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Field Evaluation of Some Recently Developed Selections for High Nodulation and Value of Nodulation Variants of Chickpea

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Abstract

Naturally occurring plants with large differences in nodulation capacities (including nonnodulation) within chickpea cultivars have been reported. Two cultivars, ICC 4948 (= G 130) and ICC 5003 (= K 850), from which such nodulation variants were identified during the mid-1980s, were susceptible to fusarium wilt a widely occurring soilborne disease, caused by *Fusarium oxysporum* f. sp. *ciceri*. This made multilocational evaluation of the nodulation variants difficult in some important chickpea-growing areas. Studies reported here suggest that high-nodulating variants can be identified from advanced breeding chickpea lines that were reported as tolerant to fusarium wilt. The potential value of these and previously reported nodulation variants for quantification of N₂ fixation, additional residual effect owing to additional N₂ fixation by the high nodulating selections, improving soil health, and better understanding of the symbiotic process have been discussed. The potential to develop stress-tolerant symbiotic chickpea lines has been indicated through successful identification of high mineral-N tolerant symbiotic selections.

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ICRISAT Conference Paper no. CP 1318.

Rupela, O. P. 1997. Field evaluation of some recently developed selections for high nodulation and value of nodulation variants of chickpea. Pages 305-317 in *Extending nitrogen fixation research to farmers' fields: proceeding of an International Workshop on Managing Legume Nitrogen Fixation in Cropping Systems of Asia, 20-24 Aug 1996*, ICRISAT Asia Center, India (Rupela, O. P., Johansen, C., and Herridge, D. F., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Introduction

Chickpea (*Cicer arietinum* L.) is an important cool-season legume of the semi-arid tropics, and is considered to sustain cropping-system productivity. Among other factors, this is due to its ability to fix atmospheric N_2 in its root nodules, and the beneficial effects on subsequent crops. It is nodulated by *Bradyrhizobium* sp. (*Cicer*) bacteria (Gaur and Sen 1979, Jordan 1984). Although the extent of nodulation and N_2 fixation vary among cultivars of legumes (Nutman 1981, Phillips et al. 1985), high-nodulating and high N_2 -fixing genotypes are not necessarily high yielding, as has been reported with common bean (*Phaseolus vulgaris* L.) cv. Dunadja (Hardarson et al. 1984) and some Korean lines of soybean (*Glycine max* (L.) Merr.) (Herridge and Betts 1988). Similar examples may have prompted some scientists to think that maintaining nodules and their N_2 -fixing functions diverts significant resources of energy to roots, resulting in low legume yields compared with those of cereals (Arnon 1980). On the other hand, groundnut (*Arachis hypogaea* L.), pigeonpea (*Cajanus cajan* Millsp.), and chickpea lines devoid of nodules (i.e., genetically nonnodulating), developed from nonnodulating plants occurring naturally in landraces or in segregating populations, when supplied with abundant fertilizer N, produced yields similar to those of nodulated plants (Nambiar 1990, Rupela 1992, Rupela and Johansen 1995).

Variants of bacteria and plants developed by recombination, induced mutation or selection can serve as basic material in better understanding events of a given biological process at biochemical and molecular levels (Crank et al. 1993, Schuller et al. 1988, Yudkin and Offord 1973). Natural occurrence of nonnodulating plants in cultivars developed through hybridization and by selection from landraces of chickpea (Rupela 1992), has been reported to be genetically controlled (Singh et al. 1992, Singh and Rupela, in press). This led us to further explore the occurrence of plants with large differences in nodulation capacities within chickpea cultivars (Rupela 1994) because these could be useful in better understanding of the N_2 fixation process. In greenhouse (Wani et al. 1995) and field tests (Rupela et al. 1995, Rupela et al. 1997), the relative differences for nodulation between high- and low-nodulating selections within cultivars were consistent on a Vertisol soil at ICRISAT Asia Center. This paper discusses further work on developing more nodulation variants of chickpea, and the potential value of nodulation variants, in general.

