EVALUATION OF N_2 FIXATION BY NODULATION-VARIANTS OF CHICKPEA IN INDIA

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Abstract

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Five nodulation-variants of chickpea (*Cicer arietinum* L.) cv. ICC 5003, delineated on the basis of visual ratings ('S1' for minimum nodulation to 'S5' for maximum), were used to investigate the optimum levels of nodulation and N_2 fixation for growth and yield. Two field experiments were conducted, with fertilizer N (enriched in ^{15}N) applied at 10 ('N1') or 100 kg ha^{-1} ('N2') on contrasting soils in different years; plants were evaluated for nodulation, growth, N_2 fixation and yield. Experiment 2 included high-nodulating (HN) and low-nodulating (LN) selections of cvv. ICC 4948, ICC 14196 and Kourinsky. Non-nodulating selections of chickpea were included as references to quantify N_2 fixation. In both experiments the trends in amounts of N fixed by the five selections at N1 were similar when assessed by ^{15}N-enriched and by N-difference methods. The percent N derived from N_2 fixation (estimated from ^{15}N data) correlated with yield and amount of N fixed in Experiment 2, but not Experiment 1. The relative nodulation differences were consistent across locations; the S4 and S5 (and HN) lines were generally superior to S1 and S2 (and LN) for nodulation, N_2-fixation, total dry matter and grain yield. Nodule number and mass correlated signficantly ($P<0.05$) with total dry matter, grain yield, total N and quantity of N_2-fixed in both experiments.

1. INTRODUCTION

Chickpea (*Cicer arietinum*) is an important cool-season legume of the semi-arid tropics 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 to beneficially affect subsequent crops. It is nodulated by *Bradyrhizobium* sp. (*Cicer*) bacteria [1, 2]. Although the extent of nodulation and N_2 fixation varies among cultivars [3, 4], high-nodulating and high-N_2-fixing genotypes are not necessarily high yielding, as has been reported for common bean (*Phaseolus vulgaris* L.) cv. Dunadja [5] and some Korean lines of soybean [*Glycine max* (L.) Merr.] [6]. Similar examples may have led some breeders to think that maintaining nodules and their N_2-fixing functions diverts significant resources of energy to roots, resulting in low legume yields compared to cereals [7]. On the other hand, groundnut (*Arachis hypogaea* L.), pigeon pea [*Cajanus cajan* (L.) Millsp.] and chickpea plants devoid of nodules (i.e. genetically non-nodulating) when supplied with abundant fertilizer N produce yields similar to those of nodulated plants [8, 9, 10].

What is optimum nodulation and N_2 fixation for a legume? It is an important question, but difficult to answer. The extent of nodulation and N_2 fixation of a given cultivar may vary with moisture, nutrient availability, and temperature of the root and shoot. Large plant-to-plant differences in nodulation capacity have been observed within bred cultivars and land races [11], with the natural occurrence of non-nodulating plants [9]. Chickpea cv. ICC 5003 (= K850 = 850-3/27) was found to have plants with a range of nodulation capacities; when rated visually on a '1' (minimum nodulation) to '5' (maximum nodulation) scale [12], about 70% of 4,965 plants observed had a rating of '4' and only 1% had ratings of '1' and '5'. Selected plants of rating '1' (designated S1) to '5' (S5) were developed into separate accessions using pure-line selection procedures. The reasons for wide-ranging nodulation capacity within a cultivar are not clear, and need to be studied.

These nodulation-variants offer the opportunity to address the question of optimum nodulation and N_2-fixation capacity in chickpea. In an experiment with pots containing a vertisol, the different nodulation ratings were found to be generally stable [13, 14]. This paper reports performance of these selections in field experiments at two locations set up to address the following questions.

- Does the nodulation response of the five selections hold across locations?
- Does nodulation capacity correlate with amount of N fixed?
- Do the high-nodulating selections yield more?

2. MATERIALS AND METHODS

2.1. Nodulation selections and experimental sites

The five nodulation-variants (selections S1 to S5) of chickpea cv. ICC 5003 were initially developed on an entisol at the research farm of the College of Agriculture, Gwalior (26°N) India, 1987-90 [11]. In the post-rainy season of 1991-92, one representative of each selection was studied at the Agricultural Research Station Durgapura (near Jaipur), Rajasthan (27°N), India (Experiment 1). This provided a different agroclimate and soil type, and perhaps a different rhizobial population, from those at Gwalior. A non-nodulating (Nod^-) selection of ICC 5003 (ICC 5003 NN) was included as a non-fixing reference. The soil at the site was sandy, pH 8.2, EC 0.72 dS m^{-1}, mineral N 7.0 mg kg^{-1} soil and total N 359 mg kg^{-1} soil, with 5.2 x 10^3 chickpea rhizobia g^{-1} soil in the top 15 cm at the time of sowing. Minimum temperatures at Durgapura range between 3 and 13°C and maximum temperatures between 22 and 34°C during the growing season.

In the post-rainy season of 1992-93, the five selections were evaluated on a vertisol at the ICRISAT Asia Center (IAC, 18°N), India, along with the parent cultivar (Experiment 2). The vertisol site of this experiment had pH 8.2, EC 0.25 dS m^{-1}, mineral N 9.8 mg kg^{-1} soil and total N 549 mg kg^{-1} soil, with 8.9 x 10^3 chickpea rhizobia g^{-1} soil in the top 15 cm at the time of sowing. Included were high-nodulating (HN, visual rating '3' to '5') and low-nodulating (LN, visual rating '1' to '2') selections (one each) of cvv. ICC 4948, ICC 14196 and Kourinsky, along with their parents, and one non-nodulating (NN) selection each of ICCV 2, ICC 4918 (= Annigeri), ICC 4993, and their parents. The Nod^- chickpea lines were included as non-fixing references. Winter at IAC is mild and short, and the average temperatures for October to February range from 28 to 30°C during the day and from 15 to 20°C at night.

2.2. Experiment 1

Two soil N levels, achieved by application of ammonium sulphate at 10 kg N ha^{-1} (designated 'N1') and 100 kg N ha^{-1} ('N2'), were the main plots, and five nodulation-variants and the parent cv. ICC 5003 were the sub-plot treatments of a split-plot design with four replications. The N2 plots had 39 mg mineral N kg^{-1} soil in the top 60-cm soil profile, when measured eight days after N application at the time of sowing. The sub-plot size was 4 x 2.4 m (9.6 m^2).

