Venkateswarlu 1993.

Similarly, total N uptake up to 60 DAS and not the N at harvest was significantly correlated with yield and NHL These results indicate that the N accumulated up to 60-70 DAS is more important for seed yield than the total N at harvest.

Spanish cultivars were more efficient in partitioning of N and dry matter than the Valencia and Virginia types although the total N uptake was comparable or even higher in the Virginia types. This can be explained by the N accumulation pattern in these types. Virginia cultivars accumulate more nitrogen later in the growing season owing to hypocotyl nodulation (Venkateswarlu et al. 1991) leading to poor N partitioning. Virginia cultivars were also inferior in remobilizing N from leaves. The leaf NRE was 74% in Girnar 1, followed by 65% in JL 24, and 48% in M 13. We consistently observed lower NHI and NRE values in Virginia genotypes than in Spanish types over three consecutive years (B Venkateswarlu, unpublished data). The poor N-partitioning efficiency of Virginia cultivars was perhaps one of the reasons for the lower seed yields, despite higher nodulation and N_2 fixation. Therefore, selection for further improvement of nodulation may not lead to higher yields in Virginia types. Such possibilities are greater with Spanish cultivars due to the linear relationships between N partitioning (NHI), dry matter partitioning (HI), and seed yields in these cultivars. However, the high nodulating lines from Virginia cultivars and the high NHI lines from Spanish cultivars can perhaps be used as parents for improvement of N_2 fixation and seed yield.

Intravarietal Selection for High Nodulation

As nodule biomass and ARA at 58 DAS correlated significantly with NHI and kernel yields, efforts were directed towards identifying plants with high nodule biomass at 60 DAS. As a first step, plants with high nodule number and mass were identified at harvest. Seeds from these plants were collected and maintained as single plant progenies. The nodulation and plant dry matter status of these progenies were verified in the subsequent generation at 60 DAS in 1992. The high nodulating selections generally had higher nodule mass than their respective cultivar means (Table 4).

Table 4. Performance of groundnut progenies selected for high nodule biomass[1]60 DAS, rainy season 1991, CRIDA, Hyderabad.

Variety	Number of[1] selections made at harvest in 1991	Number of lines with higher nodule mass than the cultivar mean	Number of lines with higher pod yield than the cultivar mean	Number of lines with both high nodule mass and high pod yield
Girnar 1	42	28 (189)2	22 (232)3	16[4]
TMV2	36	26 (205)	24 (225)	20
JL24	32	14 (172)	13 (195)	8

1. Plants with high nodule number were identified at harvest in 1991. Seeds from these plants were maintained as single-plant progenies. The nodulation and plant dry matter status of the progenies were verified at 60 DAS in 1992.
2. Figures in parentheses represent the cultivar means for nodule mass (mg plant^{-1})
3. Figures in parentheses are cultivar means for pod yields (gnr^{2}).

Efforts are in progress to correlate such above-ground plant attributes as shoot dry mass, percentage and total N content of leaves at different nodes, foliage color using Munsel color charts, and the leaf-rating method devised by Binford and Blackmer (1993), with nodule biomass at 60 DAS. The aim is to predict nodule biomass on the basis of some simple criteria without having to destroy the plant. Arunachalam et al. (1984) reported that nodule biomass and nitrogenase activity 30 days after flowering (about 60 DAS) have good predictive value for a number of plant-growth parameters at harvest. Preliminary data suggested that these approaches are likely to succeed in fields poor in N fertility.

Thus, there is obvious scope for host-plant selection and breeding for high BNF in rainfed groundnut. Selection for high nodule mass at 60 DAS may lead to an indirect selection for high NHI and NRE, and seed yield. Adequate plant-to-plant variability within a cultivar exists for these traits. Selection for high BNF has greater scope in Spanish than in Virginia types.

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ICRISAT Reports

Expectations from BNF Research: Research Administrators[9] Point of View

M C S Bantilan[1], C Johansen[2], and D McDonald[3]

Introduction

The oil crisis of the early 1970s and the consequent escalation of prices of nitrogenous fertilizers sparked off a boom in BNF research that lasted through to the early 1980s. Optimistic claims were made about substitution of fertilizer N with biologically fixed N, and funds poured in to support research. Today, however, there seems to be little residual effect of this BNF boom in farmers' fields, in South Asia at least. One reason for this, of course, is that the oil crisis spurred the discovery of vast new oil and gas reserves, and prices of N fertilizers generally stabilized at levels affordable (with or without government subsidy) to all but the poorest of farmers. Another reason is that adoption of BNF technology by fanners has not been significant.

In India in particular, there have been several large-scale schemes to introduce Rhizobium inoculation for the major legume crops, but there is little evidence of its widespread adoption by farmers. This situation exists despite evidence from many experiments showing significant responses to inoculation, and calculations of economic viability. This contrasts with the situation in countries such as Australia, where such technology has been widely adopted. But there the circumstances are different, with mainly the introduced temperate legume species requiring specific strains of rhizobia. Moreover, such countries have large-scale, mechanized, and commercialized farming systems in which it is easier to introduce *Rhizobium* inoculation procedures. The only example of large-scale, sustained adoption of *Rhizobium* inoculation technology that we are aware of in Asia is that of soybean in Thailand (see Toomsan et al., pages 17-23 this Report). Here also, there appears to be a need for specific rhizobia for soybean.

In the light of such unfulfilled promise in Asia, it is not unnatural that research administrators are somewhat wary of new proposals for BNF research targeted at improving the lot of small, resource-poor farmers. In agriculturally important legumes, BNF research has hitherto been overwhelmingly directed towards

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Rhizobium inoculation technology, with the aim of enhancing N_2 fixation through the addition of superior Rhizobium strains. Other options, such as manipulation of agronomic practices to favor BNF or genetic alteration of the plant to increase symbiotic activity, have received less attention. This paper attempts to summarize the reasons for the earlier shortcomings, suggests a holistic approach to BNF research, and makes specific suggestions on approaches to future research and development.

Shortcomings of Inoculation Technology

There are various reasons for the limited adoption of Rhizobium inoculation technology by farmers in the tropics in general and in South Asia in particular.

Assessment of 'need-to-inoculate'. Recommendations on inoculation are often of a universal nature, to be applied across diverse environments and legume species, although there are marked, well established site-to-site differences in inoculation response. It is sometimes argued that, as all such differences in response cannot possibly be known or understood, inoculation may be regarded as an 'insurance policy' with a low premium. However, only affluent farmers are prepared to buy such insurance. Even if the cost of a packet of inoculum is low, there are unavoidable costs in terms of time and skill required for inoculation at the usually busy time of sowing. If a positive response to inoculation is not reasonably assured, farmers are not likely to want to invest their time and effort in it, let alone their money. Therefore, for effective extension of BNF technology, it is necessary to define situations in which a positive response to inoculation is, or is not, probable.

The main factors affecting response to inoculation are:

- The absence or inadequate numbers of rhizobia in the soil, native or introduced, that can effectively nodulate the target legume. Tropical legumes are largely promiscuously nodulated by the cowpea-group Rhizobium (or Bradyrhizobium) which are ubiquitous in soils where these legumes normally grow. Hence the limited response of these legumes to Rhizobium inoculation (Date 1977).
- Even moderate levels of soil mineral N inhibit nodulation (Harper and Gibson 1984), which is not overcome by rhizobial inoculation.
- There are wide variations among and within legume species in their ability to meet their own N needs through fixation.
- Other plant growth-limiting factors strongly interact with nitrogen fixation.
- Rhizobium inoculation procedures may damage seeds and thus reduce seedling emergence.

The INLIT (International Network of Legume Inoculation Trials) approach (Davis et al. 1985) of NifTAL (Biological Nitrogen Fixation for International Development), University of Hawaii, remains a valid approach to determine the need-to-inoculate. It consists of a noninoculated control, an inoculated treatment, a treatment with 'optimum' N fertilizer, and the presence or absence of another major limiting factor for the legume (usually phosphorus). As multilocational field trials are expensive, various preliminary tests can give an indication of the likely response. An example is the use of simple models relating inoculation responsiveness to the MPN of effective rhizobia and level of soil mineral N (Singleton et al. 1992).

Inadequate demonstration of inoculation technology. Activities in BNF technology have often remained within the discipline of soil microbiology, with inadequate interaction with other disciplines, let alone extension personnel. There is little evidence that the demonstration and extension process for BNF technology has been thoroughly planned and effectively applied in farmer's fields.

Quality control of inoculants. In the tropics, there are few inoculant production systems producing Rhizobium inoculum of consistently good quality over a reasonable period of time. Shortcomings and remedies in this respect have been described by Thompson 1984, and Thompson 1991.

Difficulties in using Rhizobium inoculants. In high temperatures typical of tropical and subtropical environments, Rhizobium inoculants in carrier packets tend to lose their viability, even if their numbers had been adequate initially. In these regions, the normal sowing times of legumes fall at the beginning and end of a long-day rainy season (in order to grow the crops on residual soil moisture). These are normally hot periods during which exposure of rhizobial cultures to high temperatures is almost unavoidable, even if refrigeration is available. Moreover, if the inoculum is a nonsterile one, the high temperatures may favor competitors to Rhizobium. More work is needed to develop procedures that minimize the adverse effects of high temperature.

Economics of Rhizobium Inoculation Technology

Calculations of the economic viability of inoculation technology have indicated high rates of return (e.g., Verma and Bhattacharyya 1992), but such calculations often have deficiencies. For example, production costs are often subsidized by government agencies, and personnel costs are sometimes ignored. Actual costs are therefore underestimated. Nonmonetary costs and miscalculation of returns based on inoculation responses extrapolated over regions have been referred to earlier in this paper. A more thorough and conservative accounting is desirable in order to convincingly present the likely returns on investment in Rhizobium inoculation technology.

The Research-Adoption-Impact Continuum

Proposals for BNF research must be considered in the light of the entire continuum from basic research to impact assessment. Given the increasing scarcity of resources, the bottom line for any research undertaking is more and more its impact,

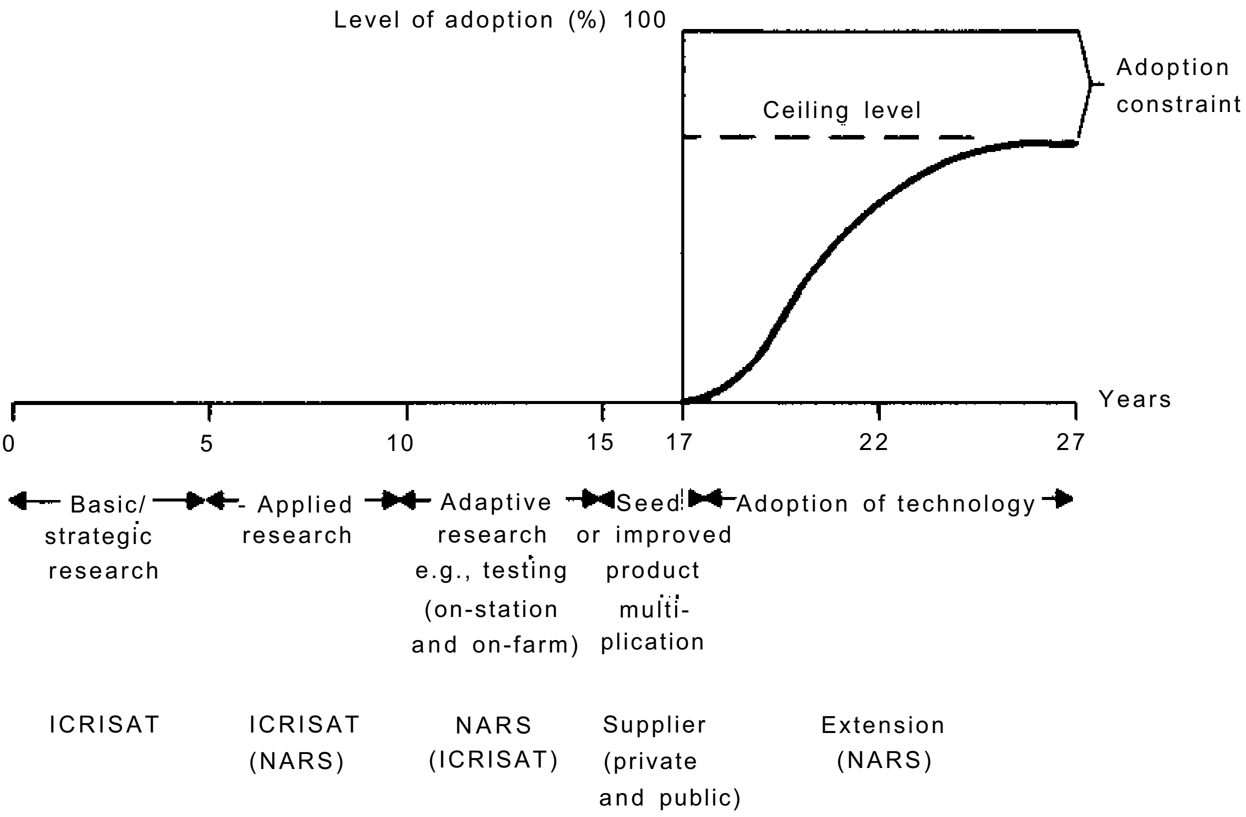


Figure 1. Schematic representation of the research, development and adoption process over time, indicating relative involvement of ICRISAT and national agricultural research systems (NARS).

or likely impact. To facilitate impact assessment, both ex-post and ex-ante, an understanding of the whole research process is essential

The research-evaluation continuum may be systematically viewed by using a general framework as outlined in Figure 1. The framework traces the development of the different components of the research process, its output, and logical consequences. The conceptualization of the framework starts with the consideration of research investments that fund the implementation of research projects. The new knowledge/technology generated is expected to bring forth changes in the production and consumption environment by making more of the commodity available in the market. To be more specific, the application of science-based technologies resulting from BNF research is expected to bring about increases in crop yields. Research on BNF is also expected to improve the efficiency of inputs through better agronomic practices and crop management. Ultimately, the changes in the production and consumption environment are translated into improvement in the welfare of farmers who use the technology as well as that of consumers who use the final products.

Before the final benefits of research accrue to the producers and consumers, two important conditions must be met. First, the research undertaken must be successful in achieving its objectives. This introduces the notion of probability of success or relative research capability. Second, the potential increase in production promised by a new technology is ultimately achieved only when it is adopted by farmers. This condition necessitates the consideration of the rate of technology adoption and the factors constraining it.

However, the measurement of the welfare gain to society is incomplete if it does not take into account the externalities which the technology involves. The externalities may be negative or positive. Classic examples of a negative externality are soil erosion in agriculture and the detrimental effects of chemical-based technology. The latter example includes the deleterious effect of pesticides on the health of farmers and their families, the transmission of chemical residues through the food chain to consumers, the toxic effect of chemicals on animals like fish, shrimp, frogs, and helpful insects in the farmers' fields, the contamination of ground and surface waters, and the reduction of soil microbial populations that help sustain soil fertility.

The positive externalities are incorporated in this framework through the concept of spillover effects. Three types of spillover effects are considered. The first type involves the across-location spillover effect in which a technology developed through research for one product in a specific location can be adapted to improve the production efficiency of the same product in other locations (geopolitical or agroecological).

The second type of spillover effect concerns the across-commodity applicability of the technology developed. For example, a cultural management technique developed specifically for groundnut may also be applicable to other legumes.

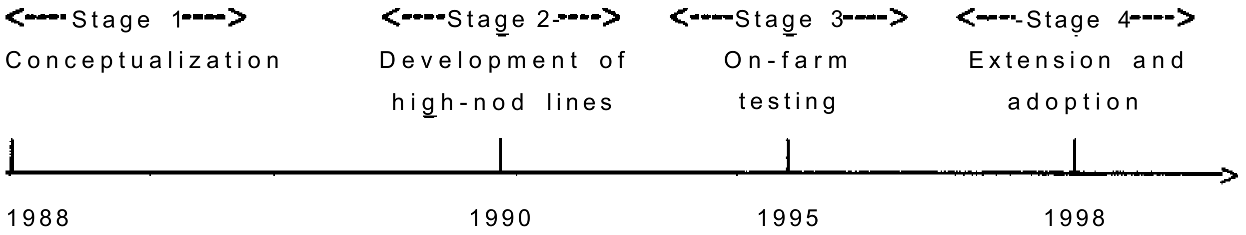
The first two types of spillover effects reflect the direct applicability of a technology, and are thus referred to as direct spillover effects.

A third type of spillover effect is referred to as the indirect or price spillover effect. A new technology (by virtue of increasing production) may have an effect on the price of a particular commodity at a particular location. In addition, it may also have an effect on the price of that commodity at another location (if the commodity is traded) and/or on the price of related commodities. This is particularly relevant when the elasticities of product demand are relatively small and/or the rate of product transformation among commodities is significant.

