Recent Advances in Pulses Research

Edited by
A. N. Asthana
Masood Ali

Publisher :

INDIAN SOCIETY OF PULSES RESEARCH AND DEVELOPMENT
Indian Institute of Pulses Research, Kanpur - 208 024, India

1997

LIMITATIONS AND POTENTIALS IN IMPROVING BIOLOGICAL NITROGEN FIXATION OF PULSES— A CRITICAL ASSESSMENT

O.P. Rupela[1] and A.L. Khurana[2]

ABSTRACT

Rhizobial inoculation is the only component of biological nitrogen fixation (BNF) technology available for on-farm use at present, and there are technical prerequisites to its successful application. Inoculation is likely to increase BNF and/or yield in several situations e.g., areas (or fields) cropped to legumes for the first time, post-rice legumes, and legumes grown in areas of very high summer temperatures. Policy support will be required to produce high quality inoculants.

High BNF lines are expected to overcome N limitations and provide high yield, both at low and high soil N conditions, and thus contribute to yield stability. Host-plant selection/breeding for enhanced BNF and yield is a highly promising area, but has received little attention from microbiologists and plant breeders working on pulses, particularly in developing countries. A blend of skills of both disciplines will be required to package BNF technology into seed. Selection of high BNF variants from released materials seems possible in different pulses. Such selections, when developed, should be used not only in crossing programmes as sources of high BNF genes, but should also be developed as cultivars after evaluation. This is strongly recommended to quicken the lengthy procedure of transferring high BNF genes into agronomically acceptable cultivars.

1. INTRODUCTION

For most agricultural research scientists and administrators biological nitrogen fixation (BNF) in legumes relates to rhizobia, rhizobial inoculants and response to their application under greenhouse and field conditions. This is perhaps because "rhizobial inoculation" is the only component of BNF technology presently available for on-farm application. However, it should be realized that informed decisions on crop husbandry and the use of legumes cultivars are very important if the benefits from BNF are to be harnessed.

---

[1] Senior Scientist, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, 502 324, Andhra Pradesh, India.

[2] Professor of Microbiology cum Principal Investigator, C.C.S. Haryana Agricultural University, Hisar 125 004, Haryana, India.

responses can be predicted through mathematical models (Thies *et al.* 1991). The native population of root nodule bacteria (RNB), the level of native mineral N in soil, and such other factors as soil moisture are important in determining the degree of response.

2.1.1 Native population of root nodule bacteria : Fields in which a given legume is grown regularly are likely to have high population (> 10^3 g^{-1} dry soil) of relevant homologous RNB, unless the soil is abnormal with problems like acidity or salinity. Displacing the high native populations of rhizobia is difficult and is only occasionally observed (Schmidt 1988). Therefore, rhizobia and rhizobia x host genotype interactions identified as effective/efficient though significant in some cases (Rai and Singh 1979), would be difficult to exploit for yield increase in all situations. In traditional chickpea growing areas in India, Rupela *et al.* (1987) observed that about 18% farmers' fields had <10^2 rhizobia g^{-1} dry soil. Similarly, Khanam *et al.* (in press) reported that 2 to 7 out of 16 farmers' fields examined has < 100 rhizobia (g^{-1} soil) of pigeonpea, lentil and chickpea in Bangladesh. Significantly improved yield due to rhizobial inoculation is expected when a field has < 10^2 rhizobia g^{-1} soil and other factors affecting BNF are optimum (Theis *et al.* 1991). Seven of the 12 trials that showed significantly improved nodulation due to inoculation did not result in significantly improved yield even when the native rhizobial population was <100 g^{-1} dry soil (Rupela and Saxena 1987). Also, it was surprising to record significantly improved nodule mass (and acetylene reduction activity) in two trials, even when the native rhizobial population was >1000 g^{-1} dry soil. This did not fit into the inoculation response model of Thies *et al.* (1991) and perhaps indicates the need to fine-tune the model.

2.1.2 Native soil N level : Soil mineral N, especially NO_3-N (Streeter 1988) rather than total N in soil adversely affects legume BNF. Pulses like chickpea seem particularly sensitive to mineral N. Rawsthorne *et al.* (1985) reported 50% reduction in chickpea nodule numbers when it was grown in pots and watered with a nutrient solution containing 1.43 mM NO_3 (=20 ppm N). In field trials at ICRISAT Asia Center, 72 to 94% reduction in the nodule mass was recorded at 50 days after sowing (DAS) in seven different chickpea cultivars (O.P. Rupela, unpublished) when mineral N in the top 30 cm of the soil profile increased from 9 mg kg^{-1} soil in the control, to 18 mg kg^{-1} soil in N-treated plots. The fact that some salts of N reduce nodulation of legumes has been known for about 100 years (Streeter 1988). Several groups are working to develop N-tolerant symbiosis in different legumes.