Furrows were opened using a bullock-drawn plough, and single super phosphate (200 kg ha^{-1}) was applied in the 30-cm rows. Each sub-plot had a microplot of 0.8 m x 1.2 m (0.8-m long, 4 rows) in the center, for ^{15}N application. The ammonium sulphate, applied as single doses, was enriched with ^{15}N at approximately 10% atom excess for N1 and 1% atom excess for N2. The required quantities were dissolved in water and 500-mL aliquots were applied to each 0.8-m-long furrow. The remainder of the each sub-plot received similar applications of unenriched ammonium sulphate. After N application, furrows were manually covered with a thin layer of soil to minimize surface movement of fertilizer during manual sowing. Peat-based inoculant (strain IC 59) containing 2.1 x 10^9 rhizobia g^{-1} was applied at 500 mg m^{-2} after mixing with local soil (1 g inoculant kg^{-1} soil); the field was then irrigated. Bunds were constructed around each plot to prevent fertilizer transfer. After eight days, on 23 October 1991, seeds were dibbled at a 20-cm spacing, on fertilizer rows spaced at 30 cm. The seeds had been coated with inoculant to provide about 2.3 x 10^4 cells per seed. Two irrigations were provided, at 32 days after sowing (DAS) and at 78 DAS. There were no serious pest problems and preventative action against *Helicoverpa* pod-borer was taken at early signs of damage, by spraying 2 L ha^{-1} of Endosulphan (35 EC) at 102 DAS. Physiological maturity was reached at about 132 DAS and final harvesting was at 152 DAS.

Plants from 0.36 m^2 were sampled at 42 DAS, and the shoots dried (70°C). Roots were excavated and acetylene reduction (AR) activities [15], nodule numbers and nodule dry weights determined. The presence of nodules on some Nod^- plants caused us to sample them again, at 92 DAS.

At final harvest, plants from 4.8 m^2 were dried in the sun and processed for total dry matter including fallen leaves, and grain yield. Of the four 0.8-m-long rows of the ^{15}N-micro plots, 0.6-m lengths of the two middle rows were harvested. Stover and seeds from the micro plots were weighed and milled separately to < 1 mm.

2.3. Experiment 2

The field used had been prepared as a long-term ^{15}N plot for screening germplasm accessions for high N_2-fixation. Soil profiles with two different N levels were created by applying ^{15}N-enriched ammonium sulphate at 10 and 100 kg N ha^{-1} (N1, N2), at approximately 10% and 1% atom excess, respectively. The ^{15}N was applied to the preceding crop of millet (*Pennisetum glaucum* (L.) R.Br. cv. ICMS 7703) sown on 8 July 1992, i.e. during the rainy season. The ammonium sulphate ^{15}N-enrichment values were . The millet crop was ratooned at 34 DAS and finally harvested at 65 DAS. It was chopped and incorporated into the plots from which it had been cut. The crop harvested at 34 DAS was retained after chopping and oven drying, and later incorporated. The field was prepared as 60-cm ridges after another 45 days when the incorporated millet had apparently decomposed.

There were no micro plots; ^{15}N had been applied to the whole area. The N-treatments formed main plots of the split-plot design, with four replications. The twenty-one chickpea entries (see **2.1.**), including the five nodulating variants (S1 to S5) of ICC 5003 used in Experiment 1, were the sub-plot treatments. The sub-plot size was 2 x 1.2 m.

The experiment was sown on 31 October 1992, on 60-cm ridges, with two rows per ridge spaced at 30 cm, and 10 cm between plants. Single super phosphate (250 kg ha^{-1}) was manually applied before sowing. The experiment was sprinkler-irrigated (38 mm) on the day after sowing, and a flood irrigation was made at 54 DAS. Weeding was done at 25 and 68 DAS and Endosulphan (35 EC, 2 L ha^{-1}) was sprayed against insects at 34 and 81 DAS. Some areas were affected by wilt caused by *Fusarium oxisporum* f. sp. *ciceri*, and data from such were excluded from statistical analysis. Net plot size for the final harvest was 1.8 m^2. Most entries matured at about 95 DAS and were harvested between 101 and 104 DAS.

Plants of cv. ICC 5003 and its five nodulation-variant selections (S1 to S5) were excavated from a 0.36 m^2 area at 42 DAS, for determinations of nodule number and nodule mass. For the other lines, plants from only two replications were similarly sampled from 0.36 m^2. The final harvest was from a 1.8-m^2 area of each sub-plot between 101 and 104 DAS, before excessive leaf-shedding had occurred. The plants were placed in cloth bags and dried (70°C), followed by threshing for assessment of seed yield and stover mass. Sub-samples of stover and seed from each sub-plot were milled separately to < 1 mm.

2.4. Calculations for assessment of N_2 fixation

The ^{15}N-enriched ammonium sulphate solutions were prepared separately to produce enrichments of approximately 1% and 10% atom excess [16]. Analysis using a Jasco 150 emission spectrometer gave values (in solution) of 0.924 and 8.723% atom excess for Experiment 1, and 0.989 and 9.225% atom excess for Experiment 2. Ground samples of stover and seed from the micro-plots were analyzed for ^{15}N at the Joint FAO/IAEA Soil Science Unit, Seibersdorf, Austria, using a VG 602 mass spectrometer.

In Experiment 1, at least 25% of nominally non-nodulating reference plants in N1 had nodules when observed at 92 DAS, whereas those at N2 were practically devoid of nodules. Also, yields (total dry matter 5.97 t ha^{-1} and grain yield 1.8 t ha^{-1}) were close to those of the best-growing chickpea at N1, therefore we ignored the ^{15}N-enrichment data generated with the nodulation-variants at N2, using only the non-nodulating plants at N2 as reference with the A_N-value method to calculate fixed N at N1 [17]:

$$N\ fixed\ (kg\ ha^{-1}) = (A_{N\ (legume)} - A_{N\ (reference)}) \times \%NFU_{(legume)}$$

$$where\ A_N\ (kg\ ha^{-1}) = \frac{(100 - \%NdfF)}{\%NdfF} \times Rate\ of\ N\ applied$$

$$\text{where } \%NFU = \%\ N\text{-fertilizer utilization} = \frac{\%NdfF \times \text{total } N}{\text{Rate } N \text{ applied}}$$

$$\text{where } \%NdfF = \frac{\%^{15}N \text{ atom excess}_{(crop)}}{\%^{15}N \text{ atom excess}_{(fertilizer)}} \times 100$$

In Experiment 2, the Nod$^-$ selections from cultivars ICCV 2, ICC 4918, and ICC 4993 were free of nodules and were used as reference with the ^{15}N-dilution method to calculating %Ndfa at N1 and N2:

$$\%Ndfa = \left(1 - \frac{\%^{15}N \text{ atom excess}_{(legume)}}{\%^{15}N \text{ atom excess}_{(reference)}}\right) \times 100$$

$$N \text{ fixed } (kg\ ha^{-1}) = \frac{\%Ndfa}{100} \times \text{Total } N \text{ yield}$$

Means of the N-yield data (N uptake by all above-ground plant parts) of the non-nodulating line(s) were deducted from the N yield of a given entry to arrive at the quantity of N fixed by the N-difference method. The net plot size for final harvest was only 1.8 m^2 in Experiment 2, therefore those data would be most appropriately expressed as kg m^{-2}, but, in order to compare the two experiments, they are presented as t ha^{-1}.