Genetic Variation in Nodulation Capacity Within Advanced Breeding Lines of Chickpea

The high-nodulating (HN)/ N_2 -fixing lines developed from two cultivars, ICC 4948 (= G 130) and ICC 5003 (= K 850), through pureline selection (Rupela 1994) were subsequently evaluated for stability of their N_2 -fixation traits at eight locations in

four countries during 1994/95 and 1995/96 postrainy seasons (Dudeja et al., these proceedings). Their nodulation was found to be consistently superior to the low-nodulating (LN) selections from the same cultivar at all the locations except where problems of fusarium wilt (caused by *Fusarium oxysporum* f. sp. *ciceri*) and variable plant stand occurred. The HN selections generally yielded higher than the LN selections at these locations. However, the high N_2 -fixing variants were of limited value because of their susceptibility to the disease.

In the 1991/92 and 1992/93 postrainy seasons, we initiated a screening program to examine the occurrence of plants with large differences in nodulation capacities within advanced breeding lines, reported tolerant to fusarium wilt. A maximum of 353 plants of each of 86 advanced breeding lines or recently released cultivars, and some germplasm accessions were examined at physiological maturity. Seed from the selected plants were used to produce single plant progenies which were evaluated/advanced (Rupela 1994) for high or low nodulation after selection from its parent line (an advanced breeding line or a germplasm line). During the early stages of screening/evaluation for 2-3 years, the selections were generally grown in nonreplicated 4-m single rows. Promising selections developed from the screening program were evaluated for nodulation and yield (total dry matter and grain) in a replicated test in the 1995/96 postrainy season, and compared with their parents.

The experiment had 84 entries as subplots (4 m \times 0.6 m) of a split-plot design. Sixty-four of these were selections developed for high or low nodulation from 17 parent lines. A given parent line and its selections were designated as a group, and there were thus 17 groups in the experiment, each having 1-10 selections. A high-nodulating chickpea line ICC 5003 was included as a control. All of these were evaluated on a Vertisol for the first time, and had three replications at two contrasting soil N levels taken as main plot treatments.

Low (N1) and high (N2) soil N levels, representing those that can be found in farmers' fields, were created by applying 0 (N1) and 100 kg N ha⁻¹ (N2) as urea to the preceding sorghum. A total of 868 mm rain fell between N application and sowing of chickpea on 1 Nov 1995, when the mineral N concentration in the top 15 cm of the soil profile in the N2 plots was 1.7 times (15.0 mg N kg⁻¹ soil) higher than in N1 plots (8.7 mg N kg⁻¹ soil). Observations on nodule number and nodule mass per plant of the entries were made between 42 and 48 days after sowing (DAS), by sampling 30 (10 \times 3 replications) plants of each entry. Total dry matter and grain yield were assessed on 1.8 m² plots between 86 and 108 DAS. Long-duration chickpea lines are known to suffer yield reduction in peninsular India because of high temperatures at pod filling (Saxena 1987). The experiment involved entries of short- to long-maturity durations. To simplify discussion, only data on total dry matter yield are presented here. A specially developed statistical analysis program

using Genstat 5 version 3.2 allowed comparisons of the selections among themselves, with their parents, with other parents as a group, and with the control cultivar ICC 5003.

Three of the 17 advanced breeding lines (ICCV 91016, ICCV 91019, and ICCV 91026) had HN selections that were significantly ($P < 0.05$) better than their parents (Table 1). Some selections in these three advanced breeding lines and in ICC 4958, a germplasm accession, were significantly ($P < 0.05$) better than others within their respective groups. The superiority in nodule mass (in some cases, at N1 and in others, at N2) of these ranged from 64% in CP 92252 (parent ICCV 91016) to 293% in CP 92296 (parent ICCV 91019). All of these produced more total dry matter than their parents and/or the other selections in their groups. The increase in total dry matter of the selections (forming significantly superior nodule mass) over their parents ranged from 5% in CP 92252 at N1, to 65% in CP 92296 at N2. In most cases, however, the increase was not statistically significant ($P < 0.05$).