In Haryana, an important chickpea growing state of India, during a survey based on 10 x 10 km grids, Grewal (1990) found that 60% of farmers' fields had

<40 to 85 mg available N kg^{-1} soil, and described these fields as extremely low in N. In another study, Singh *et al.* (1992) reported that available N in farmers' fields in Haryana ranged from 38 to 272 ppm (mg N kg^{-1} soil) and described them as low to medium. In both the studies, the available N was measured by the alkaline permaganate method which includes mineralizable N along with mineral N. Singh *et al.* (1992) also measured NO_3-N by chromotropic acid method (Sims and Jackson 1971) and NH_4-N by Nessler's reagent method (Yuen and Pollard 1952) after 2M KC1 extraction of soil samples in 1:2 soil to solution ratio. In these studies, mineral N (NH_4-N+NO_3-N) ranged from 37 to 75 ppm in farmers' fields. Available N in the samples ranged from 40 to 141 ppm. The N levels above 20 ppm (mg kg^{-1} soil) are obviously high for chickpea because they suppress BNF. Thus mineral N levels observed as suppressive for legume BNF can be present even in areas reported/believed to be low in N.

2.1.3 Abiotic and biotic factors. Such abiotic stress factors as drought and high temperature that adversely affect the growth and yield of pulses, also adversely affect their nodulation and nitrogen fixation. As in the case of high soil N, the symbiosis between legumes and rhizobia is more sensitive to stress factors than rhizobia as saprophytes. Obviously when these factors are limiting, nodulation and BNF would be adversely affected, even if the native population of relevant efficient rhizobia is high, or rhizobial inoculants have been applied. A case study on nodulation failure in rhizobial inoculation trial on a Vertisol field highlights the importance of soil moisture at an appropriate stage of symbiosis development. The field was solarized during the summer of 1985. This reduced the population of native chickpea rhizobia to <100 g^{-1} soil. Marginal soil mositure (19%) at sowing allowed good emergence but not good nodulation. The following irrigation did not help, perhaps because by the time it was applied active root hairs were too far away from adequate numbers of rhizobia. Removing the first-sown plants and re-sowing the same treatment plots following irrigation, clearly brought out the treatment differences. The inoculated plants in the second sowing had 150% more nodules than the uninoculated plants. Though nodules on control plots also increased, the number of nodules was significantly increased by inoculation. This experiment highlights the importance of adequate soil moisture when legumes are sown, if BNF is to be effective, particularly in rainfed agriculture. It also gives an insight into the problem of inconsistency of response to inoculation.

Nodules of legumes are eaten by insects. The larvae of the weevil, *Sitona macularius* (Marsham) damage lentil nodules in the West Asia/North Africa (WANA) region (Tahhan and Hariri 1982). *Metopina* larvae feed on chickpea nodules (Sithanantham and Rupela 1986), and the larvae of *Rivellia angulata* damage pigeonpea nodules (Kumar Rao and Sithanantham 1989) in peninsular

India. Cowpea nodules have also been reported to be damaged by chloropidan larvae (Nair 1978). However, information is not available on the extent of damage and the variation in damage over seasons on farmers, fields in different regions. Nambiar *et al.* (1990) indicated the possibility of controlling the damage caused by *Rivellia* to pigeonpea nodules by developing rhizobial strains containing toxin-producing genes of *Bacillus thurigiensis* var. *israeliensis.*

2.1.4 Inoculant quality : In many developing countries quality of available inoculants can be poor. An excellent quality inoculant should have $>10^8$ rhizobia g^{-1} carrier. In India, few manufacturers passed this test, irrespective of whether they were private or government institutions, when the quality was monitored in 1979/80 (O.P. Rupela, unpublished). Thirteen years later the quality of inoculants is still poor, according to a 1992 report submitted to the Food and Agriculture Organisation of the United Nations on the quality of inoculants (Project IND/86/003) produced under the aegis of the National Biofertilizer Development Centers (NBDC) (J.A. Thompson, Australia, personal communication).

This is also confirmed by a report of another expert from India employed by NBDC (K.R. Dadarwal, C.C.S. HAU, Hisar, personal communication). It must be realized that the inconsistency of response to inoculation highlighted above is further complicated when the inoculant quality is poor. Procedures for preparing high-quality inoculants in developing countries are available (FAO 1991, Thompson 1984) but the will to execute them seems missing.

2.1.5 Economics of inoculation : The cost per hectare of applying (without subsidy) seed-coated inoculants purchased from private manufacturers in India was calculated in December 1993 to be Rs. 80 (US $ <3) at the most, and was Rs. 120 (US $ <4) if applied as liquid (Table 1). This may not be a great investment for some farmers because an extra grain yield of only 12-15 kg ha^{-1} would be enough to offset the cost. But it would be an expensive input for some other farmers, particularly the resource-poor farmers of rainfed areas. However, these reservations apply to any purchased input. Further, biofertilizers are not very expensive when compared to the recommended practice of applying 20 kg N ha^{-1} to legumes (Table 1). Also, the results of inoculation trials conducted on farmers' fields by scientists/extension agencies (Table 2) are generally favourable. If the data in Table 2 are true then the inputs as in Table 1 would be a worthwhile investment. If the proposed investment is too high for resource-poor farmers then society and the Government need to provide policies to help them. The economics stated above are only related to financial investments. The use of biofertilizers, and the development of high BNF cultivars have dimensions of "Green Economics" that are generally ignored. The use of excessive N fertilizers can result in soil

Table 1 : The cost of inoculating one hectare field of legumes in India compared to that of applying 20 kg N ha^{-1} as urea