3. RESULTS

3.1. Experiment 1

Plants of selections S4 and S5 were best nodulated and difficult to differentiate visually. Means (of the two N-levels) for nodule number and mass of S3, S4, and S5 plants were significantly ($P<0.05$) different from each other (Tables I, II). Also, the N $\times$ selection interactions were significant ($P<0.05$). Mean (of all the five selections) nodulation (both nodule numbers and mass) at N2 was less than half of that at N1. Differences in the five selections were also apparent for acetylene reduction (AR) activity at 42 DAS: barely detectable in S1 and S2 plants, and increasing from S3 to S5 (Table III).

TABLE I. NODULE NUMBER OF NODULATION-VARIANTS S1-S5 OF CHICKPEA CV. ICC 5003 AT TWO N-LEVELS, AT 42 DAS (EXPERIMENT 1)

N level	Nodule number S1	S2	S3	S4	S5	Mean
	(per plant)					
N1	1	0	20	31	37	18
N2	0	0	9	11	16	7
SE			±3.0 (3.2)[a]			±2.3
Mean	1	0	14	21	26	
SE			±1.1			

[a]To compare means for same level of N.

TABLE II. NODULE DRY WEIGHT OF NODULATION-VARIANTS S1-S5 OF CHICKPEA CV. ICC 5003 AT TWO N-LEVELS, AT 42 DAS (EXPERIMENT 1)

N level	Nodule dry weight S1	S2	S3	S4	S5	Mean
	(mg $plant^{-1}$)					
N1	2	1	43	74	78	40
N2	0	0	19	21	32	14
SE			±6.5 (6.6)[a]			±5.5
Mean	1	0	31	47	55	
SE			±2.8			

[a]To compare means for same level of N.

Later (92-100 DAS), all plants of all ratings had nodules at N1, and where nodules were few (< 10 per plant) some were large, approximately 2 cm in diameter. At N2, some plants did not nodulate (data not shown). About 25% plants of the non-nodulating line of ICC 5003 had nodules at N1, but at N2 fewer than 5% plants were nodulated.

Germination and plant establishment were good. However, growth was poor in patches. At 42 DAS, when plants were first sampled for nodulation, poor plant growth was apparently associated with root-knot nematodes (*Meloidogyne incognita* and *M. javanica*).

Total dry-matter (all above-ground parts) yields of the five selections at N2 were significantly ($P < 0.05$) superior to those at N1 (Table IV). Total dry-matter yield generally increased with better nodulation (from S1 to S5) both at N1 and N2, but the differences were non-significant. Mean grain yield (2.19 t ha^{-1}) of the five selections at N2 was also significantly ($P < 0.05$) greater than that at N1 (1.73 t ha^{-1}) (Table V). Also, grain yield increased with nodulation capacity (from S1 to S5) both at N1 and N2. Mean (of the two N levels) yield of the high-nodulating (S4 and S5) selections was significantly superior to those of low-nodulating (S1 and S2) selections. Interactions between N levels and nodulation ratings were non-significant.

The average N yield (of the two N levels) of the five selections increased with nodulation (Table VI). The N yields of the high-nodulating selections (139, 141 kg ha^{-1}) were significantly ($P < 0.05$) superior to those of low-nodulating selections (101, 106 kg ha^{-1}). With the exception of S4, the nodulation-variants contained more N at N2 than at N1; the interaction with applied N was non-significant.

All five selections had similar values for %Ndfa both in stover and grain (Table VII). The %Ndfa values of selections S4 and S5 were only marginally (2 to 3%) higher than those of S1 and S2, although substantially more N was fixed by the high-nodulating selections; S4 and S5 had 32% more fixed N in stover and 67% in grain than did S1 and S2.

When assessed using the N-difference method, the high-nodulating selections had 77% more fixed N in stover, and 117% in grain, than did the low-nodulating selections (Table VII). On a total-N basis, the high-nodulating selections fixed 53% more N when measured by the A-value method and 100% more when measured by the N-difference method, compared to the low-nodulating selections (Fig. 1). The S4 plants fixed marginally more N than did the S5 plants.

3.2. Experiment 2

The S1 plants of cv. ICC 5003 formed the fewest nodules and S5 the most at 42 DAS (Table VIII). Fewer nodules (44% averaged over all entries) were formed at N2 than at N1. The parent cv. ICC 5003 formed 29 nodules at N1, similar to S3.

The HN selections of cvv. ICC 4948, ICC 14196 and Kourinsky formed (average of the two N levels) 1.8- to 4-fold more nodules than did the LN selections of their respective cultivars (Table VIII). The number of nodules formed at N2 for all of the nodulating lines were substantially lower than those at N1, except for ICC 4948 HN. All of the non-nodulating selections were devoid of nodules at both N1 and N2. Plants of ICC 5003 S5 formed the highest number of nodules per plant at both N1 and N2.

TABLE III. ACETYLENE REDUCTION ACTIVITY OF NODULATION-VARIANTS S1-S5 OF CHICKPEA CV. ICC 5003 AT TWO N-LEVELS, AT 42 DAS (EXPERIMENT 1)

N level	AR activity S1	S2	S3	S4	S5	Mean
	(μmoles C_2H_4 plant^{-1} h^{-1})					
N1	0.1	0.0	4.1	5.0	7.1	3.3
N2	0.1	0.0	0.7	0.8	1.3	0.6
SE			±1.19 (0.94)[a]			±0.66
Mean	0.1	0.0	2.4	2.9	4.2	
SE			±0.85			

[a]To compare means for same level of N.

Nodule mass (mg plant^{-1}) of selection S1 from ICC 5003 was least, and increased with nodulation rating to S5 (Table VIII). At N1, the parent line ICC 5003 formed a nodule mass of 92 mg plant^{-1} close to that of S3 (88 mg). The HN selections of the three cultivars ICC 4948, ICC 14196, and Kourinsky had substantially greater nodule mass than those of the respective LN selections. The HN selections from these three varieties were better nodulated than were their parents at both N levels, with the exception of ICC 4948 at N1. The mean nodule mass at N2 (all entries) was 31 mg plant^{-1}, 47% less than that at N1. Nodule mass of all entries was less at N2 than that at N1 (range 9-95%) except for ICC 4948 HN, with which nodule mass was 33 mg plant^{-1} at N1 and 87 mg plant^{-1} at N2.

The highest total dry-matter yields were obtained with ICC 5003 S5 at N1 and N2, and were significantly ($P<0.05$) superior to those of selections S1 and S2 (Table IX). At N1, the yield was similar to that of its parent, whereas at N2 it was substantially (34%) superior to that of the parent.