At the high soil N level, nodule mass of all chickpea lines (control, parents, and selections alike) was substantially reduced (by 53% on overall mean basis and by 49% in the control cultivar). But some selections, such as CP 92297 (parent ICCV 89230), CP 91368 (parent ICCV 89230), CP 92012 (parent 91019), and CP 92095 (parent ICCV 89305), formed nodule mass similar to or higher than that of the control at N1, and at N2, had <40% reduction. Such other selections as CP 92298 (parent ICCV 89230) and CP 92296 (parent ICCV 91019) showed 43–60% reduction in nodule mass at N2 from that at N1, but still had higher nodule mass (by 35–41%) at N2 than that formed by the HN control at N2. All these selections produced 5–65% higher total dry matter than their parents, and 14–39% higher than the control cultivar at N2. This is important because the correlation between the total dry matter and nodulation, though nonsignificant, was negative in this experiment. The study strongly indicates that it is possible to select for HN (and high N₂-fixing) plants from agronomically accepted varieties. Also, these selections can produce a total dry matter similar to, or higher than their parents. The possibility of selecting HN lines at high soil N is also indicated. Such lines should depend more on nodule-fixed N than their parents, particularly when the soil N concentrations are suppressive to nodulation/N₂ fixation. As a result, they should produce high yield and/or spare soil N for the subsequent nonlegume crop.

Value of Nodulation Variants

Nonnodulating Lines As Reference for N₂ Fixation Quantification Studies

Reliable measurement of N₂ fixed by a legume is important if this N is to be managed appropriately. Principal assumptions of ¹⁵N-based methodology are that the reference plant lacks the ability to fix N₂ and that the ¹⁵N/¹⁴N ratio of its tissues is the same

Table 1. Mean values on nodule mass and total dry matter of single plant progenies selected for large differences in nodulation capacities within advanced breeding lines of chickpea, ICRISAT Asia Center, post-rainy season 1995/96¹.

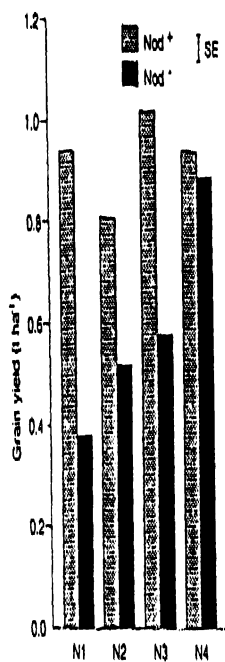
Parent	No. of selections	Type of means ²	Nodule mass (mg plant ⁻¹)			Total dry matter (t ha ⁻¹)			Nodulation improvement ³ (%)
			N1	N2	Mean	N1	N2	Mean	
ICCV 89230	2	a	117 ± 15.9	74 ± 15.9	96 ± 10.1	2.2 ± 0.15	2.4 ± 0.15	2.3 ± 0.10	4
		b	114 ± 12.3	55 ± 12.3	85 ± 7.1	2.6 ± 0.12	2.4 ± 0.12	2.5 ± 0.07	-
ICCV 89302	6	a	129 ± 15.9	78 ± 15.9	103 ± 10.1	2.0 ± 0.15	2.4 ± 0.15	2.2 ± 0.10	-
		b	128 ± 9.2	62 ± 9.2	95 ± 4.1	2.1 ± 0.08	2.4 ± 0.08	2.3 ± 0.04	-
ICCV 91016	5	a	76 ± 15.9	21 ± 15.9	49 ± 10.1	2.1 ± 0.15	2.4 ± 0.15	2.3 ± 0.10	64, N1
		b	79 ± 9.5	39 ± 9.5	59 ± 4.5	2.0 ± 0.09	2.4 ± 0.09	2.2 ± 0.05	129, N2
ICCV 91019	3	a	36 ± 15.9	21 ± 15.9	29 ± 10.1	1.5 ± 0.15	1.9 ± 0.15	1.7 ± 0.10	293, N1
		b	92 ± 10.8	52 ± 10.8	72 ± 5.8	2.0 ± 0.10	2.4 ± 0.10	2.2 ± 0.06	273, N2
ICCV 91026	5	a	72 ± 15.9	47 ± 15.9	60 ± 10.1	1.8 ± 0.15	2.4 ± 0.15	2.1 ± 0.10	88, N1
		b	85 ± 9.5	26 ± 9.5	56 ± 4.5	1.9 ± 0.09	2.4 ± 0.09	2.2 ± 0.05	-
ICC 4958	2	a	65 ± 15.9	21 ± 15.9	43 ± 10.1	2.3 ± 0.15	2.9 ± 0.15	2.6 ± 0.10	41, N1
		b	60 ± 12.3	33 ± 12.3	47 ± 7.1	2.0 ± 0.12	2.7 ± 0.12	2.4 ± 0.07	99, N2
ICC 5003 (Control)			91 ± 15.9	46 ± 15.9	69 ± 10.1	1.3 ± 0.15	2.2 ± 0.15	1.8 ± 0.10	