Description	Inoculant (without subsidy) (Rupees)	20 kg N ha^{-1} (with subsidy) (Rupees)
Cost of product (city market)	50.00 (2.5 packet ha^{-1} Rs. 20.00 $packet^{-1}$)	126.00 (Rs. 2.90 kg^{-1} urea)
Transport cost, to and from city (20 km distance, involves travel by bus)	20.00	40.00 [1]
Labour cost application	10.00 (1/4 day for treating seed)	40.00
	40.00 (1 day, if applied as liquid, to carry 600 L water, 5 mL $seed^{-1}$)	
Total cost	80.00 (US $ 3.9) pl. check the = value of (seed application)	206.00 (U S $ 6.6)
	110.00 (U S $ 3.9) pl. check the = value of (liquid application)	

[1] Assuming that a farmer will go to the city to buy inputs, the bus fare would cost him Rs 20.00. About 500 g inoculant can be carried easily by hand, but he would have to pay the equivalent of one person's fare for a 50-kg bag of fertilizer.

acidification, N leaching losses, and the eutrophication of water bodies. Industrially advanced countries that consume large quantities of N fertilizers are now being forced to invest in research programs on managing soil nitrate (Franco 1992). The use of BNF as N resource does not involve the environmental pollution associated with N fertilizers, provided legumes are appropriately managed. Because BNF can cause soil acidification, care must be taken when growing legumes in acid soils.

2.1.6 Rhizobial inoculants (biofertilizers) *versus* nitrogen fertilizers : Legume plants can meet their N needs through both BNF symbiosis and N fertilizers, or through native soil N. However, it is important to realize that rhizobial inoculants are live material and, therefore, must be handled with far more care than that provided to fertilizer N, both during transport and use. Shelf life (at least 3 months) is longer in sterile inoculants (prepared using sterilized carriers, bacteria other than rhizobia absent) than in nonsterile inoculants (which contain other

Table 2 : Response to inoculation of pulses on farmers' fields in India.

Crop	Region State	Period	Yield range of control (kg ha^{-1})	Percentage change with inoculation (range)
Chickpea	Bihar	1992-93	540-740	34-62
	Haryana	1982-84	660-1425	2-25
	Karnataka	1989-93	420-1130	18-48
	Madhya Pradesh	1989-93	600-658	18-24
	Maharashtra	1988-93	845-1500	7-28
	Punjab	1989-93	730-1980	4-25
	Rajasthan	1988-93	440-2000	8-50
	Tamil Nadu	1989-90	750	21
	West Bengal	1986-87	504-935	13-30
Pigeonpea	Haryana	1979-81	1000-1200	10-20
	Karnataka	1987-93	850-1295	9-15
	Madhya Pradesh	1987-93	410-710	16-22
	Maharashtra	1990-93	345-692	4-25
	Tamil Nadu	1992-93	-	14-0
Mungbean	Maharashtra	1990-93	720-1035	4-13
	Punjab	1990-93	368-375	8-25
	Rajasthan	1987-93	320-1093	8-25
Urdbean	Bihar	1992-93	390-550	12-19
	Maharashtra	1991-92	320-360	11-17
Lentil	Bihar	1987-92	260-710	6-21
	Punjab	1988-91	240-370	12-25

Plot size in most cases was about 200 m^2 for each treatment. Improved cultivars of the different areas were used, nodulation in most cases was not observed.

micro-organisms alongwith rhizobia). Sterile inoculants can be stored for at least three months at temperatures between 25^{0}C and 30^{0}C (Khurana and Sharma 1979). Logically, if sterile carrier based inoculants are prepared and transported when ambient temperature is <30^{0}C, refrigerated transport may not be needed in most tropical countries, at least for part of the year. The inoculants required for sowing in the rainy season (June/July) can be transported/purchased in February, March or April when temperatures are not too harsh. Farmers can satisfactorily store inoculants in earthen pots in a cool place.

High mineral N in soil particularly NO^3-N suppresses BNF (Streeter 1988). Fertilizer N, even when applied at the generally recommended level of 20 kg ha^{-1} for legumes can suppress BNF. A 15 to 43% reduction in BNF (measured by ARA) was consistently measured in a three year field trial involving two cultivars (Table 3). Nodule mass was not effected. In pigeonpea, Kumar Rao *et al.* (1981) reported a 2 to 58% reduced mass and 14 to 54% reduced ARA (measured at 20 and 60 DAS) due to application of 20 kg N ha^{-1} to an Alfisol in the rainy season of 1977. The authors are not sure if these are stray cases or a widespread phenomenon. This needs to be studied. It is realized that the recommendation to apply 20 kg N ha^{-1} to several legume crops in India was made after conducting multilocational trials for several years, but it is believed that these recommendations were solely based on yield measurements, and not on BNF measurements. It may be noted that yield/biomass did increase sometimes (Table 3) due to the application of 20 kg N ha^{-1} in the above mentioned two examples. Several publications on the N response of legumes, including some of recent years, did not record BNF-related traits (Sharma *et al.* 1992). Also, in the 1960s and 1970s, when these recommendations on N application were made in developing countries, biofertilizers were not so developed as they are at present. It is doubtful if the recommendation would now be economical without fertilizer subsidy, and when the environmental costs of producing and transporting fertilizers are considered.