Among the other entries, the total dry matter yields of the Nod$^-$ selections were generally low, and that of the locally adapted cv. ICC 4918 highest, both at N1 (with the exception of ICC4993 NN) and at N2. The HN selection of ICC 14196 (a wilt-resistant line) yielded more than did the LN selection from the same cultivar (Table IX), whereas the HN selections of ICC 4948 and Kourinsky, some of which died due to fusarium wilt, did not yield well.

Grain yields of selection S5 of ICC 5003 were highest both at N1 and N2 and significantly ($P<0.05$) superior to those of S1 or S2, although only marginally better than those of the parent (Table IX). Yield of selection S1 was lowest of the five nodulation-variants of ICC 5003 at N1, whereas at N2 its yield was similar to that of ICC 5003 S4. The non-nodulating selections gave the lowest yields (0.40 to 0.68 t ha^{-1} at N1, 0.97 to 1.05 t ha^{-1} at N2). Grain yields of ICC 14196 HN were greater (by 31 to 32%) than those of ICC 14196 LN at both N1 and N2 (Table IX). Compared to its parent, it yielded 61% more at N1 and 4% less at N2.

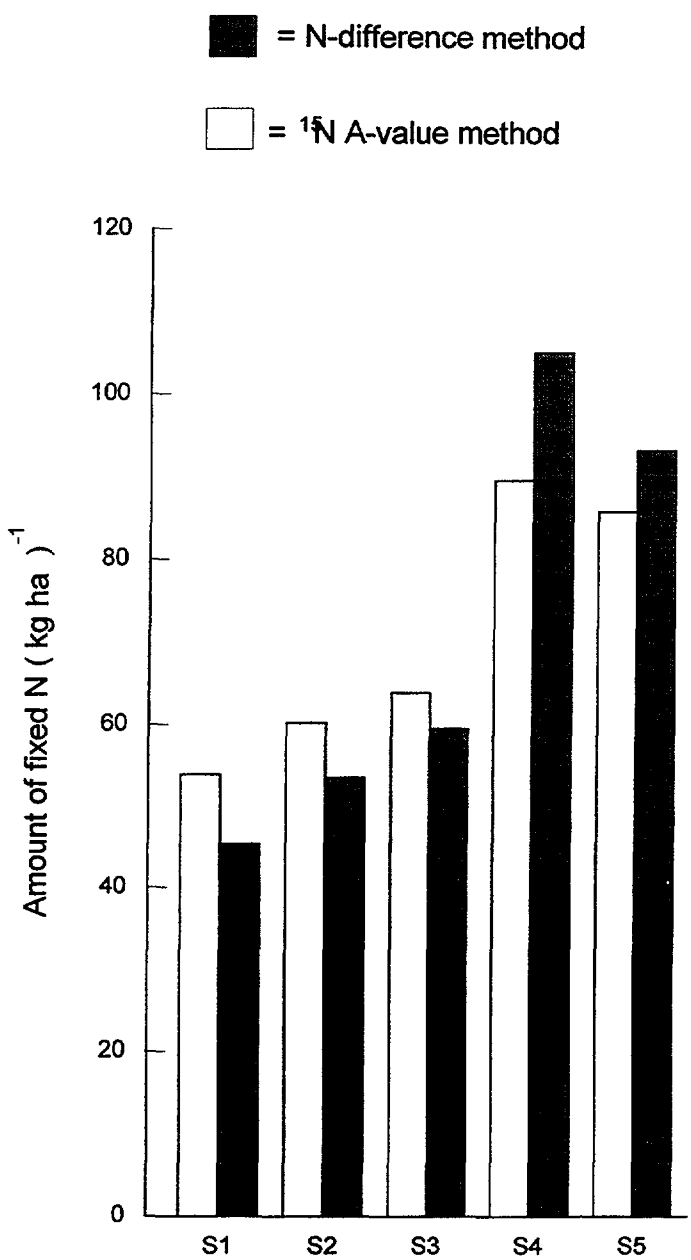

FIG. 1. Amounts of N fixed by nodulation-variants S1-S5 of chickpea cv. ICC 5003, by the A-value and N-difference methods, at N1 (Experiment 1).

TABLE IV. TOTAL DRY WEIGHT AT FINAL HARVEST OF NODULATION-VARIANTS S1-S5 OF CHICKPEA CV. ICC 5003, AT TWO N-LEVELS (EXPERIMENT 1)

N level	Total mass S1	S2	S3	S4	S5	Mean
	($t\ ha^{-1}$)					
N1	4.70	5.01	5.25	6.22	6.19	5.48
N2	6.20	5.99	6.30	6.65	6.99	6.42
SE			±0.591 (0.607)[a]			±0.233
Mean	5.45	5.50	5.77	6.43	6.60	
SE			±0.429			

[a]To compare means at same level of N.

TABLE V. GRAIN YIELD AT FINAL HARVEST OF NODULATION-VARIANTS S1-S5 OF CHICKPEA CV. ICC 5003, AT TWO N-LEVELS (EXPERIMENT 1)

N level	Grain yield S1	S2	S3	S4	S5	Mean
	($t\ ha^{-1}$)					
N1	1.22	1.57	1.67	2.09	2.11	1.73
N2	2.02	2.05	2.18	2.24	2.45	2.19
SE			±0.196 (0.175)[a]			±0.118
Mean	1.62	1.81	1.93	2.167	2.28	
SE			±0.124			

[a]To compare means at same level of N.

The total-N yields followed the trends for total dry matter and grain. The non-nodulating lines generally accumulated relatively little N, but more at N2 than at N1 (Table IX). The nodulating lines, however, did not show such a trend; only five of eighteen nodulated lines accumulated more N at N2 than at N1. The locally adapted line ICC 4918 had the highest dry matter, grain, and N yields, at N1.

Fifteen of the eighteen nodulated entries had lower %Ndfa values at N2 than at N1, both in stover and in grain (Table X), with ranges in grain of 65-83% at N1 and 61-82% at N2, and in stover of 55-78% and 45-77%, respectively. The low-nodulating selections S1 and S2 of ICC 5003 had the lowest %Ndfa values for stover both at N1 and at N2, but not for %Ndfa in grain.

When measured by the ^{15}N-dilution method, fixed N for S5 was numerically the highest of the ICC 5003 selections and substantially greater (59% at N1 and 85% at N2) than the means for S1 and S2 (Table XI). The parent line ICC 5003 fixed more (11%) than S5 at N1, but 15% less at N2. At N1, the HN selection of ICC 14196 fixed 42% more than did the LN selection, and 70% more than its parent, whereas at N2 the parent and the HN selection fixed similar quantities (62-63 kg N ha^{-1}). The locally adapted average-nodulating line ICC 4918 fixed the most N (107 kg N ha^{-1}) at N1, 48% more than did the highest-nodulating line of ICC 5003; however, they fixed similar quantities at N2. The N_2-fixation values obtained by the N-difference method were similar (r = 0.9) to those obtained by the ^{15}N-dilution method.