Another factor which has an effect on welfare gains accruing from research is government policy which can influence the production and/or consumption of a commodity, or the inputs used to produce it. Government policies can thus influence both the benefits flowing from research and their distribution.

The welfare effects of research can vary significantly with the research project, location, and commodity. The choice of a research project is likely to be influenced by the magnitude and distribution of these effects. Which of these effects are important requires clarification. For example, if two regions are part of one country and if total national welfare gain is the objective of the research institutions, then a measure of the research impact is provided by adding all the gains (or losses) of all sectors. If, however, the objective is to maximize gains to poor farmers only, the welfare gains within that subset are added to give a measure of how well the research option may satisfy that objective. Estimates of these welfare changes, if quantified, can be summarized in a form that will assist decision-makers in setting research priorities or making other allocation decisions. Other aspects that require consideration are: a) effect on income distribution and poverty; b) food security; c) human capital development; d) institution building and strengthening of national programs; e) sustainability and environmental impact; and f) implications of policy change.

It is thus clear that a whole spectrum of considerations has to be taken into account while assessing a research project. It is equally clear that a detailed understanding of the components of the research-evaluation continuum is necessary to arrive at a quantitative assessment of impact. Following is a sketch of the type of information needed to assess BNF research directed towards improving the N_2-fixing ability of chickpea (see Rupela, pages 75-83 this Report), both ex-post and ex-ante:



Stage 1 involved the development of the concept of genetic alteration of the plant for better nodulation through selection within existing cultivars. This stage led to the formulation of basic concepts and methodology for the development of the improved technology. Stage 2 involved selection of lines with superior N_2_fixing ability and their validation in on-station experiments. Stage 3 involves on-farm validation of the value of the selections. Stages 1, 2, and 3 represent the basic, applied, and adaptive research components in the development of this technology.

Stage 4 involves the demonstration, extension, and adoption of the technology among farmers. The process underlying the adoption of technologies is represented by the curve in Figure 1, in which adoption-related variables — adoption lags, rate of adoption, and ceiling level of adoption are highlighted. Introduction of a new technology does not usually lead to immediate adoption. The gestation period between the generation of a technology and its adoption varies with the sector, commodity, and even type of technology. Some farmers adopt a technology only after its effects have been convincingly demonstrated. Reluctance among farmers to adopt a technology may be due to difficulty in using it, nonavailability of the inputs required, market uncertainty, price fluctuations or preference for very low management crop technology. Thus, a sigmoid adoption curve is usually used to illustrate the adoption process; where the level of adoption is initially low, it rises at an increasing rate after sufficient diffusion is attained, and finally reaches a ceiling level of adoption. Adoption lag refers to the time interval between the introduction of a technology and the attainment of the ceiling level of adoption.

The quantitative assessment of impact is data-intensive. Data on the diverse factors involved at various stages of the research process are needed to estimate the likely impact of BNF research. An important feature of the BNF research process described above is that the expected research lag is about 10 years. This represents the time it takes for the envisioned technology to be achieved and made available to farmers. The probability of achieving the expected research results (probability of success) has to be estimated and used in measuring the impact, particularly for stages 1, 2, and 3. Estimates on the rate of technology adoption and ceiling level of adoption have to be made. The cost of implementation of the research in the first three stages should be taken into account in the assessment.

Suggestions for Attracting Administrative Support

Cost/benefit analysis. Rigorous cost/benefit projections are required to attract investment in BNF research. A prime requirement is to establish, for particular target legumes and cropping systems, the actual gains expected from improving BNF above the existing level, in comparison to achieving these gains by using mineral N fertilizer. This primarily requires assessment of the extent to which the legume can meet its needs through fixation. Essentially, need-to-inoculate studies, supplemented by more detailed studies on rate and time of application of N fertilizer, can accomplish this (although there would inevitably be some difficulties of interpretation related to fertilizer N-use efficiency and N metabolism within the plant). Also, the residual benefit of legumes, in terms of equivalents of N fertilizer applied to a subsequent crop, needs to be calculated. Further, the relative value of N derived from either fertilizer or organic sources needs to be estimated, from the viewpoint of environment protection and sustainability of cropping systems. These data provide a baseline against which to estimate gains that can be expected from improving BNF as a result of research or by direct application of known technologies. Allowing for factors such as probability of success, time lags, and ceiling level of adoption, reasonable estimates can be made for costs and benefits of a suggested research project and/or development effort.

Management and genetic options. This Working Group meeting offers an opportunity to evaluate management (primarily, inoculation technology) and genetic options for enhancing BNF, especially the new genetic options being proposed by Dr Rupela and his colleagues. If we can genetically alter the plant to better accept native rhizobia in an effective symbiosis that would both meet the legume's N needs as well as leave substantial residual N, then the aforementioned problems of inoculation technology can be bypassed.

Inoculation technology. If it is decided that further pursuit of Rhizobium inoculation technology is viable, then the shortcomings discussed earlier need to be comprehensively addressed.

Outlook for N fertilizer. The popularity of BNF research, and hence the extent of funding for it, is directly and closely related to the relative (compared with other agricultural inputs) price of N fertilizer. More emphasis should be given to comprehensive comparisons of BNF enhancement versus use of N fertilizer. This not only involves relative input costs, in relation to the benefits expected, but also the adverse consequences of use of either source of N. For example, reliance on N fertilizer can result in soil acidification, N leaching losses, and eutrophication of water bodies. Reliance on BNF can also lead to soil acidification (e.g., by proton excretion from legume roots) and inflexibility of cropping systems (particularly if legumes are a low-value cropping option).

Impact analysis. As outlined above, proposals for BNF research and development would be much more attractive to research administrators and donors if it could be clearly shown how the proposed activities fit into the research-adoption continuum. They need to be based on sound calculations of expected gains from research and other parameters of the adoption curve. Considering the past failures in adoption of BNF technology, there is scope for adoption constraint studies, to pinpoint bottlenecks. Impact analysis should be built into any proposed project. These steps do not seem to have been previously taken, but improvement of BNF would seem a readily quantifiable candidate for this suggested holistic approach.

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Expectations from BNF Research: Breeders' Perspective

Onkar Singh[1]

Introduction

The contribution of BNF to the total plant N varies widely with legume species, rhizobial population, and environmental factors. Among grain legumes, faba bean (*Vicia faba*) appears to fix the most N_2, with reports of 300 kg N ha^{-1} (LaRue and Patterson 1981). The highest estimate of fixed N_2, up to 80% of the total N, was seen in plants grown on N-poor soils (LaRue and Patterson 1981). However, the N_2 fixed under nitrogen-deficient conditions is seldom sufficient for a good yield (Bliss and Miller 1988). On the other hand, where soil nitrogen is sufficient, N_2 fixation is progressively inhibited and the comparative advantage of the In-fixing plant disappears. Therefore, it is necessary to breed genotypes that are more efficient in BNF, irrespective of whether the soils have high or low nitrogen contents. This paper considers such possibilities and discusses some questions relating to BNF research encountered by plant breeders.

Effect of BNF on Yield and Residual Benefits of Legumes

The amount of N_2 fixed by legumes in symbiosis with rhizobia varies with crop, soil type, and crop management practices, and is usually in the range of 30-300 kg N ha^{-1} $year^{-1}$ (Beringer et al. 1988). Legumes do not meet all their N requirement from N_2 fixation; estimates of BNF vary from 5% for common bean (*Phaseolus vulgaris*), to 50% for soybean (*Glycine max*) and 80% for alfalfa (*Medicago sativa*). The rest of it is taken up as nitrate from the soil. Despite many years of research, we are not sure whether BNF is more energy-consuming than nitrate assimilation. Further, high rates of N_2 fixation are not necessarily translated into enhanced seed yield. For example, good and poor N_2-fixing genotypes of mung bean (*Vigna radiata*) were found to differ substantially in their ability to remobilize N from vegetative tissue to pods during reproductive growth (Weaver and Miller 1986). Increased BNF can be useful in enhancing seed yield only in such genotypes that also have the ability to partition the increased N into their seeds.

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Intercropping or mixed cropping of legumes with nonlegumes is a common practice in the semi-arid tropics. There are reports of the positive contribution of legumes to the soil N pool for the benefit of the succeeding crops (Ward et al. pages 84-90 this Report. There are several reports that indicate an increase in the growth of nonlegumes in the presence of legumes (Eaglesham et al. 1981). Similarly, nonlegumes also can influence the effectiveness of BNF in legume crops. Hardarson (1993) has cited examples in which a legume grown in a mixed culture with nonlegumes relied more on atmospheric nitrogen than when it was grown as a sole crop. Breeders and physiologists should direct their efforts towards identifying legume varieties that are efficient not only in meeting their own N requirement but also in providing nitrogen to the companion or succeeding crops.

Methods of Estimating BNF

The most widely used methods of estimating BNF in legumes include: 1. nitrogen accumulation, 2. difference methods, 3. ^{15}N isotope methods, and 4. techniques that assess variables associated with N_2 fixation (LaRue and Patterson 1981, Weaver and Miller 1986).

Estimation of total plant N is simple and inexpensive. The procedures used to account for the contribution of soil N are known as difference methods. Comparisons of a test legume and a nonlegume, an inoculated and a noninoculated legume, and a test legume and a nonnodulating legume are generally employed but they have some limitations in certain situations. Various ^{15}N isotope methods can be used to study soil N uptake and to estimate BNF by legumes, but there are several potential sources of error in these methods, and they are expensive. Then there are indirect methods of assessing BNF from such BNF-related traits as acetylene-reduction activity (ARA), nodule number and mass plant^{-1}, leghemoglobin concentration in the nodules, visual nodulation score, and plant fresh weight. The situation is therefore confusing to a plant breeder, and there is a need to clarify which parameter/method is more reliable.

Host Plant x *Rhizobium* Interaction

The formation of N_2-fixing root nodules depends upon the interaction between compatible strains of *Rhizobium* and the roots of the legume. Plant genes expressed during nodule formation have been identified in several legumes. Similarly, *Rhizobium* genes are involved in the induction and expression of nodulation genes. The ability of the host plant and the *Rhizobium* to interact efficiently after the nodules are formed is not well understood and needs much more research. Mytton et al. (1977) provided a clear demonstration of the potential importance of *Rhizobium* x legume genotype interactions in faba bean and described a method of breeding to enhance such interactions.

It is clear that selection occurs for *Rhizobium*-legume genotype combinations that are more efficient in BNF because it is unusual to find ineffective nodules on field-grown legumes, even when the majority of the rhizobia in the soil are ineffective (Beringer et al. 1988). The question then arises as to which strategy would be more rewarding: genetic manipulation of the host plant, or the *Rhizobium*, or the interaction between them? This question was addressed by Phillips and Teuber (1985). They supported the idea of breeding the host plant for improved BNF as against the other two options. Hardarson (1993) on the other hand has observed that recent molecular biology methods have made it possible to map genes involved in N_2 fixation, nodulation specificity, competitive ability, and other characters that are important in BNF. Once superior strains are identified, transferring genes from them to other strains is expected to enhance BNF. However, molecular biology is still at the analytical stage and, hence, the next advance is most likely to come from plant breeding. Unfortunately, the role of the host plant in maximizing symbiotic performance is often overlooked and poorly understood and, therefore, needs urgent attention.

Host-plant Improvement Strategies

Genetic improvement of the host plant for increased BNF depends on the extent of genetic variability in the gene pool, the relationship among the plant traits affecting N_2 fixation, the efficiency of the screening technique and the breeding method, and favorable host-plant interaction with strains of *Rhizobium*.

Genetic variability. The genetic variability for N_2 fixation (based on indirect estimation of BNF traits) has been reported in faba bean, field pea (Pisum sativum), common bean, soybean, mung bean, and cowpea (Bliss and Miller 1988); in groundnut (Arrendeil et al. 1989); and in chickpea (Rupela and Johansen 1992). Bliss and Miller (1988), reviewing a number of studies, reported that the genetic variability for BNF traits was heritable. Acetylene-reduction activity and nodule number and mass plant^{-1} were reported to be positively correlated, suggesting that these can be used as selection criteria. Only a few studies have reported genotypic differences using direct measurement (^{15}N isotope method) of N_2 fixation in soybean, faba bean, and common bean (Bliss and Miller 1988). However, the heritability of traits is generally low, possibly due to inefficient screening/measurement methods. This must have been precisely the reason for the slow gains achieved through selection in many breeding programs aimed at genetic improvement of BNF (Arrendeil et al. 1988). Nevertheless, there seems to be sufficient grounds for initiating such breeding programs in most legumes. Progress, however, will depend upon the extent of genetic variability for BNF traits and dependable and nondestructive screening techniques that are capable of handling large breeding populations.

The question of whether to select directly for high BNF per se, or indirectly for BNF-related traits has not been considered extensively and needs urgent attention. There is also a debate on whether segregating populations should be evaluated with identified effective strains of *Rhizobium*, or with native rhizobia. The possibilities range from one extreme where plant genes prevent root nodulation by all but the desired strain to the other extreme where selected plants increase BNF in association with any effective Rhizobium. Within this range also lies the possibility of breeding legumes which form more nodules with effective rhizobia.

Nitrate tolerance. Both the host plant and the Rhizobium are affected by environmental and agronomic factors. Where grain legumes experience environmental stress, selection for both improved BNF traits and for stress tolerance are likely to be more beneficial than selecting only for BNF. Similarly, soil nitrate inhibits nodule formation and reduces N_2 fixation in the nodules that are formed. The mechanism of this phenomenon is not known, and no naturally occurring nitrate-insensitive legumes have been found. However, Jacobsen and Feenstra (1984) obtained a mutant of pea with such a trait. Nitrate-tolerant supernodulants were also obtained later in soybean (Carrol et al. 1985) and common bean (Park and Buttery 1988). The nitrate-insensitive supernodulating phenotype was found to be under the control of the shoot. However, supernodulation showed no advantage in BNF, probably due to photosynthesis being inadequate in these mutants to support a large nodule mass. Beringer et al. (1988) suggested two strategies to overcome this problem: 1. induce mutants that produce normal nodules in the presence of nitrate, or 2. induce nitrate-tolerant supernodulating mutants that have a high photosynthetic capacity. Recently, four soybean genotypes of Korean origin showing high levels of symbiotic activity in the presence of nitrate have been found (Herridge and Betts 1988). There is an urgent need to screen other legumes for such characteristics as nitrate tolerance and supernodulation, along with high yield.

Suggestions. The host legume-Rhizobium combinations occurring in the existing production systems are rarely capable of fixing the total N required for large biological/seed yields. The emphasis in the past has been on microbiological methods to enhance BNF, and these technologies have been transferred to many of the developing countries. Since a good measure of genetic variability is present for BNF traits, breeding legume host plants for increased BNF appears to be feasible. Further, evaluation of legume germplasm for BNF-related traits is needed. Mutagenesis should be used to induce nitrate-tolerant supernodulants. Breeders in collaboration with physiologists should aim at developing legume varieties that are more efficient in BNF, provide more N to companion/subsequent crops, and are nitrate-tolerant.

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Screening for Intracultivaral Variability of Nodulation in Chickpea and Pigeonpea

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Introduction

During nodulation studies of legumes, researchers will have observed varying levels of nodulation among plants within a given cultivar growing in the same field/plot. In self-pollinated legumes like chickpea, such observations can be most readily ascribed to micro-environmental variations. In outcrossing legumes, such variations may also be due to segregation for BNF traits. Plants with varying BNF within cultivar Mesilla of alfalfa, an outcrossing legume, have been reported (Duhigg et al. 1978), but such reports are lacking for self-pollinated legumes.

During a study of the natural occurrence of nonnodulating (Nod^-) plants within five chickpea cultivars, Rupela (1992) also observed plants of varying nodulation within these cultivars. The Nod^- trait, first observed in 1985 in chickpea cultivar ICC 435, was found to be consistent in subsequent generations (Rupela 1992), and controlled by a single recessive gene (Singh et al. 1992). If the Nod^- trait can be heritable, why not the trait of varying degree of nodulation? The author therefore decided to screen for this trait in chickpea. Encouraged by the successful identification of consistent nonnodulating, low-, and high-nodulating selections within chickpea cultivars (Rupela 1992, Rupela and Johansen 1992), the search was extended to pigeonpea in 1991. This paper presents salient aspects of the work done at ICRISAT Asia Center, Patancheru.

For chickpea the screening was done in the field. However, as the nodules in pigeonpea are loosely attached to the roots and are easily detached during digging of field-grown plants, we developed screening protocols for greenhouse conditions (Rupela and Johansen, in press). The strategy for identifying nodulation variants of interest was the same for both chickpea and pigeonpea, and may be relevant to other legumes as well. However, important differences in screening for nodulation variants in the two crops have been stated where relevant.