2.2 Measuring BNF

What we cannot measure, we cannot control. Parameters used to assess BNF include nodule numbers and nodule mass. These parameters can be used at most locations even in farmers' fields in developing countries, and thus are of wide application. Using acetylene reduction assay (ARA), a point-in-time measurement, and percent N derived from air (P fix) by ureide method require gas chromatograph and spectrophotometer which may be available in several laboratories of developing countries. Percentage N derived from air using ^{15}N-based method, requires very expensive equipment and may be owned by few laboratories in developing countries. The last method is considered to be the most reliable method even though it has some limitations (Witty 1983). Thus, most developing countries are left with little choice but to use simple parameters of nodule numbers and mass in on-farm situations, and in most cases, even for their research work. ^{15}N-based methods can be used by developing countries largely through collaborative arrangements with developed countries and agencies that have the resources. The International Atomic Energy Agency (IAEA), Vienna, Austria, assists developing countries through coordinated BNF programmes.

Yield improvement due to improved BNF (by inoculation or by host selection) is an obvious way to compare treatment effects and is widely used by microbiologists in developing countries, generally without supportive data on direct indicators of BNF (nodulation, ARA, P-fix). As indicated earlier in this paper, BNF is an alternative source of N for a legume plants along with mineral N in soil, or through fertilizers where these are applied. Therefore, only at experimental sites low in native N can improve BNF be expected to be reflected in improved yield. The authors believe that many legumes are efficient "scavengers" of soil N and, therefore, in control treatments, when the symbiosis is not fully functional, plants can take up soil N and thus mask the expected effect of improved BNF on improved yield. It is strongly believed that this situation can be partly addressed through the use of non-nodulating (Nod$^-$) lines of different legumes that could be used as bioindicators of soil N if they were included in different experiments, even in on-farm trials. Nod$^-$ chickpea and pigeonpea lines are now available. Obviously, considerable research work would be required to establish the suitability of a Nod$^-$ line as a bioindicator of soil N, but if this was done it should then be possible to-i) visualize the importance of nodules, ii) quantify treatment differences due to BNF and iii) assess the importance of BNF in monetary terms, even in developing countries.

In several situations improved BNF can result in improved protein content in seeds rather than in improved yield. At ICRISAT, chickpea line ICC 5003 always nodulated and fixed 2 to 4 times more N than another chickpea line ICC 4918 (Table 3). However, yield survey of trials conducted by different ICRISAT scientists during 10 years from 1976 to 1985 indicated that only in 47% of the total 29 trials ICC 5003 yielded only marginally more than ICC 4918. However, in all the seven cases, where seed protein content was measured, ICC 5003 had higher protein content than ICC 4918. This perhaps suggests that improved nodulation may improve protein content rather than grain yield, at least in some situations. N harvest (both through seed and stover) is an acceptable parameter for quantifying BNF when more dependable parameters are not available. It is particularly dependable when legumes are grown in N-starved soils.

3. POTENTIALS

3.1 Engineered/competitive rhizobia

Some research groups are using molecular biology tools to develop inoculant rhizobia that can compete successfully with native rhizobia (see excellent review by Triplett and Sadowsky 1992). There are obvious prospects of success, but this is likely to be a long-term project. Some rhizobial strains have been reported to

Table 3 : Nodule mass, acetylene reduction activity and yield of two chickpea cultivars as affected by application of 20 kg N ha $^{-1}$ at sowing in three different years[1]

		At flowering (43 - 51 DAS) Acetylene reduction activity		At final harvest	
Cultivar/ Treatment	Nodule mass (mg pl^{-1})	(uM C2H4 (pl $^{-1}$ h^{-1})	uM C2 H4 g^{-1} dry (nod h^{-1})	Grain yield (kg ha^{-1})	Stover yield (kg ha $^{-1}$)
1984/85	45	0.7	11.2	1110	650
ICC 4918	48 (+7)	0.4 (-43)	7.9 (-29)	1030 (-7)	700 (+8)
ICC 4981					
+20 kg N ha $^{-1}$					
ICC 5003	126	6.2	34.1	950	730
ICC 5003	140 (+11)	4.3 (-34)	23.9 (-30)	1020 (+7)	730 (o)
+ 20 kg N ha $^{-1}$					
SE	± 5.4	± 0.40	± 2.08	± 76	ND
1985/86					
ICC 4918	48	0.6	11.7	680	500
ICC 1918	52(+8)	0.5(-17)	10.3 (-12)	660 (-3)	530 (+6)
+ 20 kg N ha $^{-1}$					
ICC 5003	165	5.2	31.0	670	650
ICC 5003	165 (0)	4.4 (-15)	25.8 (-17)	650 (-3)	730 (+12)
+20 kg N ha $^{-1}$					
SE	± 9.2	± 0.36	± 1.99	± 43	ND
1986/87					
ICC 4918	69	0.9	13.8	1160	660
ICC 4918	63 (-9)	0.6 (-33)	9.3 (-32)	1230 (+6)	740 (+12)
+20 kg N ha^{-1}					
ICC 5003	173	4.8	27.6	980	780
ICC 5003	175 (+1)	3.6 (-17)	19.9 (-28)	970 (-1)	840 (+8)
+20 kg N ha $^{-1}$					
SE	± 9.9	± 0.39	± 2.10	± 49	ND

[1] Vertisol field having < 10 mg mineral N kg $^{-1}$ soil in top 30 cm profile, same plots received cultivars/ treatments continuously for the three years, crops grown in the postrainy season with irrigation only at sowing, N as urea was applied below seed at sowing. Data in parentheses are percentage changes in values due to the application of 20 kg N ha $^{-1}$.