4. DISCUSSION

4.1. Non-nodulating selections as reference

In Experiment 1, 25% of plants of the non-nodulating selection of ICC 5003 had nodules at N1, whereas at N2 nodules were lacking or were very small. This genotype had been reported as Nod^- based on greenhouse tests using rhizobial strain IC 59 and on field evaluation at Gwalior using the same rhizobial strain [9]. Clearly, at Durgapura, the site of Experiment 1, some native rhizobia were infective. Furthermore, on a vertisol at IAC, about 50% of this putative Nod^- selection had nodules (O.P. Rupela, unpublished), therefore it was decided not to use the ^{15}N-dilution method to assess N_2-fixation by the five selections (S1 to S5) of ICC 5003. Instead the ^{15}N A-value method was adopted, using data generated with Nod^- ICC 5003 at N2, because the few nodules formed thereon were virtually non-functional.

In Experiment 2, the non-nodulating selections from cvv. ICCV 2, ICC 4918 and ICC 4993 formed no nodules, therefore the ^{15}N-dilution method was used for quantification of N_2 fixed [17].

4.2. Nodulation, N_2 fixation and soil N

Chickpea nodules have an indeterminate growth habit [18] and may develop to about 2 cm in diameter [19]. It is likely that the formation of large nodules is associated with rhizobial genotype, and certainly nodule size is influenced by soil moisture and temperature. In Experiment 1, large nodules were observed on plants of Nod^- ICC at N1 and on S1 and S2 selections both at N1 and at N2. As a result, some plants of low-nodulating selections (S1 or S2) with few nodules had nodule dry weights close to those of their S4 and S5 counterparts (based on spot checks on vigorous versus poorly-growing plants of S1 and S2 at 74 DAS). Thus, it appears that under irrigated conditions (as in both experiments) and in light soil (Experiment 1), the expected differences in nodule mass and amount of N fixed by plants of different nodulation-capacity may be blurred. Nevertheless, the use of high-nodulating selections may have utility in environments in which the formation of only a few or no nodules would minimize the growth potential of low-nodulating selections.

In Experiment 2, HN selections of cvv. ICC 4948, ICC 14196 and Kourinsky formed more nodules with greater mass at both N1 and N2 (Table VIII) than did their LN counterparts, and were generally better nodulated than were their parents. However, the level of their superiority was influenced by N level. For example, ICC 14196 HN had 1.8-fold more nodule mass than did its parent at N1, whereas they were of similar nodule mass at N2: a 63% reduction in the case of 14196 HN and only a 29% decrease for the parent. In the case of ICC 4948 HN, instead of suppression in nodule mass, a 1.6-fold increase was recorded (from 33 at N1 to 87 mg plant^{-1} at N2), while its parent registered an 80% reduction at N2. Unfortunately, this selection is more susceptible to fusarium wilt than is its parent and ICC 4948 LN, and, as a result, its N_2-fixation was not high (Table XI). In a field experiment in the post-rainy season of 1993-94 where N2 plots had 2.5-fold more mineral N than did N1, ICC 4948 HN recorded a 71% suppression in nodule mass [20]. Therefore improvement or suppression in nodulation is dependent on the concentration of mineral-N, perhaps particularly during early plant growth, and on the host cultivar. It was apparent that, at a given soil-N level, some selections fixed more N than did their parents (Table XI). Nitrogen is known to be a dynamic nutrient, therefore strategies are required to effectively manage soil mineral-N to maximize harnessing N_2-fixation by legumes for high cropping-system productivity. Also, there is a need to better understand the interactions between soil mineral-N and nodulation/N_2-fixation to effectively identify high-fixing selections for particular soil-N levels.

TABLE VI. N YIELD AT FINAL HARVEST OF VARIANTS S1-S5 OF CHICKPEA CV. ICC 5003, AT TWO N-LEVELS (EXPERIMENT 1)

N level	Total N S1	S2	S3	S4	S5	Mean
	(kg ha^{-1})					
N1	89.5	97.6	104	149	137	115
N2	114	115	133	133	141	127
SE			±12.1 (13.1)[a]			±3.19
Mean	101	106	118	141	139	
SE			±9.24			

[a]To compare means at same level of N.

TABLE VII. PERCENT N DERIVED FROM FIXATION AND AMOUNT OF FIXED N, ESTIMATED BY THE A-VALUE AND N-DIFFERENCE METHODS, IN STOVER AND GRAIN OF NODULATION-VARIANTS OF CHICKPEA CV. ICC 5003 AT N1 (EXPERIMENT 1)

			Amount of fixed N			
Nodulation-	Ndfa		A-value		N-difference	
variant	Stover	Grain	Stover	Grain	Stover	Grain
	(%)		(kg ha^{-1})		(kg ha^{-1})	
S1	56	64	24.6	29.3	23.2	22.2
S2	56	66	21.4	38.7	19.1	34.4
S3	55	66	22.3	41.7	20.4	39.1
S4	53	68	30.3	59.2	41.0	64.1
S5	56	66	30.2	54.4	34.0	59.1
SE	$\pm$6.8	$\pm$5.7	$\pm$6.53	$\pm$5.26	$\pm$10.4	$\pm$6.27

Soil mineral-N, particularly nitrate-N, is known to suppress N_2-fixation by legumes [21]. In the two experiments reported here, mineral N was assayed in air-dried soil samples collected at sowing, with part of the mineralizable N accounted for [S.P. Wani, pers. comm.]. In Experiment 2, the mineral N levels in the top 15 cm soil profile at N1 and N2 were not disparate (9.9 vs. 12.1 mg kg^{-1} soil, respectively) at the time of sowing. Between 15 and 60 cm depths, both profiles had similar N levels. However, all of the nodulated chickpea accessions observed at 42 DAS showed suppressed nodule mass at N2 (range 9-96 %) compared to that at N1 (Table VIII). These data indicate that the plant's nodulation response is more sensitive than the measurement of mineral N in soil. Further studies are needed on response-relationships between soil-mineral N level (and form) and nodule mass/N_2-fixation by the nodulation-variants in controlled-environment conditions. Also, it will be important to determine if measuring mineral-N in fresh soil samples at sowing would be a better indicator of suppression of nodulation/N_2-fixation in chickpea.

4.3. Total dry matter and grain yield

The sites of the two experiments have contrasting environments (see **2.1.**). Generally, chickpea growth and yield are better at site 1, which is cooler with a longer growing season. The temperatures at site 1 (<30°C) also favor N_2-fixation by chickpea [19].