Screening Strategy

Biological nitrogen fixation in legumes depends on the interaction between root nodule bacteria (RNB), the host plant, and the environment in which the plants are

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grown. A good understanding of all aspects of this symbiotic process has been developed, particularly in the last two decades. The simultaneous selection of both RNB (Rhizobium or Bradyrhizobium) and host plant to improve BNF in legumes has been described by many researchers (e.g., Wynne et al. 1980, Beringer et al. 1988) as the appropriate approach. This approach, however, is difficult to apply and has not attracted many scientists. For any successful host-selection program, a quick and simple evaluation system for BNF is required. We chose to identify high-nodulating host plants for high BNF with the following assumptions:

- Native RNB generally form effective/efficient symbioses with most host plants.
- It is difficult to displace native root nodule bacteria with those selected as inoculant strains.
- The ideal moisture level for seedling emergence is also suitable for the expression of the nodulation potential of a plant, other factors being optimal.
- The nodulation potential of a host plant is probably best expressed when grown on low soil N (10 mg mineral N kg^{-1} soil or less).
- Nodule mass is highly correlated with N_2 fixation, and plants of different nodulation capacities can be selected using a visual rating scale (Rupela 1990, Rupela and Johansen, in press).

The optimum levels of the other important factors governing nodulation, such as temperature, were ensured by adjusting the sowing time of chickpea, if the screening was done in field. Nutrients (also in field) were supplied through quarter-strength N-free Anion's solution (Arnon 1938) applied at sowing at the rate of 10 mL $seed^{-1}$. Regular inoculation was provided at sowing to ensure high populations of RNB, in spite of the fact that most fields regularly growing legumes are likely to have high native populations of RNB.

Screening Nursery

Chickpea nodulation variants at low N. We first depleted the soil N of a Vertisol field by growing a cover crop of sorghum. It took four seasons (years) to reduce the mineral N level from about 20 mg kg"^{1} soil to about 10 mg kg-^{1} soil in the top 30-cm profile. This field had used for screening and evaluation of nodulation variants of chickpea since 1990. Sorghum is generally grown in the rainy season to maintain the low mineral N status at about 10 mg kg^{-1} soil. In the postrainy season, the same plots are used as a screening nursery for the identification of nodulation variants of chickpea. To identify nodulation variants at high N, a part of this field was developed as high-N plots (about 20 mg N kg^{-1} soil in the top 30-cm profile at sowing) by applying urea.

Sowing of chickpea was generally delayed until late October when the ambient maximum temperature at ICRISAT Asia Center is about 30°C or lower. Sowing was done at a spacing of 30 x 10 cm, and seeds were placed at a depth of 2-4 cm. A liquid inoculant of strain IC 59 was applied at sowing to chickpea, and sometimes also to the preceding sorghum. The inoculant was suspended at the rate of 0.1 g peat L^{-1} in quarter-strength Arnon's nutrient solution. Ten mL of this suspension was applied per seed at sowing. The inoculant generally contained > 10^8 RNB g^{-1} peat. Irrigation was applied soon after sowing.

To identify Nod^- plants at low N, we screened about 10 000 plants each of a given cultivar. The frequency of natural occurrence of Nod^- plants ranged from 120 to 490 per million in different' chickpea cultivars. We identified HN (high-nodulating) and LN (low-nodulating) selections in four chickpea cultivars after screening about 1000 plants of each.

Chickpea nodulation variants at high N. Mineral N, particularly NO_3^--N is known to suppress nodulation and nitrogen fixation in legumes (Streeter 1988) including chickpea. Rawsthorne et al. (1985) reported an approximate 50% reduction in nodule number when chickpea plants grown in pots were supplied with 1.43 mM NO_3^- (= 20 ppm N) in nutrient solution. We recorded 41-94% reduction in nodule mass in seven chickpea cultivars at 71 days after sowing when the mineral N level in a Vertisol field was increased from 9 to 18 mg kg^{-1} soil in the top 30-cm soil profile. From the literature it was apparent that there is a greater scope for developing N-tolerant symbiosis through host-plant selection than through selection of rhizobial strains.

Against this background, we planned to identify plants with high nodulation and N_2-fixing ability at about 20 mg N kg^{-1} soil. For this, we developed a field-screening nursery similar to the one developed for selecting high-nodulating plants at low N. The only difference was that this nursery had about double the amount of mineral N in the top 30-cm soil profile. The high N level was achieved by applying 100 kg N ha^{-1} as urea at least one month before sowing. This was followed by sprinkler irrigation at least twice at intervals of about 10 days. This resulted in a part of the applied fertilizer moving to a depth of about 60 cm, creating a mineral N gradient of about 20 mg kg^{-1} soil at 0-15 cm depth to about 12 mg kg^{-1} soil at 31-60 cm depth. The mineral N in nonfertilized plots was generally about 10 mg kg^{*1} soil at 0-15 cm depth and 8 mg kg^{-1} soil at 31-60 cm depth. In some years, we found mineral N ranging from 13 to 18 (average = 16) mg kg-^{1} soil when 100 kg N ha^{-1} as urea was applied to the preceding sorghum grown on a low-N (about 10 mg N kg^{1} soil) Vertisol. However, this happened with repeated application of N every year, and average levels of 16 mg kg^{-1} soil or more were found only during the second or third year of regular annual application. It was more reliable to develop high-N plots by applying N before sowing chickpea than to the preceding sorghum. It is highly likely that most fields at research stations may already have 20 mg N kg^{-1} soil or more and, therefore, can readily serve as a screening nursery without further manipulations of the kind stated above. Moreover, different soil types may need different types of handling to maintain a mineral N level of about 20 mg kg^{-1} soil. Also, legume species may differ in terms of the mineral N concentration required to suppress BNF by at least 50%.

Pigeonpea nodulation variants at low N. Screening for nodulation variants in pigeonpea was done in a greenhouse. Polythene bags (pots) (18 cm diameter) were filled with coarse river sand (1-3 mm) washed in running water. Thirty pigeonpea seeds were sown per pot at about 1 cm depth. First watering was done with quarter-strength N-free Arnon's solution in which a mixture of four pigeonpea rhizobial strains (IC 3100, IC 3195, IC 4059, and IC 4060) was suspended at the rate of 0.1 g peat L^{-1} solution. Subsequent waterings were done exclusively with the nutrient solution. The maximum temperature until nodulation observations ranged from 25° to 30°C We screened about 30 000 plants of three cultivars (ICPL 87, ICPL 227, and ICPL 83015) but failed to find even one Nod^{-} plant. However, there were several plants with contrasting nodulation ratings within each of these three cultivars (Rupela and Johansen, in press).

Visual Rating of Nodulation

Several parameters, direct and indirect, to evaluate BNF have been stated in the literature. However, a quick and simple method is required to screen a large number of plants, at least for preliminary screening. The more dependable and generally expensive methods such as the ^{15}N-based methods can be used at a later stage. Therefore, a visual rating scale was developed both for chickpea (Rupela 1990) and pigeonpea (Rupela and Johansen, in press), and used successfully as a first screen. Nodulated roots of selected plants representing low (rating 1), high (rating 5), and intermediate (ratings 2, 3, and 4) nodulation were photographed. The photograph or rating scale was referred to during evaluation for selection of plants of desired nodulation types. During confirmation studies, the nodule mass and/or acetylene-reduction activity (ARA) were determined at the vegetative stage when quantitative recovery of nodules from field-grown plants is feasible. At confirmation and in advancing generations, the use of the visual rating scale was optional, depending on the objectives of the study.

There is a need to develop protocols that can be used for inheritance studies of nodulation in chickpea and pigeonpea. Inheritance studies of nonnodulation are obviously simpler as they involve observations on just the presence or absence of nodules.

Identification of Different Nodulation Types

At physiological maturity, chickpea plants were carefully dug up and categorized for nodulation. Seeds of selected plants were retained as single plant progenies (SPP) for use during the following season.

In pigeonpea, nodulation was observed about 3 weeks after sowing. After rating of nodulation, plants of interest were transplanted into polythene bags or plastic pots (7.5 cm diameter) for seed production (Rupela and Johansen, in press). It generally took about 2 weeks to ensure that the transplanted plants had reestablished. When the plants started regrowing, they were transferred to bigger pots if seed multiplication was done in the greenhouse, or to the field if it was done there. In all the cases, more than 90% of the plants survived after transplantation.

Although SPPs of more than two nodulation types were observed within each of the several chickpea and pigeonpea cultivars, further studies were restricted to SPPs of two groups of ratings- low (rating 1 and 2) and high (rating 3 to 5).

Progenies of the selected plants were advanced after evaluation for at least two generations, both in chickpea and pigeonpea, before they were used for yield evaluation (Fig. 1). We thus used a pure-line selection procedure. In the case of pigeonpea, the selected plants were always selfed at each advancing generation (Fig. 1).

Progress Made

Using the screening procedures described, several nodulation types have been identified in chickpea and pigeonpea (Table 1) since 1985. Material from which these types have been identified is described below.

Table 1. Different nodulation types identified in chickpea and pigeonpea at ICRISAT Asia Center.

Chickpea

- Nonnodulating with native RNB (rn 6[1])
- Nonnodulating with rhizobial strain IC 59, low-nodulating with native RNB
- Low-nodulating at low N
- High-nodulating at low N
- High-nodulating at low N but low nodulating at high N
- High-nodulating at high N

Pigeonpea

- Nonnodulating with native RNB
- Low-nodulating at low N
- High-nodulating at low N

[1] rn 6 is the name of the identified gene reported by Singh et al. (1992)

1. Nonnodulating plants were identified from all the five chickpea cultivars that we studied (Rupela 1992). These were; ICC 435, ICC 4918 (= Annigeri), ICC 4948 (= G130), ICC 4993 (= Rabat), and ICC 5003 (= K 850). In pigeonpea, Nod- plants were identified from segregating populations of 6 of the 83 crosses that were studied at F_2 (Rupela and Johansen, in press).
2. High- and low-nodulating plants were identified from four out of the five chickpea cultivars that were studied. These were; ICC 4948, ICC 5003, ICC 14196, and Kourinski. Of these, the last two are kabuli types. Similarly, high- and low-nodulating pigeonpea plants have been identified from two cultivars,

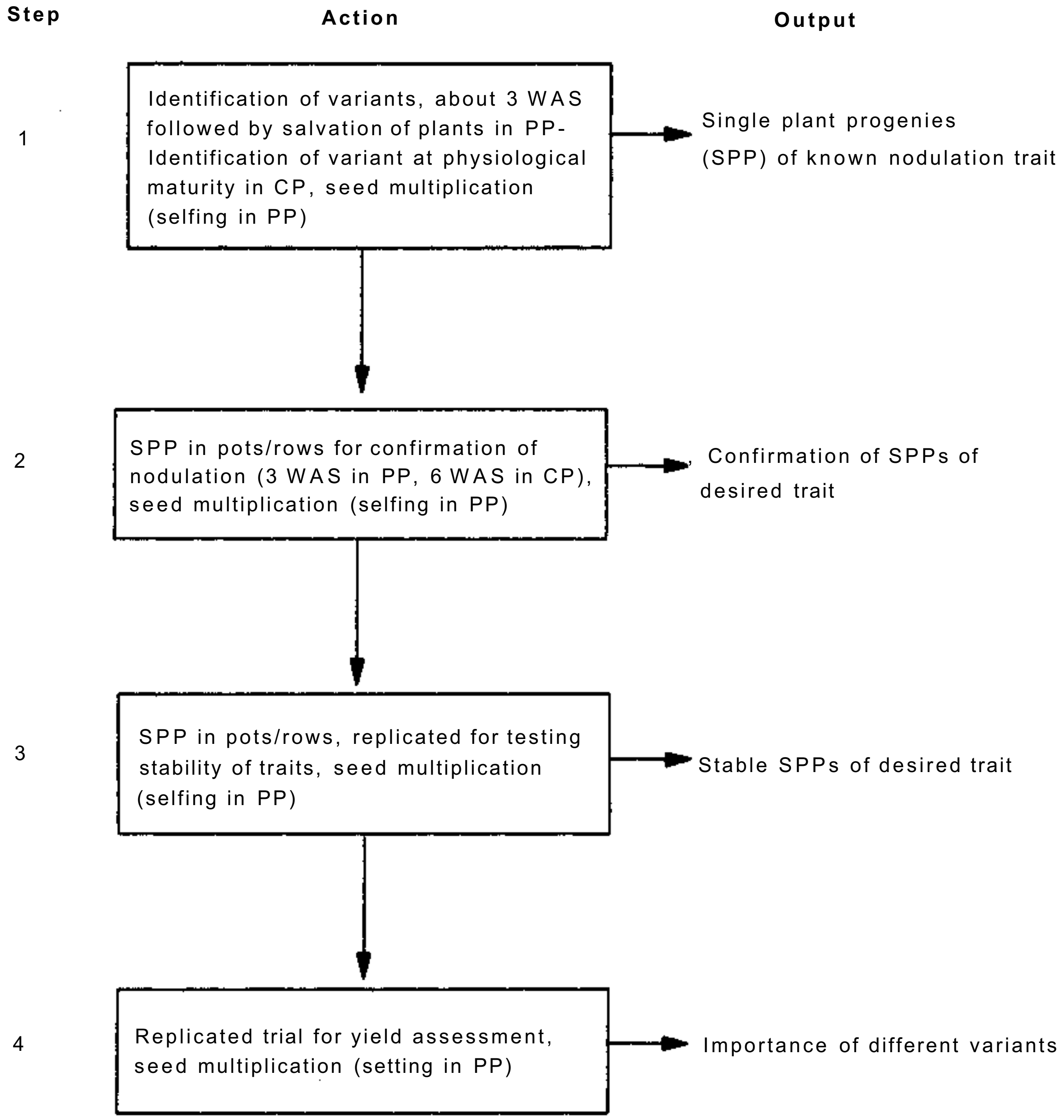

Figure 1. Protocol for selecting nodulation variants of chickpea (CP) and pigeonpea (PP). The screening nursery for chickpea can be developed in the field while for pigeonpea, evaluation has to be done in a greenhouse nursery, and seed multiplication can be done both in the greenhouse and the field.
WAS = weeks after sowing.

ICPL 87 and ICPL 227 (= ICP 1-6), and from one advanced breeding line, ICPL 83015.

3. Plants with a higher level of nodulation than most at high N (N2) were found in 81 of the 86 advanced chickpea breeding lines that were studied in 1991/92. One hundred and ninety of the 392 SPPs selected in the 1991/92 postrainy season remained high-nodulating at N2 when studied in the 1992/93 postrainy season. Thirty of the 190 highest-nodulating progenies were selected for further studies in the 1993/94 postrainy season.

Obviously, the Nod^- and the low-nodulating selections are of interest for studies of the physiology and genetics of nodulation and N_2 fixation, as well as providing a reference base in BNF-quantification studies. High-nodulating selections generally grew better than the low-nodulating ones from the same cultivar. This statement is based on small-plot (generally one row, 2 to 4-m long) data collected during evaluation of different selections. Large-plot yield trials have been conducted only with low- and high-nodulating selections of ICC 4948 and ICC 5003. In the 1991/92 postrainy season, the high-nodulating selection of cultivar ICC 4948 produced 31% more grain yield than its low-nodulating selection at low mineral N (Fig. 2). Its yield was superior even at high N. In the 1992/93 postrainy season, the nodule mass of high- and low-nodulating selections remained greatly different, the ICC 4948 high-nodulating selection yielded 10% more than the low-nodulating selection, and only 1% more than the unselected bulk of ICC 4948. Such a year-to-year variation in the yield of a given cultivar is not unexpected. The high- and low-nodulating selections of cultivar ICC 5003 yielded similarly when tested in 1991/92 (Fig. 2). In a previous pot trial, the root length density of low-nodulating ICC 5003 was 32 m $plant^{-1}$ which was two times more than that of the low-nodulating ICC 4948. Perhaps cultivar ICC 5003 could scavenge the soil N more efficiently than ICC 4948 due to its high root length density, and as a result, both the high- and low-nodulating selections of ICC 5003 yielded similarly.

These studies thus suggest a great scope for enhancing BNF in legumes through host-plant selection. Identification of abiotic stress tolerant symbiosis in legumes through host-plant selection seems promising, as indicated by the successful selection of high-nodulating chickpea plants at high N.

The high-nodulating selections are also likely to be high N_2-fixing. This was apparent from the improved yield of the high-nodulating selections in Figure 2 and from unpublished studies. BNF quantification studies on high- and low-nodulating selections using ^{15}N-based methods are in progress.