ND= Not determined.

be competitive e.g, Viking 1 of *Phaseolus*, NS 92 of groundnut and WU 95 of *Trifolium* (Schmidt 1988). But their performance has been variable, perhaps due to factors discussed in the section on "Inconsistency in Response...". Antibiosis, mobility, speed of nodulation, cell surface characteristics and nodulation efficiency are considered important characters that may make a strain more competitive than others. However, the authors believe that level of response to inoculation is dictated largely by the numbers of native rhizobia in soil and in the inoculant (Theis *et al.* 1991, Weaver and Frederick 1974). Native rhizobia have an obvious spatial advantage as they are well dispersed in the soil profile. The inoculant strain is applied only in the seed zone and may have less access to the growing roots/root hairs for establishing symbiosis with the host. Overcoming this disadvantage would require a major breakthrough, and the authors feel that such a breakthrough is unlikely in the near future.

3.2 Exclusion or restriction of nodulation by native rhizobia

This is an interesting approach to harness BNF through inoculation, based on the assumption that native rhizobia are less efficient than inoculants (we disagree with this assumption). Even if native rhizobia are ineffective, increase in BNF efficiency is likely by exclusion (i.e., by developing host cultivars immune to infection by native rhizobia) or by restriction (infection of host-plant only by efficient native rhizobia or laboratory-engineered strains). Kipe-nolt *et al.* (1992) reported a possible case of 'restriction' in wild accessions of *Phaseolus* beans. It is likely that materials identified as Nod$^-$ at one location may be nodulated by specific native rhizobia at another location. Such strains may serve as genotype- and/or location-specific compatibles, thus overcoming the problem of ineffective native rhizobia, if and where present. However, no such strains-host compatibles have been identified to date.

3.3 Management options to harness BNF

Sufficient information is available that when applied in managing legumes should result in enhancing their BNF capacity. For example, deep sowing reduces nodulation and BNF in groundnut (Nambiar and Srinivasa Rao 1987) and chickpea (O.P. Rupela unpublished). If so, why not sow shallow when moisture is not limiting? Legume cultivars vary in BNF capacity. Use of high BNF lines should be favoured without compromising greatly on yield. N application can reduce BNF in legumes. In a cereal-legume intercrop farmers, where possible, apply N to increase cereal yield. This can potentially reduce BNF capacity of the legume, and the reduction can be minimized by spacing legume and cereal sufficiently far apart and restricting N application to the cereal. Other crop husbandry practices that can help in harnessing the BNF ability of legumes are: i) growing legumes on a poor N soil, ii) making sure that the soil is not deficient in nutrients such as P and Fe, iii) applying water, if available, in optimum amount at sowing, to allow potential nodulation of cultivars.

3.4 Host-plant selection

3.4.1 Intercultivaral variability : Large genotypic differences in BNF traits such as number of nodules, nodule mass, ARA values, P fix have been reported in chickpea, pigeonpea, *phaseolus* beans and cowpea. Though this information has been available for at least a decade in some pulses, there are no active research programmes exploiting this variability to enhance BNF (except for *Phaseolus*, Bliss 1933). The reason for this apparent limited interest seems to be the low priority given to BNF research by legume breeders.

3.4.2 Intracultivaral variability : In the early 1980s, examination of segregating populations of a cross between high and low nodulation chickpea lines revealed a variability within the F_2 population from Nod$^-$ to high nodulation (Rupela and Saxena 1987). At the same time, variability within parents was very large and, therefore, further efforts to develop high-BNF, and high-yielding chickpea lines were terminated. Subsequently, during a search for Nod$^-$ chickpea plants (Rupela 1992), the occurrence of plants of contrasting nodulation identified within chickpea cultivars were developed into purelines. These variants were stable, and thus heritable, and were observed in landraees and bred cultivars of chickpea (Rupela and Johansen1992). This was true also in cultivars and advanced breeding lines of pigeonpea (Rupela and Johansen, in press).

Simple screening procedures were used to establish intracultivaral variability in chickpea and pigeonpea (Rupela, in press; Rupela and Johansen, in press). Maximum variability was recorded in a bred chickpea cultivar ICC 5003 (=K 850 = 850-3/27), where plants of each of the five visual ratings from 1 (=minimum nodulation) to 5 (=maximum nodulation) were isolated. The extent of nodulation fluctuated with mineral N status. However, the selections of rating 1 and 2 (low nodulating) nodulated statistically lower than those of rating 3, 4, and 5 (high nodulating). In two cultivars, ICC 5003 and ICC 4948 (=G130), high nodulating selections gave significantly higher yields than low nodulating selections, when tested in large (12.6m^2), low-N Vertisol plots at ICRISAT Center. On high N soil (about 18 mg mineral N mg^{-1} soil), however, the yield differences between high and low nodulating selections were lower.

From available information on chickpea and pigeonpea (Rupela, in press; Rupela and Johansen 1992, Rupela and Johansen, in Press) it appears that crop growth environments at locations where breeding material is developed do not favour (and may even select against) BNF capacity. Strong N scavenging ability of the roots may cause plants of contrasting BNF capacity appear within otherwise homogeneous material; this aspect needs to be examined. It should also be possible to select high BNF variants from existing, agronomically acceptable cultivars (see section: Stress-tolerant BNF symbioses).