TABLE VIII. NODULE NUMBER AND DRY WEIGHT OF NODULATION-VARIANTS OF CHICKPEA AND THEIR PARENT CULTIVARS, AT TWO N-LEVELS, AT 42 DAS (EXPERIMENT 2)

Genotype	Nodule number N1	N2	Mean	Nodule mass N1	N2	Mean
	(per plant)			(mg plant^{-1})		
ICC 5003 S1	4	3	3	11	7	9
ICC 5003 S2	22	1	11	46	2	24
ICC 5003 S3	30	19	25	88	74	81
ICC 5003 S4	38	23	30	102	47	74
ICC 5003 S5	41	31	36	129	102	119
ICC 5003	9	8	19	92	19	55
ICC 4948 HN	22	29	25	33	87	60
ICC 4948 LN	6	7	6	23	21	22
ICC 4948	16	4	10	46	9	28
ICC 14196 HN	30	10	20	103	38	70
ICC 14196 LN	14	5	10	73	15	14
ICC 14196	11	10	11	58	41	49
KourinskyHN	33	13	23	127	38	82
Kourinsky LN	17	8	13	53	24	38
Kourinsky	30	7	19	105	22	63
ICCV 2	22	15	18	117	29	73
ICCV2 NN	0	0	0	0	0	0
ICC 4993	23	12	17	62	47	55
ICC 4993 NN	0	0	0	0	0	0
ICC 4918	17	18	17	57	83	70
ICC 4918 NN	0	0	0	0	0	0
SE	±6.5 (6.6)[a]		±4.7	±19.0 (19.2)[a]		±13.6
Mean	18	10		59	31	
SE	±0.9			±3.0		

[a]To compare means at same level of N.

Better plant growth and greater yield may be expected at N2 than at N1, which was true for all entries at site 1 (Tables IV, V), but not at site 2 where at least 10 of the 18 nodulated lines produced less foliage and grain at N2 than at N1 (Table IX). The yield of non-nodulating lines was consistently higher at N2 than at N1. The overall (average of all entries) yield at N2 was marginally lower than at N1. It seems that temperature stress at site 2 interacted differentially with the various entries. This, along with the interactions between soil N with N_2 fixation, suggests the need for investigation of how the two sources of N (symbiosis and soil) are differentially affected by soil temperature.

Long-duration varieties of chickpea are known to yield poorly at site 2 [22] because pod development coincides with unfavorably high ambient temperatures. It is expected that plentiful N (from fixation or soil) would delay maturity, and therefore yield at site 2 would be adversely affected. Lower grain yields at N2 than at N1 have been recorded in other studies [20, O.P. Rupela unpub.].

4.4. High nodulation versus yield

When evaluated in pots [13] nodule numbers of selections S1 to S5 of ICC 5003 were well delineated at low soil-N, providing a broad range of nodule mass (mg plant^{-1}, < 10 by S1, 12 by S2, 56 by S3, 69 by S4 and 79 by S5). Therefore, it was hoped that we were closer to answering the question, "What is optimum nodulation and N_2 fixation?" particularly by using ^{15}N-based methods. However, it seems that more factors influence this trait in field conditions than in the glasshouse. Also, the data reported here were affected by nematodes in Experiment 1 and small plot size in Experiment 2. As a result, only large differences in nodulation capacity, e.g. S4/S5 vs. S1/S2 of ICC 5003 or ICC 14196 HN vs. ICC 14196 LN, were significantly different, reflecting the trends in grain yield and N_2-fixation.

Reported "super-nodulating" mutants of soybean [23, 24] and of common bean [25] produce yields lower than their normal-nodulating parents. In contrast, our high-nodulating selections generally produced yields at least marginally higher than did their parents. Of the five variants of ICC 5003, even S5, with the highest nodulation so far recorded for chickpea, produced yields not lower than those of its parent, which should remove any apprehension from the minds of skeptics that selection (not mutation) for high nodulation will lead to yield reduction.

It has been observed repeatedly, including in these experiments (data not shown), that N_2-fixation efficiency (as measured by AR activity per nodule weight) of low-nodulating selections is greater than that of high-nodulating selections. As a result, 100% nodule-mass differences in low- and high-nodulating selections may result in fixed-N differences of only 30%. Furthermore, differences in yield (a result of several factors including N nutrition) may be only 5-10% in favor of the high-nodulating selections. Nevertheless, promotion of high-nodulating variants is worthwhile as a buffer in conditions of stress in which low-nodulating lines would lack nodules. In addition, high-nodulating selections may contribute 20 kg ha^{-1} (assessment based on nodule mass at 42 DAS in Experiment 1) or more biomass (as nodules) of high N-content (at least 4% N), beneficial to soil health.

TABLE IX. DRY MATTER, GRAIN AND N YIELDS OF NODULATION-VARIANTS AND PARENT CULTIVARS, TWO N-LEVELS (EXPERIMENT 2)

Genotype	Total dry matter (t ha^{-1})			Grain yield (t ha^{-1})			Total N (kg ha^{-1})		
	N1	N2	Mean	N1	N2	Mean	N1	N2	Mean
ICC 5003 S1	2.50	3.00	2.75	0.92	1.33	1.12	44	77	60
ICC 5003 S2	2.36	1.64	2.00	1.08	0.59	0.84	45	26	36
ICC 5003 S3	3.51	2.70	3.11	1.49	1.18	1.33	69	63	66
ICC 5003 S4	4.10	3.18	3.64	1.88	1.40	1.64	80	57	69
ICC 5003 S5	4.30	4.18	4.24	1.94	1.63	1.79	82	81	81
ICC 5003	4.30	3.12	3.71	1.71	1.37	1.54	89	70	80
ICC 4948 HN	2.35	4.14	3.25	0.82	1.94	1.38	41	78	60
ICC 4948 LN	2.67	3.72	3.19	0.97	1.46	1.21	49	69	59
ICC 4948	4.06	3.64	3.85	1.85	1.56	1.71	84	64	74
ICC14196 HN	4.88	4.38	4.63	1.97	1.73	1.85	92	81	86
ICC 14196 LN	3.91	3.50	3.70	1.49	1.32	1.41	69	60	65
ICC 14196	3.83	4.25	3.04	1.22	1.80	1.51	62	81	71
Kourinsky HN	3.62	2.82	3.22	1.30	1.10	1.20	64	50	57
Kourinsky LN	4.37	4.21	4.29	1.49	1.68	1.59	86	79	82
Kourinsky	5.06	2.82	3.94	2.07	1.09	1.58	94	48	71
ICCV 2	4.37	4.22	4.29	1.88	2.01	1.95	84	84	84
ICCV 2 NN	1.09	2.58	1.84	0.40	0.97	0.69	13	45	29
ICC 4993	4.14	4.12	4.13	1.43	1.50	1.46	75	76	75
ICC 4993 NN	2.38	2.71	2.55	0.68	1.05	0.87	40	49	45
ICC 4918	5.03	4.80	4.92	2.23	1.91	2.07	138	90	114
ICC 4918 NN	1.71	2.73	2.22	0.61	1.04	0.83	29	50	39
SE	±0.676(0.662)[a]		±0.468	±0.378(0.373)[a]		±0.264	±15.2(15.1)[a]		±10.7
Mean	3.55	3.45		1.39	1.37		66	64	
SE	±0.206			±0.097			±3.5		

[a]To compare means at same level of N.