Acknowledgements

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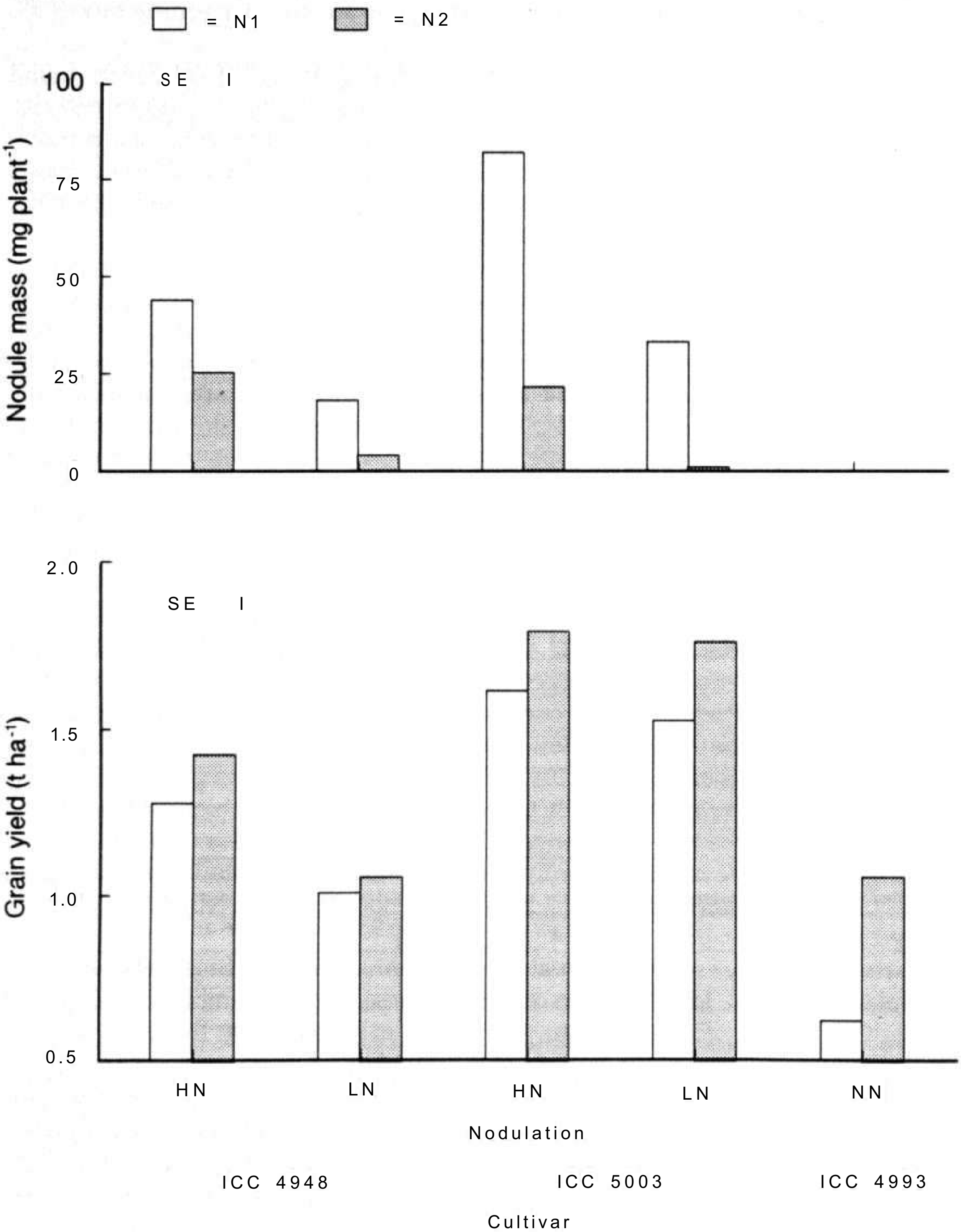

Figure 2. Nodule mass at 45 days after sowing and grain yield of chickpea cultivars of different nodulation ratings: HN = high nodulating, LN = low nodulating, NN = non-nodulating; grown at two mineral N levels in soil: low N (NI, about 10 mg kg^{-1} soil) and high N (N2, about 20 mg kg^{-1} soil); postrainy season, 1991/92, Vertisol, ICRISAT Asia Center. Both N levels and nodulation were significantly different (P = 0.05) for the above parameters. Their interactions were also significantly different for nodule mass.

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Contribution of Legumes in Cropping Systems: A Long-term Perspective

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Introduction

It is widely believed that legumes maintain or improve soil fertility because of their N_2-fixing ability. In support of this argument, the substantial amounts of N_2 fixed by legumes are cited. However, in assessing the long-term contribution of legumes in a cropping system, we need to consider not only the amount of N_2 fixed by legumes, but also the overall nitrogen balance of the cropping system.

Net N Balance of Legume Crops

In order to assess the contribution of legumes in a given cropping system, a proper estimation of the fixed nitrogen is essential. It must be remembered that it is a common practice for farmers to remove legume plant material from the field for use as fodder. In such cases, only nodulated roots and fallen plant parts are returned to the soil. However, in most studies, the amount of fixed nitrogen in the roots and fallen plant parts is not taken into account while quantifying BNF.

The net nitrogen balances calculated for several cultivars of pigeonpea grown at ICRISAT Asia Center, Patancheru, and of chickpea grown at Gwalior, Madhya Pradesh, India, indicated that all the varieties depleted soil nitrogen (Table 1). Nambiar et al. (1988) observed that groundnut fixed 190 kg N ha^{-1} season^{-1} at Patancheru. However, the crop showed a negative net N balance as 20-40% of its N requirement came from soil and fertilizer. Such negative N balances are more likely for legumes grown on high-fertility soils. Positive net N balances of up to 136 kg ha^{-1} have been observed by Peoples and Crasswell (1992) in several legume crops following seed harvest. However, when crop residues were removed from the field, the net N balances ranged from -27 to -95 kg ha^{-1} in groundnut, -28 to -104 kg ha"^{1} in soybean, -24 to -65 kg ha"^{1} in green gram, -25 to -69 kg ha^{1} in cowpea, and -28 kg ha^{-1} in common bean. These results show that legumes also mine soil N as do cereals. However, total plant N yields are far higher for legumes

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Table 1. Net nitrogen balances calculated for pigeonpea cultivars grown at Patancheru, and chickpea cultivars grown at Gwalior, India.

Cultivar	Total plant N uptake (kg ha^{-1})	Estimated plant N derived from fixation (kg ha^{-1})	Net N balance (kg ha^{-1})[1]
Pigeonpea[2]			
Prabhat	69	4	-49
UPAS 120	92	27	-39
T21	108	43	-39
BDN1	118	53	-32
Bhedaghat	101	36	-20
JA275	78	13	-33
Bhandara	108	43	-22
NP (WR) 15	114	50	-27
Chickpea[3]			
Annigeri	110	31	-77
G130	104	26	-75
ICC 435	102	29	-72
ICCC 42	88	23	-64
ICCV6	107	30	-76
K850	104	40	-63

1. Net N balance calculated as Total plant N uptake - (N derived from BNF + N derived from fertilizer + N added to soil through plant roots and fallen plant parts).
2. N derived from fixation calculated for roots also.
3. N derived from fixation calculated only for above-ground plant parts.

Source: Kumar Rao and Dart (1987). O.P. Rupela, ICRISAT, personal communication 1993.

than for cereals. From these results, it is concluded that when plant material is removed from the field, legumes in general slow the decline of, rather than enhance, the N fertility of the soil.

Residual Effects of Legumes

Notwithstanding the negative N balances, there have been consistent reports on the residual benefits of legumes. In a long-term crop-rotation experiment in progress since 1983 at ICRISAT Asia Center, such benefits to the succeeding sorghum crop have been observed consistently (Fig. 1). Improvement in cereal yields following monocropped legumes ranged from 0.5 to 3.0 t ha^{-1}, which were 30 to

350% higher than the yields in cereal-cereal cropping sequences (Peoples and Crasswell 1992).

Nitrogen effects. The benefits of legumes to succeeding nonlegume crops are quantified in terms of the fertilizer N equivalent or fertilizer replacement value (FRV). This concept does not distinguish between BNF and the 'N-conserving effect' of legumes. The FRV methodology has been widely used but it probably overestimates the N contribution of legumes as it confounds non-N rotation effects with N contribution. The FRV method gave an estimate (125 kg ha"^{1}) that was almost twice the observed value (65 kg ha^{-1}) when sorghum was used instead of maize as the test crop (Blevins et al. 1990). In order to circumvent the problems encountered with nonisotopic methods, the ^{15}N methodology has been used to measure the residual effects of legumes. Based on estimates obtained through this methodology, Hesterman et al. (1987) argued that the amount of N credited to legumes in a crop rotation in north central USA may have been inflated by as much as 123% due to the use of the FRV method. Using the ^{15}N methodology, it

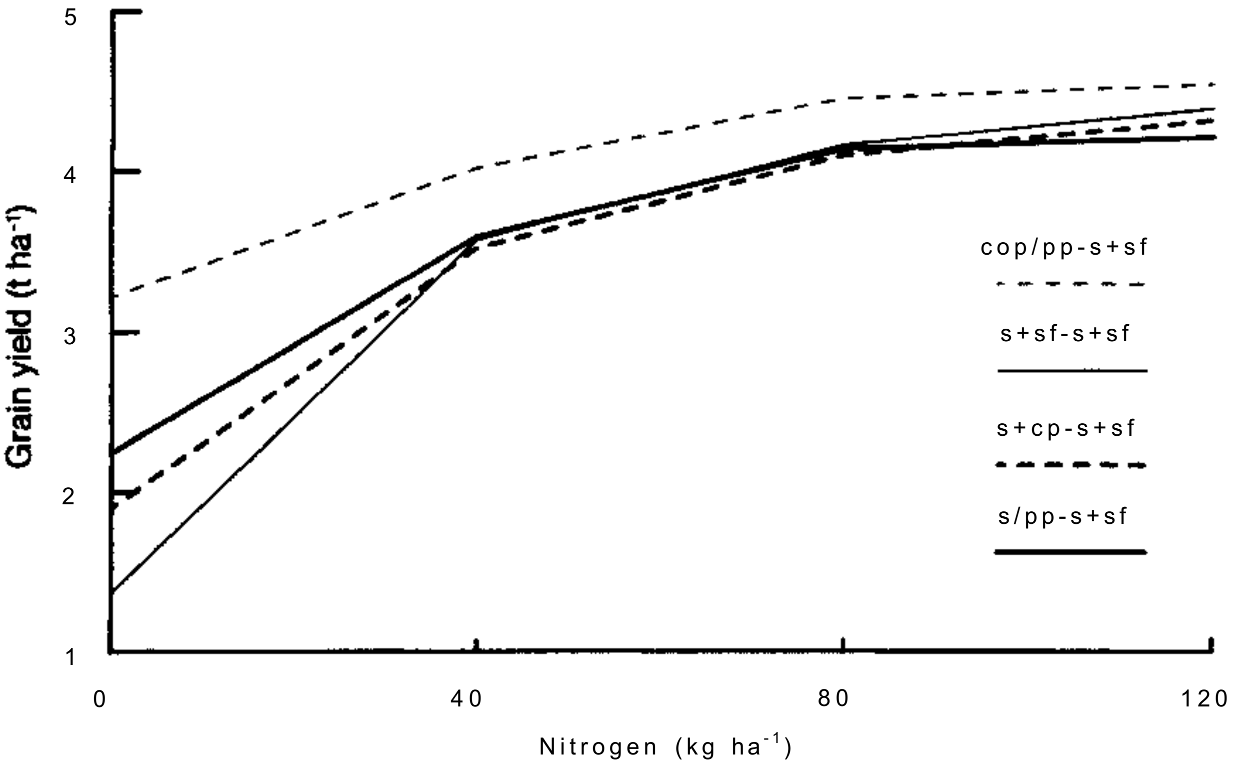

Figure 1. Mean grain yield of sorghum grown succeeding different cropping system in rainy season, 1983-92, ICRISAT Asia Center, Patancheru. (- = 2 year crop rotation, / = intercropped, + = sole crop grown during postrainy season, s = sorghum, pp = pigeonpea, sf = safflower, cp = chickpea, cop = cowpea.)

was reported that only 7-28% of the ^{15}N in legume crops is taken up by the succeeding grain crop (Ladd et al. 1983, S P Wani, unpublished results).

In the long-term experiment being conducted at ICRISAT Asia Center (with 2-year crop-rotation treatments), surface (0-20 cm) soil samples collected after the harvest of the 9th season's crop showed a higher mineral N content in soil under pigeonpea-based cropping systems than nonlegume-based cropping systems. Further, the N mineralization potential (N_0) of soil samples taken from pigeonpea-based cropping systems was almost twice that of the fallow + sorghum (F+S) treatment. Similarly, the 'active N fraction', the quotient of N_0 and N_{total} and expressed as a percentage, varied between 9 and 17% with higher values observed for soils under pigeonpea-based cropping systems. However, such results were not observed in chickpea-based cropping systems. Soil samples collected from the same field after 10 years indicated a substantial increase in total soil N in the case of pigeonpea-based systems (Table 2). In nonlegume-based or chickpea-based systems, there was a decline in total soil N.

Sorghum grown in pots filled with surface (0-20 cm) soil samples collected from the ICRISAT experiment after the harvest of the ninth year crop showed the effect of cropping history on plant growth. Sorghum yields were 36-63% higher in pigeonpea-based cropping systems than in the sorghum + safflower (S+SF-S+SF) treatment. In chickpea-based cropping systems, sorghum yields were 18-24% lower than the S+SF-S+SF plot yields. Using the ^{15}N methodology and the S+SF-S+SF treatment as control, it was estimated that 8.4-20% of the total plant N of

Table 2. Total soil N (ug g^{-1} soil) in soil samples taken from different cropping systems, ICRISAT Asia Center, 1983 and 1993.

	Soil depth			
	0--15 cm		15-30 cm	
Cropping system[1]	1983	1993	1983	1993
S/PP-S+SF	559	629	437	480
S+CP-S+SF	540	517	407	443
C/PP-S+SF	543	645	419	501
S+SF-S+SF	537	530	397	438
F+S-F+S	563	491	422	426
F+CP-F+S	567	507	399	446
M+S-M+S	558	559	422	461
F ratio	NS[2]	**[3]	NS	**
SE	±18.4	±13.2	±15.0	±14.4

1. S = sorghum; PP = pigeonpea; SF = safflower; CP = chickpea; C = cowpea; F = fallow; M = mung bean; / = intercrop; + = sequential crop; and - = rotation.
2. NS = Not significant.
3. ** = P < 0.01.

sorghum grown in soil taken from pigeonpea-based cropping systems was derived from N that was either fixed previously and had accumulated, or from soil N that was made available due to the presence of pigeonpea in the rotation. Also, the 'A' values for soil from the pigeonpea-based cropping system were higher by 26 to 76 mg pot^{-1} (5-13 kg N ha^{-1} equivalent) than that of the S+SF-S+SF treatment. The FRV for these treatments using soil from the S+SF-S+SF treatment ranged from 65 to 161 mg pot^{-1} (24-28 kg N ha^{-1} equivalent). These results indicate that increased sorghum yields in pigeonpea-based cropping systems are partly due to increased soil N availability, but that all the benefits cannot be explained in terms of N effects (S P Wani unpublished results).

Non-N effects. The overall benefits of legumes are not fully explained when only their BNF effects are considered. The other likely benefits include increased availability of nutrients other than N (through increased total soil microbial activity and/or increased activity of such specific groups of microorganisms as vesicular arbuscular mycorrhizae or plant growth promoting rhizobacteria), improved soil structure, enhanced level of growth-promoting substances, and reduced pest and disease incidence. The extent of these benefits are dictated by site, season, and crop sequence.

Reduced Legume Yields in Rotation

Generally, cropping-system trials in the tropics are conducted for short periods. Very few long-term trials are monitored. In the long-term trial at ICRISAT Asia Center, pigeonpea yields were observed to have declined (T J Rego unpublished results). To identify the causes for the fall in yields, experiments were conducted in the greenhouse. We confirmed lower yields when pigeonpea was grown in pots filled with soil from field plots of pigeonpea-based systems than when it was grown on soil from F+S-F+S plots. We noticed that the decreased pigeonpea yields were due neither to the increased incidence of fusarium wilt, nor to the increased number of parasitic nematodes (S P Wani unpublished results). They may be due to an allelopathic effect. This needs further research.

Improving the Contribution of Legumes in Cropping Systems

Although legumes have the ability to fix atmospheric nitrogen, it cannot be assumed that the inclusion of any legume in a cropping system will ensure significant contributions to the N cycle. As is evident from published reports, most legumes deplete soil N when plant material is removed from the field. To derive maximum benefits from legumes, we must take a holistic approach and understand the entire BNF and N-cycling system.