1. Data for selected parents where selections were significantly different ($P < 0.05$) from parents or among themselves for nodule mass.

2. a = mean values for the parent, b = mean values for selections from the parent.

3. Percentage of increase in nodule mass of a selection at N1 or N2 over its parent.

4. - = no improvement.



as soil mineral N; and the legume and reference plants explore soil N pools of identical composition for the same length of time (Peoples, Turner, et al., these proceedings). Nonnodulating isolines of legumes are the preferred reference (control) lines for their N₂ fixing isolines (Fried et al. 1983) and are thus of limited application. ¹⁵N-based methods are considered reliable (Danso et al. 1993) for quantification of N₂ fixed by legumes. These methods require use of mass spectrometers, which are generally inaccessible to most BNF researchers in developing countries. The ¹⁵N-enrichment method also requires use of expensive labelled N-fertilizer.

Use of ¹⁵N natural abundance method requires determination of isotope discrimination between ¹⁵N and ¹⁴N (B-value), which occurs during N₂ fixation. B-value of -1.65‰ for 100% N₂-dependent greenhouse-grown chickpea plants has been determined, and is expected to apply in most situations (Peoples, Turner, et al., these proceedings). The delta value of nodulated lines in some of our

unpublished experiments was as low as -2.58. If we use a B-value of -1.65‰, this will mean >100% N₂ fixation, which is physiologically impossible. Peoples, Turner, et al. (these proceedings) suggested to arbitrarily nominate such low values as 100%, i.e., designate B-value as -2.58‰. Thus the ¹⁵N-based methods are not without difficulties. While highlighting problems and misconceptions with the ¹⁵N-based methods, Danso et al. (1993) stated that even though not perfect, these methods are still the most reliable to measure N₂ fixation, and should provide near-accurate results when used carefully. Nonnodulating chickpea lines responded to increasing levels of soil mineral N (Fig. 1) when grown on a Vertisol at IAC. It needs to be widely examined if these would be of value for the difference method of quantification of N₂ fixed by nodulated chickpea lines. If found suitable, it would obviate the need of expensive ¹⁵N fertilizers and equipment to determine ¹⁵N.