3.4.3 Breeding for BNF : Advances have been made in developing high-BNF, high-yield lines of clover, alfalfa and *Phaseolus* bean. There are few published estimates of heritability for BNF traits in pulses (Bliss and Miller 1988). Environmental and genotype x environmental effects on BNF are usually large, and this may deter scientists from working in this area. Bliss (1993) suggested that sufficiently high replication can reduce the effect of non-genetic (environmental) factors, and improve the precision of selection at various stages. Progress has been demonstrated by developing high-BNF high-yield *Phaseolus* lines using simple parameters like nodule number, nodule mass and nodulation score (Rosas and Bliss 1986). It must be possible to use similar parameters to develop high-BNF high-yield lines of other pulses also. Bliss (1993) recommended mass selection and family selection for maximum genetic gains for retaining high BNF and essential agronomic traits in *Phaseolus* bean. Inter-and inter-cultivaral variability for nodulation should be exploited for sources of high BNF genes. In addition, high BNF variants from released cultivars or advanced breeding lines should also be considered for release. This could eliminate the time-consuming step of transferring high BNF traits to otherwise desirable backgrounds.

3.5 Stress-tolerant BNF symbioses

The need to identify stress-tolerant symbioses has been emphasized previously in this paper. For effective selection, screening large population is more important than precision of the screening procedure, at least at the early stages of selection. More dependable screening/evaluation procedures can be used at later stages.

The isolation and evaluation of rhizobial strains is particularly difficult, and requires microbiologically acceptable procedures and greenhouse facilities. Rhizobia able to form high temperature tolerant symbioses (temperature is a stress factor for BNF of chickpea in peninsular Indian environments) were identified at ICRISAT Center (O.P. Rupela, unpublished) using the following method. Soil samples from areas known to have high soil temperatures were collected and mixed with the 103 different rhizobial strains available at ICRISAT. The soil was then used as potting medium. Plastic buckets (18 L volume) filled with the potting medium were sown with chickpea cultivar ICC 5003 and placed in four water baths. Temperatures were maintained at 25, 30, 32, and 35°C for 8 hours a day, during daytime, and were allowed to reach ambient for the remaining 16 hours each day. The results indicated that soil temperatures higher than 25°C adversely affected nodule mass and ARA (Rupela and Saxena 1987). Nodules were formed at all the four temperatures, but the number of nodules, their development (nodule mass) and their functions (ARA) were adversely affected at soil temperatures about 25°C. There were apparently functional (pink) nodules even at 32°C.

Rhizobia were isolated from these pink nodules and evaluated at 32°C for BNF traits. It was found that these strains were indeed superior to the standard strain ICC 2002, which was efficient only at 25°C (ICRISAT 1983). It was thus possible to quickly identify high temperature tolerant symbioses in short-time; screening all the 103 strains at these temperatures will have taken many cycles of experiment because of limited capacity of the water baths. The screening procedure followed seems analogous to enrichment technique for isolating desired bacteria from a large population.

Nodule development and functions are adversely affected by high mineral N in soil (See details in the subsection on `Native Soil N level`). It has been suggested by Gibson and Harper (1985) that N-tolerant symbioses may be more readily developed through host-plant selection than through the selection of rhizobial strains. After developing an appropriate field screening method to identify N-tolerant symbiosis (Rupela, In press), 9182 plants from 86 wilt-resistant, high-yielding advanced breeding lines were evaluated in the post-rainy season 1991/92, and 392 out of the 9182 plants were identified as high nodulating at high mineral N at physiological maturity. The selections were expected to have all traits of the present material, which obviously requires confirmation. When tested in the post-rainy season 1992/93, 190 of the 392 single plant progenies remained high nodulating at high mineral N (about 20 mg N kg^{-1} soil). The 30 highest nodulating progenies of these 190 are being studied in the post-rainy season 1993/94 at low and high N conditions. Some of the progenies were indeed high nodulating both at low and high soil N when observed at 42 DAS.

3.6 Variants as research tools

Legumes use two sources of N. Young plants use seed or soil N (or fertilizer N) initially, until nitrogen fixation through nodules is established (generally called the complementary stage between mineral N and BNF). After the nodules are established, BNF is suppressed due to high soil N, if present (an antagonistic stage for the two sources of N). Field studies on nitrogen nutrition are, therefore, complex and difficult. Appropriate studies on nodulation variants Nod^-, low nodulating, high nodulating and of high N tolerant symbiosis, reported in the preceding section, can help develop a better understanding of N metabolism in legumes. Sagan *et al.* (1993) used Nod and super-nodulating mutants of field grown peas to analyze nitrogen nutrition. They reported that N-deficiency induced flowering termination, and that the source of nitrogen (mineral N versus symbiotically fixed N) had little effect on yield.

In ureide-producing legumes the xylem sap composition has direct relationship with the BNF capacity of a given cultivar. In pigeonpea, this relationship has been used to quantify BNF (Peoples *et al.* 1989) by different cultivars. In amide-producing legumes such as chickpea, a xylem sap-based BNF quantification method similar to that for pigeonpea is unavailable. We are very hopeful that a better understanding of N metabolism using the nodulation variants may lead to developing such a method for amide-producing legumes.

4. CONCLUSION

Rhizobial inoculation is the only existing component of BNF technology presently for on- farm application. Microbiologists need to support this technology with information on economics including `green economics` and with impact analysis vis-a-vis nitrogen fertilizer. Only then will it be possible to convince farmers, extension agencies and research administrators of its value. The use of rhizobial inoculation together with high BNF, high yield legumes seems a promising package for harnessing the benefits of legume BNF in a cropping systems perspective. Long-term trials highlighting the value of high BNF (through the use of efficient rhizobial strains and/or high BNF, high yield cultivars) should help promote the use of this renewable/natural N resource.