Host-plant improvement. Variability exists in legumes for the amount of N_2 fixed and for the proportion of plant N derived from BNF. We need to identify legumes and genotypes that yield more, and derive a large part of their N requirement from fixation. For example, compared to chickpea, pigeonpea returned a large amount of fixed N to soil through nodulated roots and fallen leaves. Similarly, there is a need to identify genotypes that can fix well under adverse soil conditions such as high soil N, soil acidity and alkalinity, Al and Mn toxicity, waterlogging, high and low soil temperature, etc. The natural occurrence of nonnodulating plants within chickpea genotypes indicates a need to ensure that their proportion in that genotype does not increase. Most plant breeding and testing work is done on research stations where soil mineral N is invariably higher than in farmers' fields. Nonnodulating and low-nodulating plants are therefore not discriminated against when selecting and testing improved genotypes. This has been demonstrated in chickpea and pigeonpea (see Rupela pages 75-83 this Report) and may also be true for other legumes. To avoid this, appropriate procedures must be adopted in breeding and testing programs.

Improved crop management. Appropriate crop and soil management practices should be followed to ensure maximum BNF contribution by legumes. For example, reduced BNF due to high mineral N in soil can be managed either by immobilization of the soil N through addition of organic material with a high C / N ratio or through reduced tillage. In intercropping situations in which application of fertilizer N is essential for obtaining high cereal yields, an appropriate form of fertilizer, e.g., slow-releasing formulations or organic N, should be used. Also, suitable methods of fertilizer application, e.g., placement of fertilizer in cereal crop rows rather than broadcasting and mixing in soil, must be followed. Appropriate amendments with nutrients other than N which might limit legume growth—and in turn BNF — should be applied.

Rhizobial inoculation. Under field conditions, response to rhizobial inoculation in traditional legume-growing areas has not been consistent. Situations which need inoculation should be identified and efforts must be focussed on such areas. Research for selection of efficient strains and identification of specific host-bacteria combinations must continue. The important constraints limiting the exploitation of inoculation technology are: 1. poor quality of the inoculants; 2. lack of knowledge about inoculation technology among extension personnel and farmers; 3. ineffective inoculant delivery systems; and 4. lack of appropriate policy support by governments that would favor use of inoculants by farmers.

Conclusion

In addition to the ability of a legume to fix atmospheric nitrogen, its contribution in a cropping system is due to its N sparing effect, the break-crop effect, and enhanced soil microbial activity. A dependable methodology to quantify the benefits derived from these different factors may be difficult to evolve, and will require long-term studies. However, a legume-based rotation is generally more sustainable than a rotation without a legume. Informed decisions to enhance the BNF of a legume crop, and thus its contribution in the cropping system, are essential. This can be achieved by using legume cultivars with high N_2-fixing ability, by ensuring a high population of efficient homologous rhizobia in the soil, and by employing appropriate agronomic practices for high BNF and high yield.

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Declining Yields in Cereal Cropping Systems: Can the Introduction of Legumes Help Arrest the Decline?

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Introduction

One of the prerequisites for sustainable agriculture is the maintenance and/or improvement of soil fertility. However, the intensive and exploitative farming systems that are being used to meet the growing food needs of an increasing population have resulted in declining crop yields and shrinking of the agricultural resource base, in both irrigated lowlands and rainfed uplands (Harrington 1991). This paper discusses some of the issues related to the decline in agricultural productivity due to inappropriate land-use systems, and the potential role of legumes in reversing this trend.

Influence of Cereal Cropping on Soil Productivity

Monocropping. In southern Queensland, Australia, continuous cropping and cereal cultivation on soils that previously supported native vegetation resulted in reduced organic matter content, lower nutrient-supplying capacity, and increased bulk density (Dalai et al. 1991). The lower the clay content, the greater was the rate of loss of organic matter under cultivation, and the larger the replenishments required to maintain organic matter at a steady level (Table 1). This situation may be similar for any cropping system involving cereals and legumes. However, there are few studies on this aspect.

Dalai et al. (1991) also reported that under cereal cultivation over several decades, soil organic N declined at a mean rate of 31-51 kg N ha^{-1} per year in a number of Australian soils (Fig. 1). In consequence, degradation of the soil structure and decreased soil aggregation were observed, along with declines in cereal yield and protein content.

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Table 1. Rate of addition of organic materials required to maintain the soil organic matter level at equilibrium or steady state, Queensland, Australia.

Soil series[1]	Great soil group	Clay content <%)	Soil texture	Rate of addition (t ha^{-1} $year^{1}$)
Waco	Black earth	72	Clayey	1.4
Thallon	Gray brown, and red clays	59	Clayey	0.8
Langlands-Logie	Grey brown, and red clays	49	Clayey	1.6
Cecilvale	Gray, brown, and red clays	40	Clayey	4.6
Billa Billa	Gray, brown, and red clays	34	Loamy clay	5.4
Riverview	Red earth	18	Sandy loam	29.2

[1] Dominant natural vegetation on each soil series: Dichanthium sericeum, Eucalyptus microtheca, Acacia harpophylla, Eucalyptus populnea, Casuarina cristata, and Eucalyptus melanophloia, respectively.

Reproduced with permission from Dalai et al. 1991.

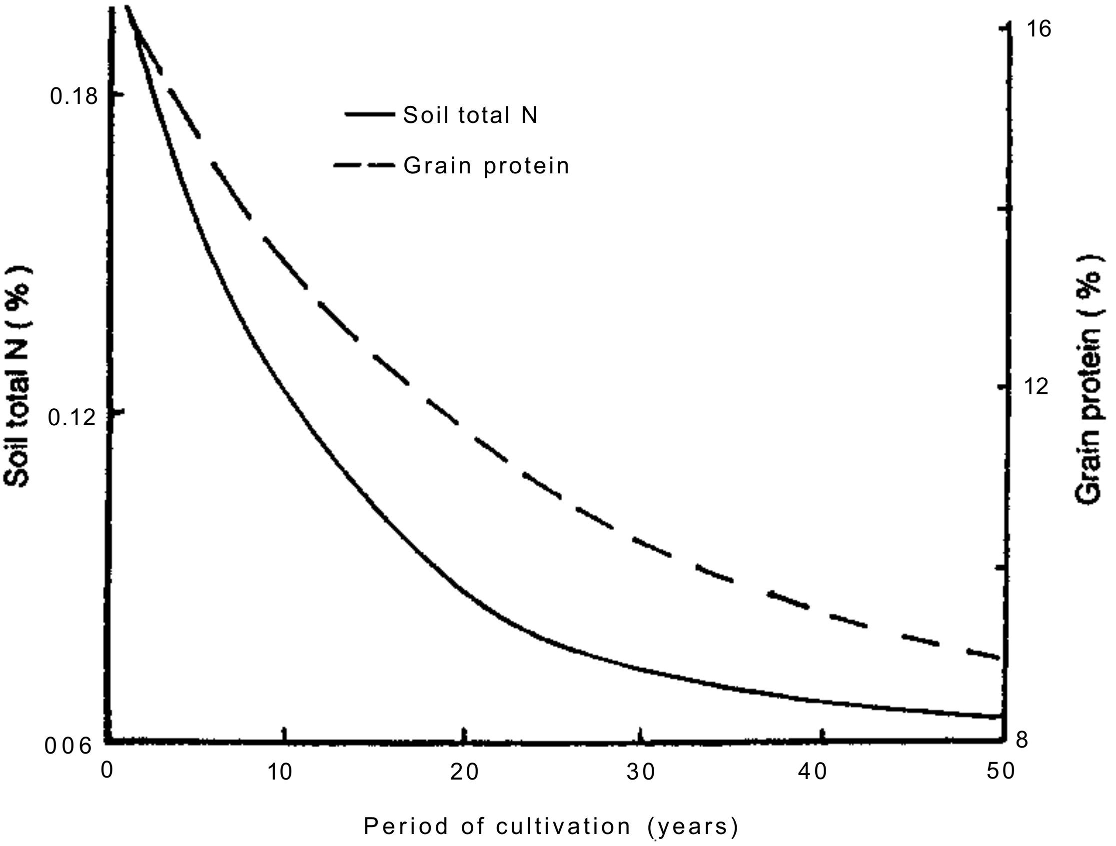

Figure 1. Decline in soil nitrogen and wheat grain protein under cereal cultivation over several decades in Australia.

1. Soil total N (%) = 0.068 + (0.201 - 0.068) exp (-0.086 yr).

2. Grain protein (%) = 8.0 + (16.5 - 8.0) exp (-0.0433 yr).

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When cereals were cultivated on the same field (monoculture) for 7 years in Pulaway, Poland, Niewiadomski and Grejner (1984) found that in the seventh year, grain yield was 41% lower in winter wheat, 31% in spring wheat, and 11% in spring barley than in rotations with legumes. In a long-term study (1973-84), Krejcir and Labounek (1988) observed similar results in barley. Stojanovic (1985) reported that application of fertilizers hardly alleviated the negative effects of long-term monoculture of wheat and maize, and suggested that cereals should be cultivated in rotation with legumes. Torres et al. (1988) reported that depletion of soil fertility in infertile upland areas with acidic soils was exacerbated by cereal monocropping without nutrient application, and recommended diversification into cereal-legume crop sequences to derive important nutrient cycling advantages.

Double cropping. Rice-wheat cropping is a dominant agricultural system in Bangladesh, China, India, Nepal, and Pakistan. It is estimated that rice-wheat rotation is practised on more than 23 million ha in Asia; 10.3 ha in China (Wang and Guo 1993), 11.3 million ha in India (Singh and Paroda 1993), 1.5 million ha in Pakistan and 0.5 million ha each in Bangladesh and Nepal (Singh and Paroda 1993). About 28% of the rice and 36% of the wheat areas of the five countries taken as a whole are under rice-wheat cropping. Several other countries in the region, including Bhutan, Japan, Democratic Peoples Republic of Korea, and Myanmar also have pockets of rice-wheat cropping. Other traditional rice-producing countries in the region, as Indonesia, the Philippines, Thailand, and Vietnam, have also been trying to produce wheat commercially in rice fallows, due to the increasing demand for wheat.

Concerns have been expressed about the sustainability of rice-wheat cropping systems (Abrol and Gill 1994). Continuous rice-wheat cropping is said to be overexploitative of the natural resource base. Under intensive rice production systems, particularly irrigated rice, yields generally stagnate or even decline over time (Kijne 1994). There are a number of reports that factor productivity and input-use efficiency are declining under intensive rice-based cropping systems, including the rice-wheat system (Singh and Paroda 1993). Higher input levels are required in order to maintain yields, as is indicated by farm-level data from Indonesia, the Philippines, and Thailand. The deterioration in productivity was found to be associated with the deficiency of secondary nutrients and micronutrients such as sulfur and zinc.

The profitability of rice-wheat cropping, when adjusted for currency depreciation, probably declined in many areas during the 1970s and 1980s, in spite of a decrease in production costs. Such declines in profitability are probably greater where support prices are so low as to be a disincentive for farmers to adopt or continue rice-wheat cropping as is the case of wheat in Pakistan and Bangladesh (Kijne 1994).

In Bangladesh, average wheat yields have declined since the peaks of 1983 and 1988. In the Terai region of Nepal reports from various sources give cause for concern, although no single set of data unambiguously confirms any long-term decline in productivity. In the Punjab province of Pakistan, this has been a familiar pattern: rapid growth in rice yields between the mid-1960s and mid-1970s, followed by a plateau and then a decline in the 1980s (Harrington et al. 1990).

Moreover, there are indications that the productivity of the resources devoted to the rice-wheat system is also decreasing. Water-induced land degradation (e.g., salinization, sodification, ground-water depletion) in the western part of the rice-wheat belt of India has become a major problem. Such problems as the gradual loss of soil fertility may also be occurring. In addition, the damage caused by certain pests, diseases, and weeds seems to be intensifying. Many of these processes (e.g., nematode build up) are obscure and puzzling. The consequence of all these processes, however, is clear: higher levels of inputs are needed merely to maintain crop yields. Total productivity is declining (Harrington et al. 1990).

Decreased productivity due to continuous cereal double-cropping has also been reported in other land-use systems. In the Chiang Mai Valley of northern Thailand, increasing food demands since 1960 led to the expansion of double- and triple-cropping. But the result was that soils which had adequately supported agriculture for over a thousand years began to show deficiencies and lower crop yields (Rerkasem and Rerkasem 1988).

Taylor (1984) reported that a maize-millet-sorghum rotation over 4 years on granitic soils in the Upper region of Ghana also resulted in declined sorghum and millet yields after one complete rotation.

Pillai et al. (1987) observed decreased crop yields in long-term field trials (1977-83) in India. Rego and Burford (1992) reported loss of soil N (25 ug g^{-1} of soil N) and decreased productivity in a continuous double-cropping (sorghum-safflower) system in long-term fertility trials on Vertisols at 1CRISAT Asia Center, India.

Can the Introduction of Legumes Help Arrest the Decline in Soil Fertility?

Depletion of soil fertility adversely affects crop yields and grain quality mainly on account of the reduced nutrient supply from the soil organic matter. The organic matter content of a soil depends upon the relative rates at which organic materials (crop residues, animal and green manures, and organic waste) are added to the soil and are depleted through decomposition. The greatest challenge to arable agriculture in the long term is the maintenance, and preferably, improvement of soil fertility. The management options in this regard include: application of N fertilizer; use of zero or minimum tillage in order to reduce the loss of organic matter through decomposition; and use of grain and pasture legumes in crop rotations. However, the increasing population pressures and decreasing landholdings do not allow Asian farmers to bring their cultivated lands under pasture legumes or to practice zero tillage because of the lack of mechanization, meagre resources, and fragmented landholdings. Moreover, resource-poor farmers cannot afford chemical fertilizers. Introducing grain legumes appears to be the logical solution. Several researchers have, therefore, suggested diversification into cereal-legume rotations to overcome the problem of reduced yields (Kang and Juo 1986, Singh and Paroda 1993).

Benefits of legumes. The beneficial effect of legumes on soil fertility and on other nonleguminous crops has long been a subject of interest to research workers. It is generally accepted that N_2 fixation by legumes retards soil N depletion and that a major part of the N_2 fixed becomes available, directly or indirectly, to the associated or succeeding crop (Giri and De 1979, Jones 1974). However, no uniform methodology has been adopted to assess the extent of these benefits, and opinions differ on the mechanism of N transfer from the legume to the associated or succeeding crop (Herridge 1982).

Rego and Burford (1992) reported that a grain legume intercropping system (cowpea/pigeonpea) benefited the succeeding sorghum crop to the equivalent of 40 kg fertilizer N ha^{-1} consistently for 8 years, while a cereal/grain legume system (sorghum/pigeonpea) and a cereal-grain legume sequential system (sorghum-chickpea) gave benefits equivalent to 25 and 10 kg fertilizer N ha^{-1}, respectively. A continuous sorghum/pigeonpea system increased soil N content by 140 ug g^{-1} and a continuous sorghum-chickpea system by 25 ug g^{-1}. In a 3-year comparison of legume-wheat and fallow-wheat sequences with a sorghum-wheat sequence, Shinde et al. (1984) found that first two sequences generally performed better than the latter at a medium level of N fertility management. In a continuous crop rotation for 4 years, the total soil N increased under all rotations in which a legume was included, but not under a maize-wheat-fallow system, with the highest build up of N being observed in rotations with groundnut (Jadhav 1990). Winter grain legumes enhanced the N and P status of the soil compared with cereal or fallow, and increased the yield and N uptake of succeeding maize (Ahlawat et al. 1981).

Similarly, the grain yield of wheat was significantly higher when it followed sorghum intercropped with cowpea or groundnut than when it followed sole sorghum (Waghmare and Singh 1984). The grain yield of maize was significantly higher when it was intercropped with black gram, cowpea, and green gram than when it was grown as a sole crop or intercropped with groundnut. In addition, maize intercropping with black gram, green gram, groundnut, or cowpea was found to increase the soil N content more than sole cropping. In general, in soils with lower levels of N, inclusion of legumes in the cropping system resulted in increased soil N content (Das and Mathur 1980).

Current transfer of N from legume to the associated crop. It is often assumed that legumes provide some N benefit to the associated crop in an intercropping system. In medium-duration pigeonpea, nodulation, acetylene reduction activity and percentage of nitrogen derived from the atmosphere (Ndfa) tended to be higher in intercropping with sorghum than in sole pigeonpea (Ito et al. 1993). Reddy et al. (1985) reported very little transfer of nitrogen from legume to nonlegume in intercropping systems, maize/groundnut, sorghum/cowpea, and sorghum/pigeonpea. They emphasized that it is difficult to prove N_2 transfer under field conditions as the nitrogen effects are often confounded with the other effects of intercropping. Although evidence of the transfer of symbiotically fixed N_2 to an associated crop has not been directly obtained by feeding labelled ^{15}N to the legume, there have been reports (Van Kessel et al. 1985) of direct hyphal linkage of mycorrhizae allowing transportation of nutrients between two root systems. Therefore, it is possible that the N_2 fixed in a legume is transferred through such a hyphal linkage to an associated nonlegume, but the nature and quantity of this transfer have not been substantiated under field conditions. However, Kumar Rao et al. (1987) reported that there was no evidence of any immediate benefit from the N_2 fixed by a legume (pigeonpea) to the associated nonlegume (sorghum). The negligible transfer of N_2 in intercropping systems could be due to the fact that i) legumes are a minor component in the system, and ii) in most grain legumes the fixed N_2 is harvested in the seed.