Additional Residual Effect of the High-Nodulating Selections

It is widely reported that the yield of cereal crops following legumes is significantly greater than after non-legumes (Ladha and Kundu, these proceedings). The question of whether the additional nodulation/N₂ fixation of the HN selections would result in any additional residual effect was studied on a Vertisol at IAC (Rupela et al. 1995). Plants of widely different nodulation capacities occurring in a chickpea variety ICC 4948 (= G 130), and later developed into separate lines were compared in a field experiment on chickpea-sorghum cropping system. In this experiment, ICC 4948 HN formed significantly ($P < 0.05$) greater nodule number (36 per plant at N1 and 16 per plant at N2) and nodule dry mass (74 mg per plant at N1 and 21 mg per plant at N2) than did ICC 4948 LN or its parent ICC 4948 (= G 130). Biomass and grain yield produced by the HN selection were, however, only 9-10% higher than 4.33 t of biomass, and 2.05 t of grain yield per ha, of the LN chickpea line ICC 4948 LN. A significantly higher nodule mass of the HN selection did result in significantly higher grain N concentration and consequently 17-25% higher N yield than the LN selection and the parent (range 109-116 kg N ha⁻¹) at the low soil N level (N1). After the chickpea was harvested on 24 Feb 1993, hybrid sorghum CSH 6 was sown on 24 Jun 1993. Sorghum was harvested on 16 Oct 1993, and stover yield, grain yield, and N content were measured. Sorghum grown after the HN chickpea produced 6% higher stover yield and 7% higher grain yield at low soil N than those grown after the LN selection from ICC 4948 (Table 2), which yielded 3.7 t of stover and 2.29 t of grain per ha. The total N yield, both of chickpea and sorghum from plots having a HN selection was 13% greater than those growing a LN selection, which was 153 kg ha⁻¹ at the N1 level. Differences between the two treatments for grain and stover yield were, however, not statistically significant. The treatment differences due to the three nodulation types

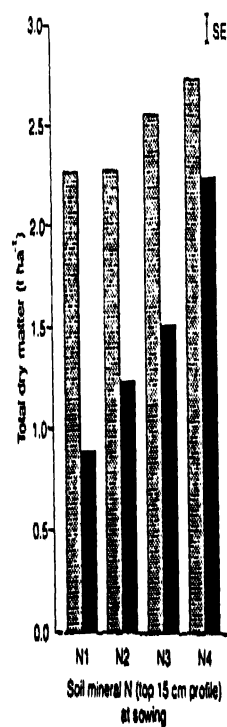


Figure 1. Mean (5 years, 1990/91 to 1994/95) total dry matter and grain yield of five nodulated (Nod⁺) chickpea cultivars and nonnodulating (Nod⁻) selections from each of them, Vertisol, post-rainy season, ICRISAT Asia Center. Mean mineral N concentration (mg g⁻¹ soil) in top 15 cm soil at the time of sowing chickpea was 11 (N1), 13 (N2), 16 (N3), and 26 (N4). No N was applied to chickpea. Preceding sorghum (rainy season) received 0 (N1), 40 (N2), 80 (N3), and 160 (N4) kg N ha⁻¹ in two split doses in all the 5 years, except in 1992 when 0 (N1), 80 (N2), 160 (N3), and 320 (N4) kg N ha⁻¹ was applied. Twenty kg P ha⁻¹ as single super phosphate was applied to chickpea at sowing in 1990/91, 1992/93, and 1994/95. Data are from nonreplicated demonstration plots (8m x 7.2m), year was used as replication for statistical analysis.

Table 2. Stover, grain, and N yield of sorghum (CSH 6) grown after chickpea, and total N yield of chickpea + sorghum, ICRISAT Asia Center, rainy season 1993.

Chickpea line grown before sorghum ¹	Grain ²			Stover			N yield of chickpea + sorghum (kg ha ⁻¹)											
	N1	N2	Mean	N1	N2	Mean	N1	N2	Mean									
ICC 4948 HN	2.38	2.65	2.52	4.01	4.16	4.08	173	160	166									
ICC 4948 LN	2.29	2.42	2.36	3.70	3.99	3.84	153	193	173									
ICC 4948	2.40	2.20	2.30	4.26	3.74	4.00	152	127	139									
ICC 4993 Nod	1.68	1.95	1.81	3.30	3.06	3.18	32	72	52									
SE	±0.249(±0.238) ³			±0.168			±0.367(0.272)			±0.193			±12.0(±10.5)			±7.4		
Mean	2.19	2.31		3.81	3.74		128	138										
SE	±0.140			±0.124			±7.9											

1. HN = high-nodulating, LN = low-nodulating, Nod = nonnodulating.

2. N1 = low soil N; N2 = high soil N.

3. Values in parentheses are SEs to compare means within the same N-level.

Source: Rupela et al. 1995.

were appreciable at the high soil N level (N2) where chickpea nodulation was greatly reduced in the 1992/93 postrainy season.