TABLE X. PERCENT N DERIVED FROM FIXATION IN STOVER AND GRAIN OF NODULATION-VARIANTS OF CHICKPEA AND PARENT CVV., AT TWO N-LEVELS (EXPERIMENT 2)

	Ndfa					
	Stover			Grain		
Genotype	N1	N2	Mean	N1	N2	Mean
	(%)					
ICC 5003 S1	55	56	56	75	68	72
ICC 5003 S2	55	45	50	77	72	75
ICC 5003 S3	75	57	66	80	61	70
ICC 5003 S4	77	62	70	79	74	77
ICC 5003 S5	79	77	78	82	82	82
ICC 5003	71	69	70	78	79	78
ICC 4948 HN	67	63	65	72	68	70
ICC 4948 LN	63	67	65	65	76	70
ICC 4948	78	73	76	79	76	78
ICC 14196 HN	76	73	74	81	78	80
ICC 14196 LN	75	67	71	78	71	74
ICC 14196	57	66	61	71	80	75
Kourinsky HN	71	60	66	79	72	76
Kourinsky LN	77	65	71	83	76	80
Kourinsky	74	68	71	78	77	77
ICCV 2	73	60	67	79	70	74
ICC 4993	79	72	75	81	79	80
ICC 4918	72	67	70	79	77	78
SE	±8.1(7.4)[a]		±5.2	±5.5(4.9)[a]		±3.5
Mean	71	65		77	74	
SE	±3.8			±2.7		

[a]To compare means at same level of N.

TABLE XI. ESTIMATES OF FIXED N, USING ^{15}N AND DIFFERENCE METHODS, BY NODULATION-VARIENTS OF CHICKPEA AND PARENT CULTIVARS, AT TWO N-LEVELS (EXPERIMENT 2)

	Total amount of fixed N					
	^{15}N-dilution			N-difference		
Genotype	N1	N2	Mean	N1	N2	Mean
	(kg ha^{-1})					
ICC 5003 S1	38	49	43	15	42	28
ICC 5003 S2	44	21	33	17	-[a]	4
ICC 5003 S3	54	59	57	40	28	34
ICC 5003 S4	64	41	52	52	22	37
ICC 5003 S5	65	65	65	53	46	49
ICC 5003	72	55	63	60	35	48
ICC 4948 HN	33	51	42	12	43	28
ICC 4948 LN	34	50	43	20	34	27
ICC 4948	66	77	71	55	29	42
ICC 14196 HN	75	62	68	63	45	54
ICC 14196 LN	53	53	53	40	25	33
ICC 14196	44	63	53	33	46	39
Kourinsky HN	50	40	45	35	15	25
Kourinsky LN	71	57	64	57	44	50
Kourinsky	71	43	57	64	13	39
ICCV 2	65	58	62	55	49	52
ICC 4993	59	72	66	46	41	43
ICC 4918	107	67	87	109	54	82
SE	±11.7(11.5)[b]		±8.11	±16.9(14.3)[b]		±10.1
Mean	45	42		46	34	
SE	±3.64			±9.7		

[a]Negative value recorded.
[b]To compare means at same level of N.

4.5. Selecting for high N_2 fixation based on nodulation versus ^{15}N-enrichment methods

In Experiment 2 at N2, ICC 5003 S1 yielded (and therefore fixed N_2) similarly with ICC 5003 S4, which is difficult to explain (Table IX). ICC 5003 S1 may be more tolerant of soil nitrate than ICC 5003 S4. As a result ICC 5003 S1 not only fixed more, it also utilized soil-N more effectively.

Measurement of $^{15}N/^{14}N$ ratios by mass-spectrometry is a sensitive procedure. The values of % ^{15}N atom excess so obtained are used with yield data to calculate the amount of N fixed. Therefore, any factor affecting yield also affects the determination of fixed N. Also, the ^{15}N methods are based on assumptions [26] including what constitutes an appropriate non-fixing reference crop. It is difficult to assess if all such assumptions are satisfied in a given experiment. Using the ^{15}N-dilution method in Experiment 2, the top three entries at N1 were ICC 4918, ICC 14196 HN and ICC 5003, and at N2 are ICC 4948, ICC 4993 and ICC 4918 (Table XI). On the basis of nodule mass per plant, the top-three entries at N1 were ICC 5003 S5, Kourinsky HN, and ICCV 2, and at N2 were ICC 5003 S5, ICC 4948 HN and ICC 4918 (Table VIII). Therefore, only ICC 4918 would be selected by both

TABLE XII. CORRELATION COEFFICIENTS BETWEEN N_2-FIXATION AND YIELD TRAITS

	Experiment 1 (n = 20)			Experiment 2 (n = 60)		
N_2-fixation trait	Total dry wt.	Grain yield	N yield	Total dry wt.	Grain yield	N yield
		(r)			(r)	
Nodule no.	0.55*[a]	0.64*	0.66*	0.32*	0.34*	0.38*
Nodule wt.	0.56*	0.70*	0.67*	0.39*	0.35*	0.36*
AR activity	0.37*	0.64*	0.39*	-[b]	-	-
%Ndfa, stover	-0.26	0.01	-0.22	0.53*	0.53*	0.47*
%Ndfa, grain	-0.11	0.25	-0.13	0.45*	0.46*	0.37*
Amount N fixed (^{15}N)	0.28	0.27	0.49*	0.90*	0.95*	0.88*
Amount N fixed (N-diff.)	0.44*	0.34*	0.66*	0.90*	0.95*	0.89*

[a]Statistically significant ($P < 0.05$).

[b]Not determined.

methods. Nodule number and mass were significantly correlated with yield traits (total dry matter, grain, and N-yield) in both experiments (Table XII), whereas the ^{15}N-based estimates of %N derived from fixation in stover and grain were significantly correlated with the yield traits in Experiment 2 but not in Experiment 1. The ^{15}N-dilution method requires the use of expensive ^{15}N-labelled fertilizer and special equipment (mass spectrometer or emission spectrometer) generally unavailable in developing countries. Selecting varieties (or plants) greatly superior in nodulation capacity with even marginally superior yields may obviate the need for ^{15}N in selection programs for high N_2-fixation. Confirmation studies, however, should be conducted using ^{15}N-based methods.