Residual effect of legumes on the succeeding crop. Another beneficial effect of legumes in a cropping system is the transfer of fixed N_2 to the succeeding crop. Using the ^{15}N isotope dilution method in a pigeonpea-cereal rotation, Kumar Rao et al. (1987) reported that the cereal derived some N_2 fixed by the preceding pigeonpea and that the residual benefit to the cereal was not only on account of the 'sparing' of soil N. The N requirement of maize following sole pigeonpea was reduced by 38-49 kg N ha'^{1} compared with maize following either fallow or sole sorghum, or a sorghum/pigeonpea intercrop. Similarly, mung bean, cowpea, and pigeonpea reduced the N requirement of a succeeding cereal crop (Shinde et al 1984). The magnitude of the residual effect depends on the preceding cropping system, preceding legume species, and the succeeding crop species. In most cases in the semi-arid tropics, residual N contribution by legume to the succeeding crop has been estimated to be equivalent to 30 to 70 kg fertilizer N ha^{-1} (Rupela and Saxena 1987, Rego and Burford 1992). Residual benefits have been assessed mainly in terms of increased grain and dry matter yields. However, legumes may also benefit succeeding crops by improving the soil structure, breaking the pest and disease cycle, and enhancing soil microbial activity.

Conclusion

Given the accelerated turnover of plant nutrients in intensive cropping, soil fertility dynamics assumes greater relevance to crop productivity than was previously considered. The problems associated with cereal mono- or double-cropping systems threaten the sustainability of food production security in populous Asian countries. Major tasks will be to pinpoint the specific areas or cropping systems that are most seriously threatened in these countries, identify the biological and physical causes of the problems, and develop, test, and promote the implementation of more sustainable, high-productivity cropping systems.

The commodity research model was ineffective in addressing post-Green Revolution system-level research issues. The productivity of a cropping system involves interactions that occur within crop rotations at specific sites. These post-Green Revolution problems require the evolution of joint research efforts among national programs focusing on system-wide constraints at specific research sites. This requires reorientation of the existing research systems. Hence, research planning should be done by multidisciplinary commodity teams that include social, biological, and physical scientists.

The planning process will also require a more detailed diagnosis and understanding of farmer-perceived constraints including characterization of the target site, and detailed analysis of the cause-effect relationships so that appropriate solutions can be developed. This improved 'systems-perspective' ecoregional model will require both short- and long-term research agendas that address not only productivity, but also the sustainability of the natural resource base.

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Soil Characteristics of Iron-chlorotic Groundnut Fields, On-farm Observations and Alleviation of the Symptoms

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Introduction

Iron (Fe) chlorosis is a major production constraint to groundnut grown on calcareous alkaline soils in many parts of the world. Although such soils are generally rich in total Fe content, the available Fe is very low due to the high pH and high buffering capacity of the soils which may impede Fe uptake in many crops (Marschner et al. 1986). Iron nutrition is of particular significance to legumes because it is involved in a range of physiological and biochemical processes associated with BNF (O'Hara et al. 1988). A recent review by Tang and Robson (1992) concluded that Fe deficiency impedes nodule formation, leghemoglobin production, and nitrogenase activity in several legumes, leading to low nitrogen concentration in the shoots. Therefore, an adequate supply of Fe is required for optimal BNF.

In a recent survey of farmers' fields in Andhra Pradesh (Vara Prasad 1993), it was reported that farmers applied 100-200 kg N ha^{-1} to groundnut in 2 to 4 splits to alleviate Fe chlorosis that was mistaken as N-deficiency symptoms. Other on-farm studies have shown that Fe chlorosis resulted in 30-45% economic yield losses (Anders et al. 1992).

In this paper, soil characteristics of farmers' fields regularly showing symptoms of Fe chlorosis of groundnut and the relationship between these characteristics are presented. The study also reports a preliminary trial to explore whether Fe chlorosis can be alleviated by applying rhizobial inoculants in combination with foliar Fe sprays.

Materials and Methods

A survey was conducted in Kurnool district of Andhra Pradesh and Nanded district of Maharashtra, and at ICRISAT Asia Center (IAC). Surface soil samples

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Potdar, M.V., Rupela, O.P., and Anders, M.M. 1994. Soil characteristics of iron-chlorotic groundnut fields, on-farm observations and alleviation of the symptoms. Pages 100-107 in Linking Biological Nitrogen Fixation Research in Asia: report of a meeting of the Asia Working Group on Biological Nitrogen Fixation in Legumes, 6-8 Dec, ICRISAT Asia Center, India. (Rupela, O.P., Kumar Rao, J.V.D.K., Wani, S.P., and Johansen, C, eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

(045 cm) were collected from 18 groundnut fields in Kurnool district, 4 fields in Nanded district, and 8 fields at IAC. The IAC fields were included in the study as a reference soil in which Fe chlorosis is occasionally observed. One bulked sample representing 10 subsamples from each field was analyzed for chemical properties related to Fe chlorosis (Table 1). Most samples were collected before sowing of groundnut, except at a few locations where they were collected when the crop was about 3 weeks old. Information on farmers' experiences with crop management and yield losses due to Fe chlorosis was collected from the farmers.

In addition, an exploratory study was conducted at two sites prone to Fe chlorosis at Banaganapalle village in Kurnool district during the 1992/93 postrainy season. A widely adapted but Fe-inefficient groundnut cultivar, TMV 2, was grown. Surface soil samples (0-15 cm) were collected before sowing and analyzed (Table 2). Most probable number counts of cowpea-group rhizobia were assessed by the plant infection method using siratro (Macroptilium atropurpureum) as a trap host (Brockwell 1980). The three main treatments were: 1. inoculation with Bradyrhizobium strain NC 92 (Rhiz), 2. recommended fertilizer practice (RFP) of 20 kg N and 22 kg P ha^{-1}, and (3) Rhiz + RFP. The subplots had two treatments:with Fe (foliar spray) and without Fe. The two sites were treated as replications. The gross plot size was 48 m^2 and net plot size 108 m^2 for each subplot. The RFP plots received 110 kg 18-46-0 grade DAP ha^{-1} (= 20 kg N and 22 kg P ha^{-1}) drilled at 0.3 m row spacing. A peat-based inoculant of rhizobial strain NC 92 was suspended in water at the rate of 1 g inoculum L^{-1} and was applied at 4 mL suspension seed^{-1} (= 225 g inoculum suspended in 225 L water ha^{-1}) with a gravity-flow applicator

Table 1. Soil characteristics of 22 farmers' groundnut fields in Andhra Pradesh and Maharashtra states of India and 8 experimental fields at ICRISAT Asia Center, postrainy season 1992/93.

	Farmers' fields[1]		ICRISAT fields	
Soil characteristic	Mean	Rabge	Mean	Range
pH (1:2 H_2O)	8.47	8.03-9.17	7.74	5.56-8.79
EC[2] (dSm^{-1})	0.63	0.21-2.03	0.21	0.05-0.33
$CaCO_3$ (%)	16.37	6.82-33.20	4.19	2.00-6.05
Mineral N (mg kg^{-1})	32.20	12.70-87.83	13.61	4.70-26.70
Total N (mg kg^{-1})	655.21	381-1080	630.67	505-955
Available Olsen P (mg kg^{-1})	14.39	4.00-50.00	17.61	8.00-28.00
DTPA[3]-extractable Fe (mg kg^{-1})	12.05	1.48-45.92	15.39	4.06-45.60
Total Fe (%)	4.05	1.59-7.47	2.03	2.01-2.04
$\log_{10}$ MPN[4] rhizobia g^{-1} soil	3.85	1.62-6.39	3.26	1.61-5.40

1. Inclusive of 18 fields in Kurnool district of Andhra Pradesh and 4 fields in Nanded district of Maharashtra.
2. EC = Electrical conductivity.
3. DTPA = Diethylene triamine penta acetic acid.
4. MPN = Most probable number.

Table 2. Soil characteristics of two experimental sites at Banaganapalle village in Andhra Pradesh, India, postrainy season 1992/93.

Soil characteristic	Site1	Site2
Soil type	Deep Vertisol	Deep Vertisol
Degree of chlorosis	Severe	Moderate
pH (1:2 H_2O)	8.8	8.5
EC[1] (dSm^{-1})	0.6	0.6
$CaCO_3$ (%)	16.0	10.8
Mineral N (mg kg^{-1})	28.5	17.9
Total N (mg kg^{-1})	433.0	563.0
Available Olsen P (mg kg^{-1})	5.3	7.5
DTPA[2]-extractable Fe (mg kg^{-1})	5.7	6.9
Log_{10} MPN[3] rhizobial count g^{-1} soil	2.22	3.2

1. EC = Electrical conductivity.
2. DTPA = Diethylene triamine penta acetic acid.
3. MPN = Most probable number.

in the furrows just before sowing. Sowing was done on 15 Dec 1992 by hand dibbling at 30 x 10 cm spacing. Commercial-grade Fe sulfate ($FeSO_4.7H_2O$) at 0.5% (w/v) with surfactant (2 mL Teepol® detergent L^{-1} water) was applied as a foliar spray at 35, 63, and 94 days after sowing (DAS).

The crop was regularly irrigated and given optimum plant protection and cultural practices. Plant growth and yield components were recorded on 10 plants randomly selected from each subplot at pod initiation (65 DAS), pod development (94 DAS), and maturity (134 DAS). The rate of N_2 fixation was measured using the acetylene-reduction activity method (Hardy et al. 1968) at 65 DAS. At harvest, all the plants from each 10.8 m^2 subplot area were harvested, and data on plant stand and dry pod and haulm (fodder) yields recorded. Plants were visually scored for the degree of chlorosis on a 1-5 point scale at 35, 63, and 94 DAS.

Results and Discussion

Survey. Soils across the surveyed area could be classified as Alfisols or Vertisols. Most farmers indicated that Fe chlorosis was a major production constraint causing 20-70% yield losses. However, they generally interpreted the chlorotic symptoms as being due to N-deficiency and responded by applying fertilizer N. The chlorosis could occur in patches or be uniformly distributed over the entire field.

The predominant crop rotations in the surveyed areas in Andhra Pradesh and Maharashtra were paddy-groundnut, groundnut-groundnut, onion-groundnut, sunflower-groundnut, cotton-groundnut, and sorghum-groundnut. These rotations indicate that groundnut in farmers' fields in these areas was generally preceded by high N-input cash crops. At IAC, groundnut generally rotates with pearl millet and sorghum. These cereal crops at IAC may not receive such high dosages of fertilizers as those in the surveyed areas. Indeed, the farmers' fields contained about three times more mineral N than those at IAC. High mineral N in soil is known to suppress BNF of legumes (Streeter 1988) which in turn may impair the natural ability of groundnut to acquire Fe through rhizobial symbiosis (Nambiar and Sivaramakrishnan 1987). Lack of symbiotic BNF may thus be one reason why Fe chlorosis was more severe in farmers' fields than at IAC where groundnut is generally rotated with cereals. Iron-inefficient genotypes (TMV 2 in Andhra Pradesh and SB XI in Maharashtra) were the most commonly grown genotypes (Reddy 1988, Vara Prasad 1993) in the surveyed fields.

The soil samples collected from farmers' fields were more alkaline (pH 8.47) and more calcareous ($CaCO_3$ 16.37%) than the soil at IAC (pH 7.74, $CaCO_3$ 4.19%) (Table 1). Similarly, farmers' fields were rich in mineral N (12.7-87.83 mg kg^{-1}), available P (4-50 mg kg^{-1}), total Fe (1.59-7.47%), and poor to rich in available Fe (1.48-45.92 mg kg^{-1}) when compared to the soil at IAC (mineral N 4.7-26.70 mg kg^{-1}, available P 8-28 mg kg^{-1}, total Fe 2.01-2.04 mg kg^{-1}, and available Fe 4.06-45.60 mg kg^{-1}). The cowpea-group rhizobial population (log_{10} MPN g^{-1} soil) was similar in surveyed farmers' fields and those at IAC. These results suggested that calcareous soils are rich in total Fe content, but the problem seems to be its utilization by the crop.

On-farm Trial

Chlorosis symptoms. Moderate to severe Fe chlorosis occurred as early as the seedling stage at both sites, and chlorotic symptoms persisted throughout crop growth. Chlorosis was more severe and uniform at Site 1 than at Site 2. This was reflected in the poor plant growth and high plant mortality in plots receiving no Fe sprays at this site. These experimental sites had a history of Fe chlorosis. Soil at both the sites was alkaline, calcareous, Fe-deficient, and rich in mineral N.

The combined application of Bradyrhizobium and fertilizer always resulted in a higher chlorosis rating than the application of either Bradyrhizobium or fertilizer alone (Fig. 1). Iron chlorosis symptoms disappeared within 5 days following the first Fe spray at 35 DAS, and subsequent sprays significantly reduced the symptoms (Fig. 1). The visual effect of each spray persisted for about one month. The generally low chlorosis rating observed in plots treated with Bradyrhizobium strain NC 92 may be related to its siderophore-producing ability (Nambiar and Sivaramakrishnan 1987).

Nodulation and nitrogen fixation. The number of nodules, nodule dry mass, and ARA measured on an area basis were not significantly affected by Bradyrhizobium and Fe sprays alone or in combination (Table 3). The application of Bradyrhizobium alone resulted in a significantly higher specific ARA than in the RFP treatment at 65 DAS. Such an adverse effect of fertilizer N on BNF has been reported for several legumes (Streeter 1988). Soil at the experimental sites contained 18-29 mg $kg-^{1}$ of

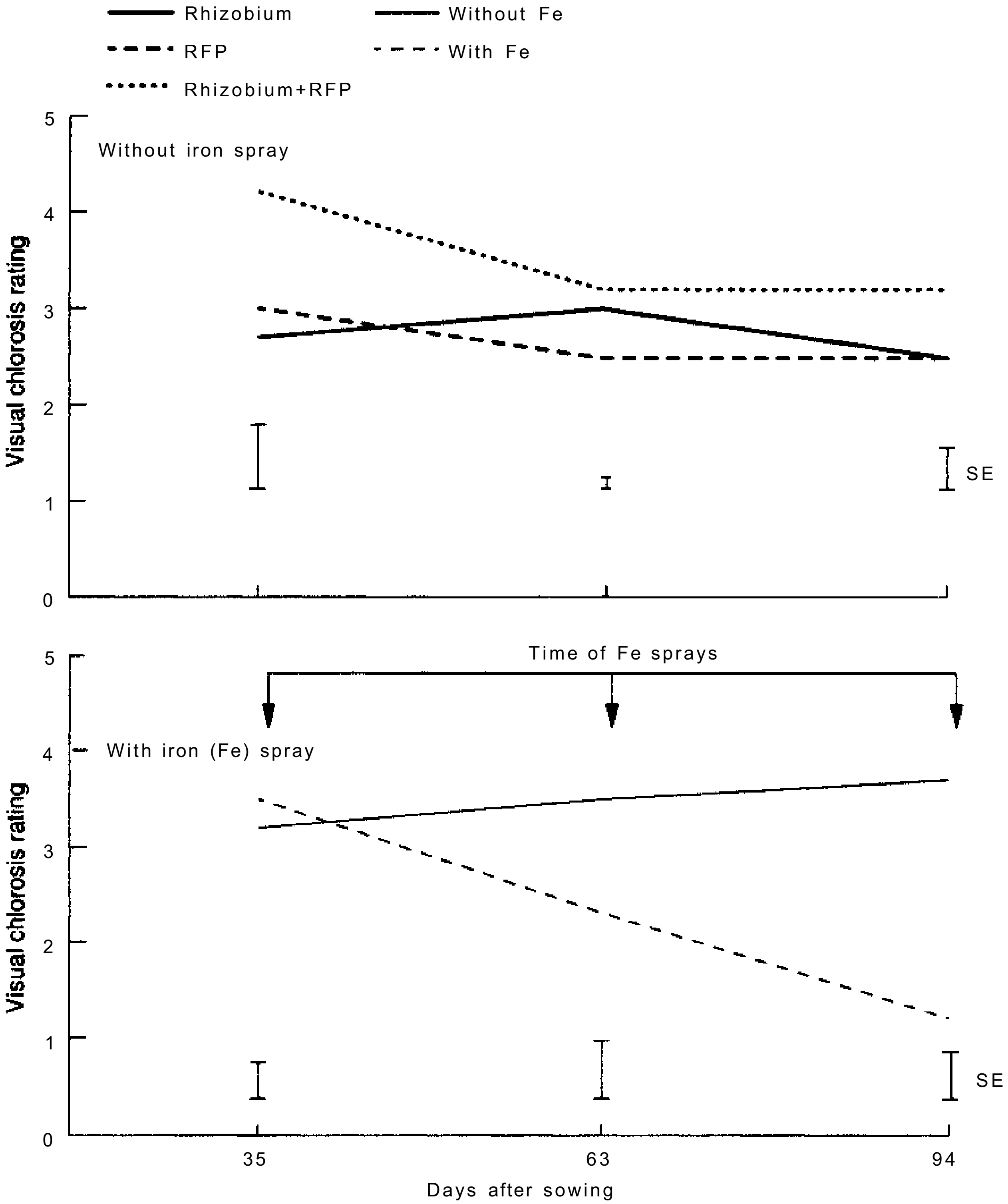

Figure 1. Mean visual chlorosis rating of groundnut under different fertilizer practices at Banaganapalle village, Andhra Pradesh. Visual chlorosis was measured on a 1-5 point scale, 1 = no symptoms (0% chlorosis) and 5 = severe (76-100% chlorosis).