From the data reported (Rupela et al. 1995), it was apparent that the use of HN selection only marginally improved the overall productivity of the cropping systems, at least in first year. At N1 level, the plots where HN chickpea selection was grown, yielded 20 kg N ha⁻¹ higher (sorghum + chickpea) than those where the LN selection was grown. The long-term significance of this small gain needs to be examined in long-term studies.

Soil Biology and High BNF Variants

The HN selection in the experiment described above produced only marginally higher grain yield (3.3-6.9%, mean of the two N levels) than that of the parent variety, and the LN selection from it. But the HN selection fixed significantly more atmospheric N at low soil N, as measured by acetylene reduction activity. In this experiment, soil microbial biomass carbon determined at flowering (46 DAS) was 1.8 times, and microbial biomass N was two times more in case of the HN than in the LN selection (Fig. 2), when grown at low (4.5 mg mineral N g⁻¹ soil)

soil N (Rupela et al. 1995). No such differences in microbial biomass were observed in these lines when grown at the high (8.1 mg mineral N g⁻¹ soil) soil N level. These results indicate that when grown at low soil N, the HN selections supported more microbial activity in the soil, perhaps by providing the necessary C through root exudate and through senescing roots and nodules. Mean (of the 2 N levels) flush C:N ratio was low (13.8) in HN plots and high (18.9) in LN plots (Rupela et al. 1995). Such an increase in microbial biomass and reduced flush C:N ratios should result in increased availability of soil nutrients in plots of HN lines.

Nodulation Variants as Research Tools

Legumes use two main sources of N, mineral N and symbiotically fixed N. Young plants use seed or soil N (or fertilizer N) initially, until N₂ fixation through nodules is established (generally called the complementary stage between mineral N and BNF). After the nodules are established, BNF is suppressed by high soil N, if present (an antagonistic stage for the two sources of N). Field studies on N nutrition are therefore complex and difficult. Appropriate studies on nodulation variants Nod⁻, LN, HN, and of high N-tolerant symbiosis, reported by Rupela (1994), can help develop a better understanding of N metabolism in legumes. Sagan et al. (1993) used Nod⁻ and supernodulating mutants of field-grown peas to analyze N nutrition. They reported that N-deficiency induced flowering termination, and that the source of N (mineral N versus symbiotically fixed N) had little effect on yield.

In ureide-producing legumes, the xylem sap composition has a 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 such amide-producing legumes as chickpea, a xylem sap based BNF quantification method similar to that for pigeonpea is not available. A better

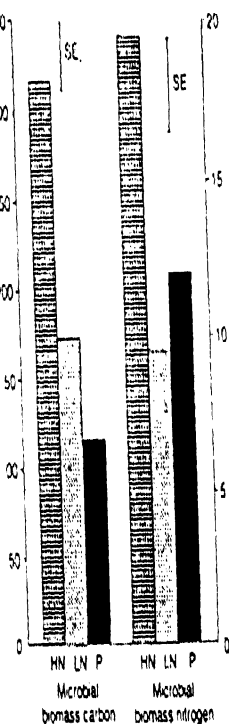


Figure 2. Microbial biomass carbon and nitrogen (µg g⁻¹ soil) in soil from low soil N level plots of chickpea lines of high (HN), low (LN) nodulation capacities and their parent ICC 4948-P. Source: Rupela et al. (1995).

understanding of N metabolism using the nodulation variants could lead to development of such a method for chickpea.

The nonnodulating lines of chickpea lack nodulation ability, but form normal root hairs (Rupela 1992). Such lines can be useful in examining if changes in flavonoids (signal factors) are responsible for the nonnodulation. Such studies may lead to understanding underlying reasons for the occurrence of LN and HN plants within cultivars.