Acknowledgments: We thank Dr C. Johansen for critical comments and Mr. P.V.S. Prasad for typing the manuscript.

REFERENCES

Bantilan, M.C.S., Johansen, C., McDonald, D. (In press). Expectations from BNF research- research administrators point of view. Report of the first meeting of the Asia Working Group on Biological Nitrogen Fixation in Legumes 6-8 Dec 1993, ICRISAT, India. Patancheru, A.P., 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Bilss, F.A. 1993. Breeding common bean for improved biological nitrogen fixation. Plant and Soil 152: 71-79.

Bliss, F.A., Miller (Jr), J.C. 1988. Selecting and breeding grain legumes for enhanced nitrogen fixation. Pages 1001-1012 in Worlds Crops: Cool Season Food Legumes (Summerfield, R.J. ed.) Kluwer Academic Publishers, London.

Brodrick, S.J. and Giller, K.E. 1991. Genotypic differences in molybdenum accumulation affects N_2- fixation in tropical *Phaseolus vulgaris* (L). Journal of Experimental Botany 42: 1339-1343.

FAO (Food and Agricultural Organization of the United Nations) 1991. Expert consultation on legume inoculant production and quality control. FAO, Rome.

Franco, J. 1992. Nitrate management program-fertilizer research and education program: progress report 1991. Communications in Soil Science and Plant Analysis 23: 2111-2134.

Gibson, A.H. and Harper, J.E. 1985. Nitrate effect on nodulation of soybean by *Bradyrhizobium japonicum*. Crop Science 25:497-501.

Grewal, M.S. 1990. Application of geostatistics to spatial studies of essential and non-essential elements of Haryana soils. Ph. D. thesis, CCS Haryana Agricultural University, Hisar, India.

Herridge, D.F. and Holland, J.F. 1993. Low nodulation and N_2 fixation limits yield of pigeonpea on alkaline Vertisol of northern N. S.W. : Effect of iron, rhizobia and plant genotype. Australian Journal of Agricultural Research 44: 137-149.

Hirsch, A.M. 1992. Developmental biology of legume nodulation. New Phytologist 122:211-237.

Howieson, J.G., Robson, A.D. and Ewing, M.A. 1993. External phosphate and calcium concentrations and pH, but not the products of rhizobial nodulation genes, affect the attachment of *Rhizobium meliloti* to roots fo annual medics. Soil Biology and Biochemistry 25 : 567-573.

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), 1983. Biological Nitrogen Fixation. *In* ICRISAT Annual Report 1982, pp. 115-118. ICRISAT Patancheru, India.

Khanam, D., Rahman, H.H. and Maqbul Hossain, A.K. (In press). Use of rhizobial inoculats : On-farm experience in Bangladesh. Report of the first meeting of the Asia Working Group on Biological Nitrogen Fixation in Legumes 6-8 Dec 1993, ICRISAT, India. Patancheru, A.P., 502 324, India: Internatioal Crops Research Institute for the Semi-Arid Tropics.

Khurana, A.L. and Sharma, H.R. 1979. Suitability of wood charcoal powder as rhizobial carrier. Indian Journal of Microbiology. 19 : 178-179.

Kipe-nolt, J.A., Montealegre, M. and Tohme, J. 1992. Restriction of nodulation by the broad host range *Rhizobium tropici* strain CIAT 899 in wild accessions of *Phaseolus vulgaris* L. New Phytologist 120: 489-494.

Kumar Rao, J.V.D.K. and Sithanantham, S. 1989. Impact of nodule damage by *Rivellia angulata* on N_2 fixation, growth, and yield of pigeonpea (*Cajanus cajan* L. Millsp.) grown in a Vertisol. Biology and Fertility of Soils 7:95-100.

Moore, W.E.C., and Moore, L.H. 1992. Index of the bacterial and yeast nomenclatural changes. American Society for Microbiology, Washington, D.C.

Nair, S.K. 1970. Infestation of legume root nodules by a chloropidan larvae. Plant and Soil 50 : 493-495.

Nambiar, P.T.C. and Srinivasa Rao, B. 1987. Effect of sowing depth on nodulation, nitrogen fixation, root and hypocotyl growth, and yield in groundnut (*Arachis hypogaea* L.). Experimental Agriculture 23: 283-291.

Nambiar, P.T.C., Ma, S.W. and Iyer, V.N. 1990. Limiting an insect infestation of nitrogen - fixing root nodules of the pigeonpea (*Cajanus Cajan*) by engineering the expression of an entomocidal gene in its root nodules. Applied and Environmental Microbiology 56: 2866-2869.

Peoples, M.B. and Craswell, E.T. 1992. Biological nitrogen fixation: investments, expectations and actual contributions to agriculture. Plant and Soil 141 : 13-39.

Peoples, M.B., Hebb, D.M., Gibson, A.H. and Herridge, D.F. 1989. Development of the xylem ureide assay for the measurement of nitrogen fixation by pigeonpea (*Cajan cajan* (L.) Millsp.). Journal Experimental Botany 40: 535-542.