Table 3. Influence of Bradyrhizobium and iron sprays on nodulation, acetylene-reduction activity (ARA), and growth of groundnut at 65 DAS in farmers' fields at Banaganapalle, postrainy season 1992/93.

Treatment	Nodule number $plant^{-1}$	Nodule dry mass (mg $plant^{-1}$)	ARA (umol C_2H_4 g^{-1} nodule dry mass)	Shoot dry mass (g $plant^{-1}$)
Fertilizer practice				
Rhiz[1]	13	9	109	1.73
RFP[2]	12	9	74	1.84
Rhiz + RFP	13	7	62	2.04
SE	±1.5	±1.4	±5.0*	±0.10
Fe spray				
Without $FeSO_4$	12	8	87	1.67
With $FeSO_4$	14	9	76	2.08
SE	±0.8	±0.9	±22.4	±0.11*
Interaction				
SE	±1.8	±1.8	±27.8	±0.17

1. Rhiz = Bradyrhizobium application.

2. RFP = Recommended fertilizer practice.

* Significant at P = 0.05 level.

mineral N (Table 2) that may have suppressed N_2 fixation. Nodule initiation seems to be the stage most sensitive to Fe deficiency in many legumes (Tang and Robson 1992). In this study, the first Fe spray was applied at 35 DAS, well after the nodule-initiation stage in groundnut. Therefore, nodule number and nodule mass $plant^{-1}$ remained unaffected despite the high native population of rhizobia at Site 2 (Table 2) and rhizobial application.

Growth and yield. The application of Bradyrhizobium and Fe sprays alone or in combination had no significant effect on leaf area and plant dry mass (leaf, stem, pod, root, and total) at 65, 94, and 134 DAS (data not shown). This may be partly explained by the large growth differences between the experimental sites and the lack of enough replications.

Groundnut yield (pod, haulm, and total dry matter) and harvest index were not significantly affected by any treatment or interaction of treatments except for shelling percentage (Table 4). Foliar Fe sprays significantly increased shelling percentage over the nonsprayed control. Shelling percentage was significantly influenced by fertilizer practices and by Fe spray interaction (Table 4). The plots treated with a combination of recommended fertilizer (RFP) and foliar Fe sprays

Table 4. Influence of Bradyrhizobium and iron sprays on groundnut yield and yield components in fanners' fields at Banaganapalle, Andhra Pradesh, postrainy season, 1992/93.

Treatment	Plant stand ('000 ha^{-1})	Dry yield (t ha^{-1})			Shelling (%)
		Pod	Haulm	TDM[1]	
Fertilizer practice					
Rhiz[2]	288	0.82	2.73	3.55	66
RFP[3]	301	0.76	2.63	3.39	67
Rhiz + RFP	312	0.72	2.98	3.70	60
SE	±24.9	±0.057	±0.210	±0.249	±1.9
Fe sprays					
Without $FeSO_4$	285	0.69	2.44	3.13	61
With $FeSO_4$	315	0.84	3.13	3.97	67
SE	±17.2	±0.065	±0.239	±0.302	±0.7**
Interaction					
SE	±32.6	±0.10	±0.36	±0.44	±2.0*

1. TDM = Total dry matter,
2. Rhiz = Bradyrhizobium application.
3. RFP = Recommended fertilizer practice.

* Significant at P = 0.05, ** Significant at P = 0.01

gave the highest shelling percentage (69%), whereas the lowest shelling percentage (54%) was obtained in plots applied with a combination of RFP and Bradyrhizobium culture but without Fe sprays.

Conclusions

The decrease in chlorotic symptoms following foliar Fe sprays suggested that the observed symptoms were induced by Fe deficiency and not by N deficiency as perceived by farmers. Mineral N at the experimental sites was indeed higher than in the groundnut fields monitored at IAC where severe Fe deficiency symptoms, even in sensitive cultivars, were only occasionally observed. Nodule number and nodule dry mass $plant^{-1}$ were not significantly affected by Bradyrhizobium application or Fe sprays. We attribute this to the high soil mineral N and, therefore, the desired effect of reduced Fe deficiency symptoms due to the siderophoretic properties of the *Bradyrhizobium* strain NC 92 was not apparent. These preliminary studies need confirmation with treatments ensuring adequate establishment of the BNF symbiosis.

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Concluding Session

Recommendations

The participants endorsed the initiation of the Asia Working Group on Biological Nitrogen Fixation in Legumes (AWGBNFL) and regarded it as an opportunity to test/validate the benefits of BNF in legumes as part of the effort to develop sustainable agricultural production systems. They endorsed the Working Group concept as explained by Gowda and Ramakrishna (see page 2 this Report) and the proposed structure of the AWGBNFL (see Appendix). The participants made the following recommendations:

- Scientists from CLAN countries actively participating in the Working Group will form a core group of researchers and be called AWGBNFL members. Researchers with marginal or conditional interest at present are encouraged to join the Working Group as associate members.
- ICRISAT should provide a common platform and act as Technical Coordinator to achieve the objectives of the Working Group.
- The group observed that rhizobial inoculation of legumes is the best-bet BNF technology presently available for on-farm use. There is a need to collate the experiences of scientists who have conducted inoculation trials in different countries, including those conducted as part of international networks such as NifTAL. Areas where *Rhizobium* inoculation technology would be successful need to be identified and the technology needs to be tested on farmers' fields with a view to identifying constraints to adoption of inoculation technology by farmers.
- The group recognizes the value of long-term trials and demonstration plots for sensitizing farmers, peer scientists, and extension agencies. Members are encouraged to set up such trials/plots on various aspects of BNF at their locations. Observations on BNF-related variables should be made in on-going long-term trials where available.
- As is implied in the Working Group concept, AWGBNFL members should obtain funds from their own institutions to conduct research on mutually agreed topics. Additional funds may, however, come from donors in due course. Members are encouraged to develop proposals seeking funding. The Technical Coordinator will assist in identifying prospective funding sources and finalizing proposals for submission.

AWGBNFL Work Plans for 1994 and 1995

Introduction

In line with the Working Group concept, experiments were planned through shared vision/experience on topics of mutual interest. The following four topics were considered relevant under the agreed objectives of the AWGBNFL.

1. On-farm rhizobial inoculation trial.
2. Performance of high BNF selections of chickpea cultivars ICC 4948 and ICC 5003.
3. Determining the need to develop N-tolerant BNF symbioses in legumes.
4. Identification of nodulation variants in legumes.

The first two experiments attracted general support. Therefore, details of these two experiments were discussed and defined and are presented here. Opinion on the latter two experiments was divided. Existing workloads perhaps prevented scientists from committing to too many new experiments. It was agreed that experiment number 3 was partly covered under experiment number 1. Available nodulation variants of chickpea identified at ICRISAT Asia Center and now available for confirmation in different climatic and rhizobial backgrounds as experiment number 1 was considered a prerequisite for experiment number 4 before scientists could take up further work in this direction. It was felt that scientists can themselves develop details for experiment number 4, if interested, using the information given in the paper by Rupela (pages 76-84 this Report). Therefore, details of experiment numbers 3 and 4 are not presented here. If enough interest is found, details of these two experiments can be made available in due course, or at the time of next meeting.

All interested are encouraged to join this joint venture. Clarifications and suggestions to improve the conduct of these trials are welcome.

Experiment number: AWG 94/01

1. Title

On-farm rhizobial inoculation trial

2. Introduction and aim

During the last 15 years, agricultural research systems in several countries have been conducting inoculation trials, independently or as part of networks such as NifTAL. The response to inoculation, in terms of increased yield, has been reported to be unpredictably inconsistent. This has worried researchers and research administrators alike.

Two recent publications from NifTAL[1] have suggested that response to inoculation can be predicted, as is the case with some other agricultural inputs, and even modeled. The mineral N level and the population of homologous rhizobia are the major determining factors in the NifTAL model called 'Response', Though researchers may have the facilities to conduct response to inoculation trials on research stations and in farmers' fields, only a few may have the facilities to determine the population of relevant rhizobia in the soil. Even the procedures followed to determine mineral N in samples collected from farmers' fields seem to require an update. As a result, most on-farm trials fail to explain the reason(s) for the presence or absence of a response. Trials without back-up data do not have much value, and have obviously created the impression that responses to inoculation are inconsistent. Scientists with access to facilities to measure mineral N and rhizobial population levels at experimental sites are encouraged to take up this trial because these are the minimum observations required to understand the need to inoculate and to identify the shortcomings of inoculation technology.

On-farm trials have demonstration value. It will be a good idea to involve extension agencies in this effort right from the planning stage. Alternatively, the trial could be conducted in an area where extension agencies are already active.

3. Experimental details

3.1 Treatments

a. No inoculation (control).

b. Inoculation.

c. Adequate soil N status (100 kg N ha^{-1} as urea, half at sowing and half as top-dressing between 30 and 50 DAS).

Notes: 1. To be followed by a nonlegume (preferably a cereal) in the following cropping season.

2. Though response to N application by a legume is only occasionally observed, treatment 'c' is considered important as a reference for yield of the legume under N-sufficient conditions.

3. Uniform application of 16 kg P (=200 kg single super phosphate) ha^{-1} is to be applied at land preparation because in several fields P may be limiting BNF.

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[1] **Singleton, P.W., Thies, J.E., and Bohlool, B.B. 1992.** Useful models to predict response to legume inoculation. Pages 245-256 *in* Biological nitrogen fixation and sustainability of tropical agriculture (Mulongoy, K., Gueye, M., and Spencer, D.S.C., eds.). Chichester, UK:John Wiley and Sons.

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3.2 Design and layout

A replicated trial even in farmers' fields will be ideal. However, it was argued in the meeting that selecting a farmer's field and an experimental site is a very difficult task. Therefore, using different farmers' fields in a village as replications, as is generally done for on-farm trials, was considered sufficient.

There was a great deal of discussion on the plot size for each treatment. Many participants favored a minimum of 1000 m^2. Some felt that plots of such size may be difficult to get in some countries where land holdings are generally small. The following guidelines should, however, be followed:

- Identify an area/field where poor nodulation previously been observed.
- Select the biggest possible field (not smaller than 400 m^2) in agreement with the farmer, and divide it in half for the two treatments.

3.3 Location

Scientists from the following locations agreed to conduct this trial: CCSHAU, Hisar; IARI, New Delhi; RAK College of Agriculture, Sehore; University of Agricultural Sciences, Bangalore (all in India); BARI, Bangladesh; NARC, Nepal; and Khon Kaen University, Thailand. **Anyone else who is interested is welcome to join this effort.**

3.4 Genotypes

Legumes and genotypes of interest to the farmer, or those judged best by the scientist can be used.

3.5 Inoculant

Inoculants obtained from a reliable source and previously tested for quality must be used.

3.6 Precautions against pests, diseases, weeds

According to the agronomic practices of the area.

Note: As far as possible, all the trials in a village should be on the same crop species using the same treatments so that each trial can be considered as a replication for statistical analysis.

3.7 Special requirements

3.71 Selecting the farmers
3.7.2 Understanding their cropping system and other needs

3.7.3 Developing a rapport with the farmers and selecting the fields

3.7.4 Preparing a work calendar agreeable to the farmers

3.7.5 Applying basal doses of P and N where relevant (see Notes under 3.1)

3.8 Procedural details

Staff familiar with field trials and rhizobial inoculation technology must be present at sowing. Trials may be confined to a village, or spill over to nearby villages. The liquid method of inoculant application has been found to be the best and can be used even in farmers' fields. A handbook titled 'Methods of rhizobial inoculation' can be obtained on request from OP Rupela, ICRISAT Asia Center. Other agronomic practices including harvest practices should be those followed in the area.

3.9 Observations, records, and measurements

3.9.1 Name of the farmer, address, and size of plot for each treatment.

3.9.2 Source of inoculant, name of rhizobial strain

3.9.3 Type of soil.

3.9.4 Dates of fertilizer application, sowing, harvest, and other important operations.

3.9.5 pH, EC, mineral N (KCl extract method[2]), total N, Olsen P concentrations at experimental site at sowing.

Note: At least four spots per treatment, two depths (0-15 cm, 15-30 cm). Samples within a plot should be pooled according to depth so that there are two samples per treatment plot.

3.9.6 Soil moisture at sowing (at 0-15 cm depth).

3.9.7 Nodule number, nodule mass (measured once at about flowering). Sample size should preferably be 0.5 m^2 per plot, or a minimum of 10 plants per plot from one location, excluding border plants.

Note: Observations on ^{15}N assessment by the natural abundance method are suggested, where feasible.

3.9.8 Total biomass and grain yield of both legume and nonlegume.

Size of plot = full (leaving 1 m border on all sides).

3.9.9 Economics

Note: Stover and grain should be costed on the basis of the prevailing wholesale prices. All economic inputs must be accounted for.

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[2] Mineral N and not total N is considered most relevant and interacting with BNF in legumes. Although N is the most mobile of mineral nutrients, its assessment at sowing may give a good idea of its interaction with BNF. The KCl extract method of Keeney and Nelson (1992) [Keeney, D.R., and Nelson, D.W. 1992. Nitrogen - inorganic forms. Pages 643-694 *in* Chemical and microbiological properties (Page, A.L., Miller, R.H., and Keeney, D.R., eds.). 2nd edn. Wisconsin, USA: American Society of Agronomy] is recommended for assessment.

Experiment number: AWG 94/02

1. Title

Performance of high-nodulating selections of chickpea cultivars ICC 4948 and ICC 5003.

2. Introduction and aim

Recent studies at ICRISAT Asia Center have established wide intracultivaral differences in nodulation capacity in chickpea plants. Studies have also indicated that high-nodulating selections fix significantly more N_2 than low-nodulating selections of the same cultivar. Also, high-nodulating selections tend to yield more than the low-nodulating selections at low soil N levels. However, at N levels higher than 15 mg kg^{-1} soil, there was no such consistent trend in nodulation, biological nitrogen fixation, or yield. Multilocational trials involving selections from two cultivars are proposed, in order to assess their performance. The material may face a different population spectrum of native root-nodule bacteria and other soil and environmental factors at these locations, which should help to assess the performance of high-BNF selections.

3. Experimental details

3.1. Treatments

3.1.1 N levels = Low (N1), High (N2).

Note: Soil mineral N levels higher than 15 mg kg^{-1} soil have been found to suppress BNF in chickpea on Vertisols at ICRISAT Asia Center. After selecting a field, we grew sorghum in the rainy season to further deplete the mineral N in the soil. (Remember, it is the mineral N, more specifically $N0_3$-N, which suppresses BNF in legumes.) Application of 100 kg N ha^1 to the sorghum of high-N (N2) plots provided two contrasting mineral N levels, low (NI) and high (N2), at the time of sowing chickpea in the following postrainy season.

3.1.2 Genotypes = 8

ICC 4948: HN, LN, B
ICC 5003: HN, LN, B
ICC 4993: NN, ICC 4918 NN (reference base)

Note: HN = high-nodulating selection
LN = low-nodulating selection
NN = nonnodulating selection
B = unselected bulk.

3.2 Design and layout

3.2.1 Design : Split-plot; main plot = N levels, subplot = genotypes

3.2.2 Replications: 4

3.2.3 Plot size : 4 x 3 m (minimum)

3.2.4 Total experiment: 2 (N levels) x 8 (genotypes) x 4 (replications) x 12 m^2 (size of one plot) = 0.08 ha + alleys

3.2.5 Plant density : 30 x 10 cm

3.2.6 Layout: To be decided by the leading member at the location

3.3 Location

Depending on the interest of scientists from different countries and on the availability of seed.