Nitrogen fixation by legume-rhizobial symbiosis uses products of photosynthesis, and therefore it competes for photosynthate partitioned to economic yield (Atkins 1984, Tiwary and Heichel 1991). The lines with large differences in nodulation capacities within cultivars are putative isolines, and have been used to understand carbon budgeting in relation to N_2 fixation in chickpea. Five different selections with increasing capacity for nodulation within chickpea cultivar ICC 5003 (Rupela et al. 1997) grown in Vertisol-filled pots were used for this purpose by Wani et al. (1995). Chickpea plants grown at two mineral N levels were exposed to $^{14}CO_2$, and the distribution of ^{14}C -photosynthate in different plant parts was determined. Total plant biomass of the chickpea selections increased significantly ($P < 0.01$) with increasing mineral N and nodulation level when measured at 57 DAS. Mean percentage ^{14}C -photosynthate translocated to the roots decreased with increasing nodulation capacity, and also with increasing mineral N level. Low-nodulating selections of chickpea grown under low soil N level were unable to fulfil their N requirements through BNF as indicated by their higher root/shoot ratio compared with the HN selections. Such plants invested more carbon in increased root production so as to exploit soil N, as evidenced by the greater partitioning of ^{14}C -photosynthate to roots in LN selections.

Conclusion

Substantial progress has been made towards characterizing the role of rhizobia in symbiosis, the genes involved in signalling, and expression of *nif* genes later in the plant, but information from the plant is rather sparse. Hitherto unknown plant genes may be involved in the process of infection, elongation of infection thread, differentiation of the bacteroid, and finally, expression of the *nif* genes. Mutants affected in nodulation are the key components in understanding nodule development and determine role of plants in the symbiotic association. Naturally occurring nodulation variants within cultivars, reported for chickpea, an important legume in the semi-arid tropics, will be useful in better understanding of the symbiotic process. In addition, high nodulating/high N_2 -fixing variants with tolerance to stress factors will be

of value in sustaining production of relevant cropping systems. The nonnodulating variants can be appropriate nonfixing reference crops for quantification of N_2 fixed by nodulated chickpea by ^{15}N -based and difference methods.

Acknowledgments

This research work was partly supported by the Special Purpose Grant from the Australian Centre for International Agricultural Research (PN 95710) to ICRISAT, and is gratefully acknowledged.

References

- Arnon, I. 1980. Breeding for higher yields. Pages 77-81 in *Physiological aspects of crop productivity*. Bern, Switzerland: International Potash Institute.
- Atkins, C. A. 1984. Efficiencies and inefficiencies in the legume-Rhizobium symbiosis: A review. *Plant and Soil* 82:273-284.
- Crank, S. F., Wilson, K. J., Tewari, S., and Giller, K. E. 1993. A host specific, nitrogen fixation mutant of *Bradyrhizobium*: physiology on three host plants. *Journal of Experimental Botany* 44:1305-1312.
- Danso, S. K. A., Hardarson, G., and Zapata, F. 1993. Misconceptions and practical problems in the use of ^{15}N soil enrichment techniques for estimating N_2 fixation. *Plant and Soil* 152:25-52.
- Fried, M., Danso, S. K. A., and Zapata, F. 1983. The methodology of measurement of N_2 fixation by non-legumes as inferred from field experiments with legumes. *Canadian Journal of Microbiology* 29:1053-1063.
- Gaur, Y. D., and Sen, A. A. 1979. Cross inoculation group specificity in *Cicer* Rhizobium symbiosis. *New Phytologist* 83:745-754.
- Jordan, D. C. 1984. Family III. Rhizobiaceae. Pages 235-256 in *Bergey's manual of systematic bacteriology*, Vol 1 (Krieg, N. R., ed.). Baltimore, USA: Williams and Wilkins Publishing Company.
- Hardarson, G., Zapata, F., and Danso, S. K. A. 1984. Effect of plant genotype and nitrogen fertilizer on symbiotic nitrogen fixation by soybean cultivars. *Plant and Soil* 82:397-405.
- Herridge, D. F., and Betts, J. H. 1988. Field evaluation of soybean genotypes selected for enhanced capacity to nodulate and fix nitrogen in the presence of nitrate. *Plant and Soil* 110:129-135.