Rai, R. and Singh, S.N. 1979. Interaction between chickpeas (*Cicer arietinum* Linn.) genotypes and strains of *Rhizobium* sp. Journal of Agricultural Sciences Cambridge 92: 437-441.

Rawsthorne, S., Hadley, P., Summerfield, R.J. and Roberts, E.H. 1985. Effect of supplemental nitrate and thermal regime on the nitrogen nutrition of chickpea (*Cicer arietinum* L.) II. Symbiotilc development and nitrogen assimilation. Plant and Soil 83: 279-283.

Rosas, J.C. and Bliss, F.A. 1986. Host plant traits associated with estimates of nodulation and nitrogen fixation in common bean. Horticultural Science 21: 287-289.

Rupela, O.P. 1992. Natural occurrence and salient characters of non-nodulating chickpea plants. Crop Science 32: 349-352.

Rupela, O.P. (In press). Screening for intracultivaral variability for nodulation of chickpea and pigeonpea. Report of the first meeting of the Asia Working group on Biological Nitrogen Fixation in Legumes 6-8 Dec 1993, ICRISAT, India. Patancheru, A.P., 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Rupela, O.P. and Johansen, C. 1992. Genetic variation for nodulation within chickpea cultivars. Paper presented in the Research Coordination Meetling of IAEA "The Use of Isotopes in Increasing the Yield and Nitrogen Fixation Capabilities of Common Grain Legumes in Asia. " 13-17 July 1992, Harbin, China.

Rupela, O.P. and Johansen, C. (In Press). Identification of non-nodulating, and low and high nodulating plants in pigeonpea. Soil Biology and Biochemistry (Special volume, 1994).

Rupela, O.P. and Saxena, M.C. 1987. Nodulation and nitrogen fixation in chickpea. Pages 191-206 *in* The Chickpea (Saxena, M.C. and Singh, K.B., eds.). Wallingford. Oxon, U.K: CAB International.

Rupela, O.P., Toomsan, B., Mittal, S., Dart, P.J. and Thompson, J.A. 1987. Chickpea Rhizobium populations: survey of influence of season, soil depth and cropping pattern. Soil Biology and Biochemistry 19: 247-252.

Sagan, M., Ney, B. and Duc, G. 1993. Plant symbiotic mutants as a tool to analyse nitrogen nutrition and yield relationship in field - grown peas (*Pisum sativum* L.). Plant and Soil 153: 33-45.

Schmidt, E.L. 1988. Competition for legume nodule occupany: a down-to-earth limitation on nitrogen fixation. Pages 663-674 in world crops: cool season food legumes: proceedings of the International food Legume Research Conference on Pea, Lentil, Faba Bean and Chickpea, 6-11 Jul. 1986, Spokane, Washington, USA (Summerfield, R.J., ed.). Dordrecht, Netherlands: Kluwer Academic Publishers.

Sharma, H.M., Roy Sharma, R.P. and Mishra, S.S. 1992. Fertilizer management in legume and cereal based cropping systems. Indian Journal of Pulses Research 5: 40-44.

Sims, J.R. and Jackson, G.D. 1971. Rapid analysis of soil nitrate with chromotropic acid. Proceedings of Soil Science Society of America. 35: 603-606.

Singh, J.P., Dahiya, D.J., Kumar, V. and Singh, M. 1992. Distribution and status of different forms of nitrogen in soils of Haryana. Journal of Indian Society of Soil Science 40: 698-704.

Sithanantham. S. and Rupela, O.P. 1986. Insect damage to chickpea root nodules. International Chickpea Newsletter No. 15: 12-13.

Sprent, J.I., Minchin, F.R. and Thomas, R.J. 1983. Environmental effects on the physiology of nodulation and nitrogen fixation. *In* Temperature Legumes: Physiology, Genetics and Nodulation (Jones, D.G. and Davies, D.R., eds.), pp. 269-318. Pitman Books, London, UK.

Streeter, J. 1988. Inhibition of legume nodule formation and N_2 fixation by nitrate. Critical Reviews in Plant Science 7: 1-23.

Tahhan, O. and Hariri, G. 1982. Survey of lentil insects in northern and north eastern Syria. Lens Newsletter 9: 34-36.

Thies. J.E., Singleton. P. W. and Bohlool, B.B. 1991. Modeling symbiotic performance of introduced rhizobia in the field by use of indices of indigenous population size and nitrogen status of the soil. Applied and Environmental Microbiology 57: 29-37.

Thompson, J.A. 1984. Production and quality control of carrier-based legume inoculants. Information Bulletin No. 17. Patancheru, A.P., 502 324, India: International Crops Research Institute for the Semi-Arid Tropics.

Triplett, E.W. and Sadowsky, M.J. 1992. Genetics of competition for nodulation of legumes. Annual review of Microbiology 46: 399-428.

Witty, J.F. 1983. Estimating N_2 fixation in the field Susing ^{15}N labelled fertilizer: some problems and solutions. Soil Biology and Biochemistry; 15: 631-639.

Weaver, R.W. and Frederick, L.R. 1974. Effect of inoculum rate on competitive nodulation of *Glycine max* L. Merrill. II. Field studies. Agronomy Journal 66: 233-236.

Yuen, S H. and Pollard, A.G. 1952. The determination of nitrogen in agricultural materials by Nessler Reagent 1. Preparation of reagent. Journal of Science Food Agriculture 3: 441-447.