3.4. Genotypes

As in 3.1.2

3.5. Precautions against pests, diseases, and weeds

According to the routine agronomic practices of the area.

3.6. Special requirements

3.6.1 Procuring seed from ICRISAT Asia Center

3.6.2 Experimental site must not have the problem of fusarium wilt caused by *Fusarium oxysporum* f sp. *ciceri,* because the genotypes proposed for the trial are susceptible to this disease.

Note: We are attempting to identify materials of contrasting nodulation capacities from wilt-resistant lines.

3.6.3 Ensure low N in the field by growing a cereal crop in the rainy season to deplete soil N from NI plots. N2 plots, also growing the same cereal crop, should receive at least 100 kg N ha^{-1} in two split doses.

3.6.4 Review the need for any special equipment, items to be borrowed or purchased for the trial.

3.7. Starting date and duration

Rainy season : Jun/Jul to Sep 1994.

Postrainy season : Oct/Nov 1994 to Mar/Apr 1995.

3.8. Procedural details

3.8.1 Achieve two contrasting mineral N levels preferably by applying 100 kg N ha^{-1} to N2 plots of the preceding cereal.

3.8.2 Follow routine agronomic practices, but modify where required to achieve a nodulation environment close to optimum, at sowing of chickpea:

3.8.2.1 Sowing date should be adjusted so that ambient temperature is 30°C or less.

3.8.2.2 Optimum level of nutrients for nodulation, particularly P, should be applied.

3.8.2.3 Native rhizobia should be abundant, $>10^3$ g^{-1} soil. However, apply an efficient strain as liquid inoculant at sowing.

3.8.2.4 Soil moisture at sowing must be the optimum to achieve about 100% emergence.

3.9 Observations, records, and measurements

3.9.1 Site characterization : pH, EC, mineral N, total N, Olsen P in N1 and N2 plots, separately at sowing of chickpea.

Note: For recommended soil sampling procedure read the notes prepared by B Seeling and T J Rego (copies available from IAC).

3.9.2 Soil moisture at sowing

3.9.3 Soil type

3.9.4 Assessment of BNF traits:

Nodule niunber, nodule mass, shoot mass, at about 40-60 days. Sample size should preferably be 0.5 m^2 per plot.

Desirable : ^{15}N assessment using the natural abundance method.

3.9.5. Total biomass and grain yield of chickpea.

Note: One row on each side of a plot and 30 to 50 cm at row ends must be left as borders and excluded from yield assessment.

3.9.6. Stover N%, seed N%.

Note: A subsample from each plot for stover (chaff) and seeds at harvest should be used for the purpose.

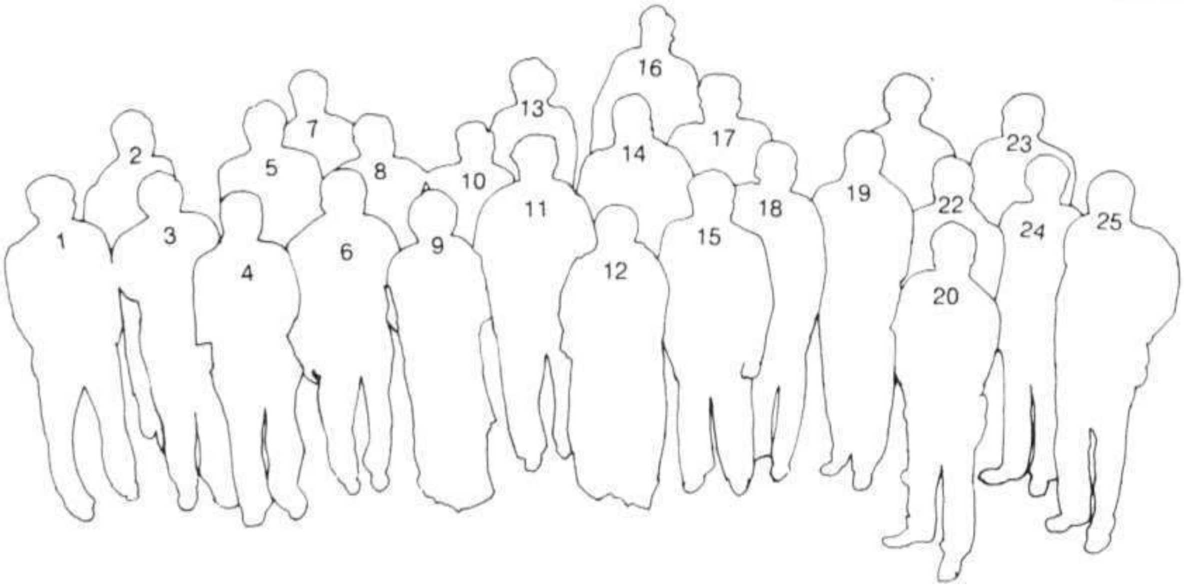
Closing

OP Rupela thanked the participants of the meeting for their contribution to improving and endorsing the scope, structure, and objectives of the Working Group. Research topics of wide interest of members were expected to be addressed through sharing resources and wisdom. It was hoped that the Working Group will act as a critical mass of scientists to promote BNF technologies through on-farm activities.

He thanked the management of ICRISAT for their support, D McDonald, C Johansen, and CLL Gowda for their support and guidance in initiating the Working Group, and wished all the visitors a safe journey home.

First Meeting
Asia Working Group
on Biological Nitrogen Fixation in Legumes (AWGBNFL)

6-8 December 1993



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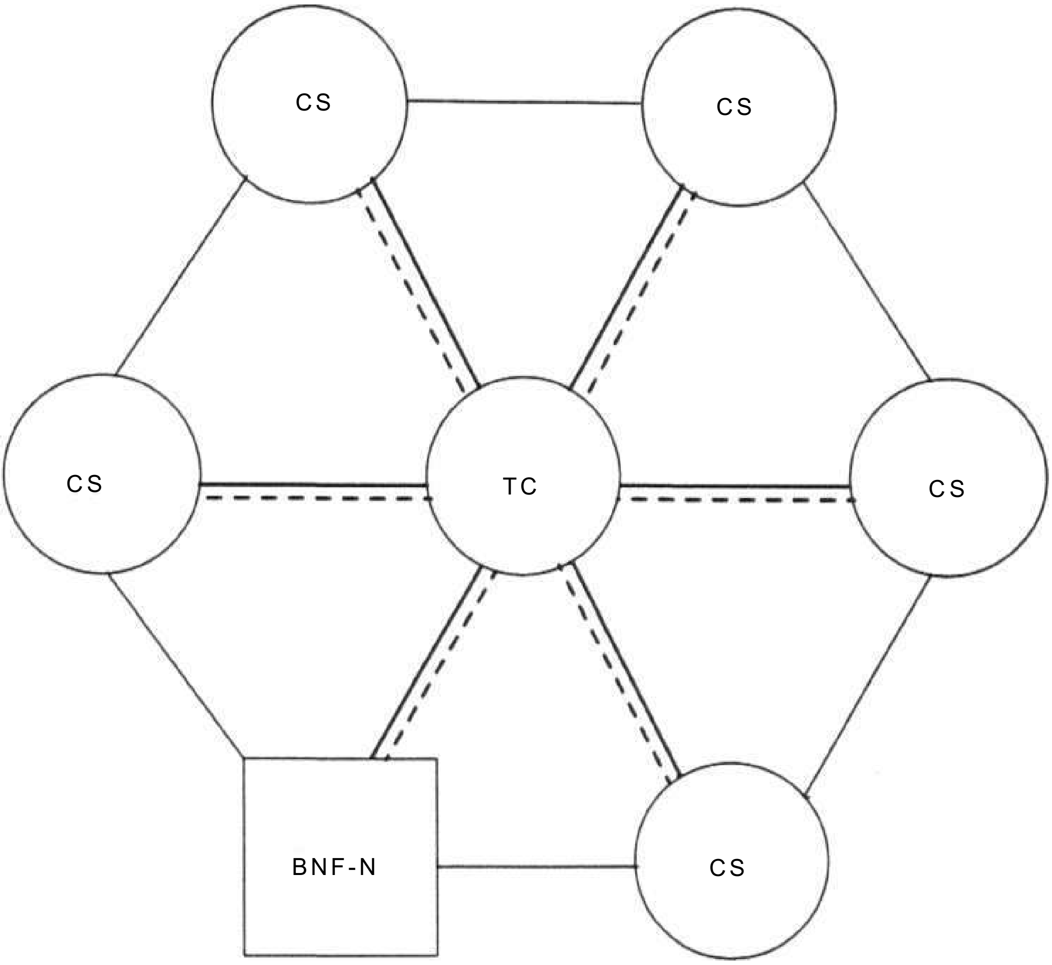
Acronyms

AGLN	Asian Grain Legumes Network
AICORPO	All India Coordinated Research Project on Oilseeds
AICPIP	All India Coordinated Pulses Improvement Project
AICRPS	All India Coordinated Research Project on Soybean
ARA	Acetylene-reduction activity
AWGBNFL	Asia Working Group on Biological Nitrogen Fixation in Legumes
BARI	Bangladesh Agricultural Research Institute
BAU	Bangladesh Agricultural University
BINA	Bangladesh Institute of Nuclear Agriculture
BNF	Biological nitrogen fixation
CCRN	Cooperative Cereals Research Network
CCSHAU	Chaudhary Charan Singh Harayana Agricultural University (India)
CLAN	Cereals and Legumes Asia Network
CRIDA	Central Research Institute for Dryland Agriculture (India)
Cv	Cultivar
DAS	Days after sowing
DOAE	Department of Agricultural Extension (Thailand)
DPR	Directorate of Pulses Research (India)
DTPA	Diethylene triamine penta acetic acid
EC	Electrical conductivity
HN	High nodulating
FRV	Fertilizer replacement value
IAC	ICRISAT Asia Center
IARI	Indian Agricultural Research Institute
ICAR	Indian Council of Agricultural Research
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
INLIT	International Network of Legumes Inoculation Trials
LN	Low nodulating
MER	Monitoring and evaluation report
MFO	Marketing and farmer organization
MOAC	Ministry of Agriculture and Co-operatives (Thailand)
MPN	Most probable number

NARC	National Agriculture Research Council (Nepal)
NHI	Nitrogen harvest index
NifTAL	Biological Nitrogen Fixation for International Development (USA)
NN	Nonnodulating
NRCG	National Research Centre for Groundnut (India)
PS	Private sector
RNB	Root-nodule bacteria
SNA	Specific nitrogenase activity
SPP	Single plant progenies
TC	Technical coordinator
VAM	Vesicular arbuscular mycorrhiza
WAS	Weeks after sowing
WG	Working Group

Appendix

Asia Working Group on Biological Nitrogen Fixation in Legumes (AWGBNFL)



TC = Technical coordinator

CS = Country scientist(s)

BNF-N = Existing networks on biological nitrogen fixation

—— = Link between country scientist(s) and coordination unit

- - - - = Linkages among country scientists and BNF networks through technical coordinator

—— = Direct linkages among country scientists and BNF networks

ICRISAT

International Crops Research Institute for the Semi-Arid Tropics
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1992

Proposal for an Asia Working Group on Biological Nitrogen Fixation in Legumes (AWGBNFL)

O P Rupela, J V D K Kumar Rao, C Johansen, S P Wani, R C Nagoswara Rao, C L L Gowda, and D McDonald

Background

Legumes have long been considered important in sustaining yields of cropping systems. Biological nitrogen fixation (BNF) is a major factor of this sustainability. With this role in agricultural production and in the nitrogen cycle, BNF is an important research aspect. Reduced funding support for BNF research in the recent past perhaps reflects lack of fulfillment of the expectations of the donor agencies. However, we believe that a comprehensive understanding of BNF has been generated in the past two decades. It is proposed to assemble components of this understanding for on-farm testing and to demonstrate its role in sustaining high yields. Previous and some existing networks on BNF emphasize rhizobial aspects. Many research results, however, strongly suggest that the host plant and environmental factors play an overriding role in this symbiotic process. Some recent experience and literature strongly suggest the need for a renewed look at this research area, particularly when sustainably high levels of production in grossly different agroecological environments are needed. We are therefore exploring the scope of initiating a working group that would follow a problem-solving approach, largely through host-plant selection. Our initial efforts suggest considerable scope for such an approach. The purpose of this communication is to solicit interest among research groups and scientists concerned with BNF in legumes in the Asia region and then, if the response is positive, arrange appropriate communication channels to initiate group activities.

Current Research Status/Knowledge

- Most fields where legumes have been traditionally grown have high populations of native rhizobia that will nodulate these legumes. Some cropping systems, such as flooded rice, drastically reduce native rhizobial populations.
- Even it is possible to identify rhizobial strains that form superior nitrogen-fixing symbioses with host-plant, when inoculated with seed such strains are usually poor competitors with native rhizobial strains in forming nodules, except when the native rhizobial population is low.
- The BNF process is sensitive to several agriculturally important abiotic factors, such as moisture, temperature, and available nitrogen in soil.

- Mutants of hosts tolerant to high mineral nitrogen and with supernodulation are known in some legumes, but they lack yield potential
- Some insect larvae feed on nodules.
- Host genotyes vary widely in nodulation traits, but the expected correlation between nodulation traits and yield is generally absent or weak.
- Nonnodulating (Nod-) lines of some legumes are known, or can be developed, to serve as references for quantification of nitrogen fixation.
- A good understanding of the nitrogen fixation process at the molecular level has been developed.

Objectives

- Validate best-bet BNF-technology on farmers' fields and use this experience to update the technology.
- Characterize BNF constraints and identify solutions through host-plant selection.
- Stimulate research to identify host plants and bacteria that will develop constraint tolerant symbioses.
- Quantify realizable benefits from BNF in different agroecological environments.
- Facilitate linkages among and between participants to achieve the above objectives.

Scope and prospects

It is possible to demonstrate the positive contributions from BNF in soils where nitrogen is limiting. Legume roots take up soil nitrogen depending on its availability, thus diminishing the possibility of demonstrating BNF contributions to increased yield. To be convincing, therefore, the BNF contributions need to be quantified for different cropping systems over long periods. This should help set realizable benefits in different agroecological environments.

Indication of declining yields over years of cropping suggests the need for a new look at nutritional aspects of crop growth in cropping systems, including the expected and realized contributions from BNF. It has been observed tha rhizobia in free-living form are more tolerant of stress factors tnan their host plants. This, coupled with the presence of inter- and intra-cultivaral differences in the extent of nodulation, suggests the possibility of identifying plants with optimum and stress-tolerant symbioses.

There is an apparent similarity in the research output and the objectives of this porposal with those of the ongoing BNF networks such as those at NifTAL, USA and the Internaional Atomic Energy Agency (IAEA), Vienna, Austria. But this proposal aims at achieving these objectives through on-farm research and by emphasizing host-plant aspects. In many countries, research and on-farm activity are handled by different agencies. We therefore expect participation of both research and extension personnel in this Working Group. Also, the proposed host plant bias of this symbiotic activity might encourage the association of different groups of researchers, such as plant breeders and physiologists, from those covered by the existing networks. We propose that this Working Group would be complementary to the efforts of the existing BNF networks.

Expected output

- Viable projects with realizable goals.
- Better awareness of existing knowledge and experience amongst BNF researchers in Asia,
- Cultivars with optimum symbioses with native and/or inoculant rhizobia.
- Generation of self reliance and expertise in the conduct of BNF research amongst the participating national programs.
- Understanding and enhancing the role of legumes in sustaining high yields of different cropping systems in a nonexploitative manner.

About ICRISAT

The semi-arid tropics (SAT) encompasses parts of 48 developing countries including most of India, parts of southeast Asia, a swathe across sub-Saharan Africa, much of southern and eastern Africa, and parts of Latin America. Many of these countries are among the poorest in the world. Approximately one-sixth of the world's population lives in the SAT, which is typified by unpredictable weather, limited and erratic rainfall, and nutrient-poor soils.

ICRISAT's mandate crops are sorghum, pearl millet, finger millet, chickpea, pigeonpea, and groundnut; these six crops are vital to life for the ever-increasing populations of the semi-arid tropics. ICRISAT's mission is to conduct research which can lead to enhanced sustainable production of these crops and to improved management of the limited natural resources of the SAT. ICRISAT communicates information on technologies as they are developed through workshops, networks, training, library services, and publishing.

ICRISAT was established in 1972. It is one of 18 nonprofit, research and training centers funded through the Consultative Group on International Agricultural Research (CGIAR). The CGIAR is an informal association of approximately 50 public and private sector donors; it is co-sponsored by the Food and Agriculture Organization of the United Nations (FAO), the World Bank, and the United Nations Development Programme (UNDP).

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