Nambiar, P. T. C. 1990. Nitrogen nutrition of groundnut in Alfisols. Information Bulletin no. 30. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 28 pp.

Nutman, P. S. 1981. Hereditary host factors affecting nodulation and nitrogen fixation. Pages 194-204 in Current perspectives in nitrogen fixation (Gibson, A. H., and Newton, W. E., eds.). Canberra, Australia: Australian Academy of Science.

Phillips, D. A., Bedmar, E. J., Qualset, C. O., and Tauber, L. R. 1985. Host legume control of *Rhizobium* functions. Pages 203-213 in Nitrogen fixation and CO₂ metabolism (Ludden, P. W., and Burris, J. E., eds.). New York, USA: Elsevier Science Publishing Company Inc.

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 (*Cajanus cajan* L. Millsp.). *Journal of Experimental Botany* 40:535-542.

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

Rupela, O. P. 1994. Screening for intracultivar variability of nodulation in chickpea and pigeonpea. Pages 75-83 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 1993, 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.

Rupela, O. P., and Johansen, C. 1995. Identification of nonnodulating, and low and high nodulating plants in pigeonpea. *Soil Biology and Biochemistry* 27:539-544.

Rupela, O. P., Wani, S. P., Danso, S. K. A., and Johansen, C. 1995. Effect of high nodulating selection of chickpea cultivar ICC 4948 on yield and soil properties of a chickpea-sorghum cropping system. *Journal of Soil Biology and Ecology* 15:127-134.

Rupela, O. P., Sharma, L. C., and Danso, S. K. A. 1997. Evaluation of N₂ fixation by nodulation-variants of chickpea in India. Pages 99-119 in Improving yield and nitrogen fixation of grain legumes in the tropics and sub-tropics of Asia (Eaglesham, A. R., ed.). Vienna, Austria: International Atomic Energy Agency.

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.

Saxena, N. P. 1987. Screening for adaptation to drought: case studies with chickpea and pigeonpea. Pages 63-76 in Adaptation of chickpea and pigeonpea to abiotic stress: proceedings of the Consultants' Workshop, 19-21 Dec 1984, ICRISAT Asia Center, India (Saxena, N. P., and Johansen, C., eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Schuller, K. A., Minchin, F. R., and Gresshoff, P. M. 1988. Nitrogenase activity and oxygen diffusion in nodules of soybean cv. Bragg and a supernodulating mutant: effects of nitrate. *Journal of Experimental Botany* 39:865-877.

Singh, O., and Rupela, O. P. (in press). A new gene that controls root nodulation in chickpea. *Crop Science*.

Singh, O., van Rheenen, H. A., and Rupela, O. P. 1992. Inheritance of a new nonnodulation gene in chickpea. *Crop Science* 32:41-43.

Tiwary, S. N., and Heichel, G. H. 1991. Carbon costs of dinitrogen fixation associated with dry matter accumulation in alfalfa. *Crop Science* 31:985-992.

Wani, S. P., Sivaramakrishnan, S., Rupela, O. P., Johansen, C., and Lee, K. K. 1995. Partitioning of ¹⁴C-photosynthate in low and high nodulating selections of chickpea. Pages 203-208 in Nuclear methods in soil-plant aspects of sustainable agriculture (IAEA-TECDOC-765): proceedings of an FAO/IAEA regional seminar, 5-9 Apr 1993, Colombo, Sri Lanka. Vienna, Austria: International Atomic Energy Agency.

Yudkin, M., and Offord, R. (eds.). 1973. Methods in studying metabolism. Pages 225-232 in Comprehensible biochemistry. London, UK: Longman Group Limited.