5. CONCLUSIONS

These two experiments with nodulation-variants did not answer the difficult question, "What is optimum nodulation and N_2 fixation for chickpea?" However, they confirmed that the relative nodulation differences in low-nodulating (S1 and S2) and high-nodulating (S4 and S5) variants were consistent over locations. Also, the high-nodulation selections generally fixed more N_2 than did the low-nodulating counterparts and gave yields at least marginally superior to those of their parents. The various selections responded differently to soil-N level. For most, nodulation was suppressed at the high soil-N levels, whereas a few lines showed enhanced nodulation at high soil-N, confirming the possibility of selecting for high-soil-N tolerant symbiosis in chickpea, as previously proposed [27].

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REFERENCES

[1] GAUR, Y.D., SEN, A.A. Cross inoculation group specificity in *Cicer Rhizobium* symbiosis. New Phytol. **83** (1979) 745-754.

[2] JORDAN, D.C. Family III. "Rhizobiaceae." Bergey's Manual of Systematic Bacteriology, Vol 1, (KRIEG, N.R. Ed.). Williams and Wilkins Pub. Co., Baltimore (1984) 235-256.

[3] NUTMAN, P.S. "Hereditary host factors affecting nodulation and nitrogen fixation." Current Perspectives in Nitrogen Fixation (GIBSON, A.H., NEWTON, W.E. Eds.). Australian Academy of Science, Canberra (1981) 194-204.

[4] PHILLIPS, D.A., BEDMAR, E.J., QUALSET, C.O., TAUBER, L.R. "Host legume control of *Rhizobium* functions." Nitrogen Fixation and CO_2 Metabolism (LUDDEN, P.W., BURRIS, J.E. Eds.). Elsevier Science Publishing Company Inc. (1985) 203-213.

[5] HANDARSON, G., ZAPATA, F., DANSO, S.K.A. Effect of plant genotype and nitrogen fertilizer on symbiotic nitrogen fixation by soybean cultivars. Plant Soil **82** (1984) 397-405.

[6] HERRIDGE, D.F., BETTS, J.H. Field evaluation of soybean genotypes selected for enhanced capacity to nodulate and fix nitrogen in the presence of nitrate. Plant Soil **110** (1988) 129-135.

[7] ARNON, I. "Breeding for higher yields." Physiological Aspects of Crop Productivity. International Potash Institute, Bern (1980) 77-81.

[8] NAMBIAR, P.T.C. Nitrogen Nutrition of Groundnut in Alfisols. Information Bulletin no. 30. ICRISAT, Patancheru (1990) 28pp.

[9] RUPELA, O.P. Natural occurrence and salient characters of nonnodulating chickpea plants. Crop Sci. **32** (1992) 349-352.

[10] RUPELA, O.P., JOHANSEN, C. Identification of nonnodulating and low and high nodulating plants in pigeon pea. Soil Biol. Biochem. **27** (1995) 539-544.

[11] RUPELA, O.P. "Screening for intracultivaral variability of nodulation in chickpea and pigeonpea." Linking Biological Nitrogen Fixation Research in Asia (RUPELA, O.P., KUMAR RAO, J.V.D.K., WANI, S.P., JOHANSEN, C. Eds.). ICRASAT, Patancheru (1994).

[12] RUPELA, O.P. A visual rating system for nodulation of chickpea. Int. Chickpea Newsl. **22** (1990) 22-25.

[13] LEGUMES PROGRAM, ICRISAT. "Variation in nitrogen fixation in a chickpea cultivar." Legumes Program, Annual Report 1992. ICRISAT, Patancheru (1993) 21-23.

[14] WANI, S.P., SIVARAMAKRISHNAN, S., RUPELA, O.P., JOHANSEN, C., LEE, K.K. "Partitioning of ^{14}C-photosynthate in low and high nodulating selections of chickpea." Nuclear Methods in Soil-Plant Aspects of Sustainable Agriculture. IAEA, Vienna (1995) 203-208.

[15] DART. P.J., DAY, J.M., HARRIS, D. "Assay of nitrogenase activity by acetylene reduction." Use of Isotopes for Study of Fertilizer Utilization by Legume Crops: Technical Report 149. IAEA, Vienna (1972) 85-100.

[16] INTERNATIONAL ATOMIC ENERGY AGENCY 1983. A Guide to the Use of Nitrogen-15 and Radioisotopes in Studies of Plant Nutrition: Calculations and Interpretation of Data. IAEA-TECDOC-288, Vienna (1983) 65pp.

[17] HARDARSON, G., DANSO, S.K.A. "Use of ^{15}N methodology to assess biological nitrogen fixation." Training Course Series no. 2: Use of Nuclear Techniques in Studies of Soil-Plant Relationships. IAEA, Vienna (1990) 129-160.

[18] SPRENT, J.I., STEPHENS, J.H., RUPELA, O.P. "Environmental effects on nitrogen fixation." World Crops: Cool Season Legumes (SUMMERFIELD, R.J. Ed.). Kluwer Academic Publishers, Dordrecht (1988) 801-810.

[19] RUPELA, O.P., SAXENA, M.C. "Nodulation and nitrogen fixation." The Chickpea (SAXENA, M.C., SINGH, K.B. Eds.). C.A.B. International (1987) 191-206.

[20] RUPELA, O.P., WANI, S.P., JOHANSEN, C. Effect of high nodulating selection of chickpea cultivar ICC 4948 on yield and soil properties of a chickpea-sorghum cropping system. J. Soil Biol. Ecol. **15** (1995) 127-134.

[21] STREETER, J. 1988. Inhibition of legume nodule formation and N_2 fixation by nitrate. Crit. Rev. Plant Sci. **7** (1988) 1-23.

[22] SAXENA, N.P. "Screening for adaption to drought: Case studies with chickpea and pigeonpea." Adaptation of Chickpea and Pigeon Pea to Abiotic Stress. ICRISAT, Patancheru (1987) 63-76.

[23] ESKEW, D.L., KAPUYA, J., DANSO, S.K.A. Nitrate limitation of nodulation and nitrogen fixation by supernodulating nitrate-tolerant symbiosis mutant of soybean. Crop Sci. **29** (1989) 1491-1496.

[24] WU, S., HARPER, J.E. 1991. Dinitrogen fixation potential and yield of hypernodulating soybean mutants: A field evaluation. Crop Sci. 31 (1991) 1233-1240.

[25] BUTTERY, B.R., PARK, S.J., DHANVANTRI, B.N. Effect of combined nitrogen *Rhizobium* strain and substrate on a supernodulating mutant of *Phascolus vulgaris* L. Can. J. Plant Sci. **70** (1990) 955-963.

[26] DANSO, S.K.A., HARDARSON, G., ZAPATA, F. Misconceptions and practical problems in the use of ^{15}N soil enrichment techniques for estimating N_2-fixation. Plant Soil **152** (1993) 25-52.

[27] RUPELA, O.P., JOHANSEN, C. "High mineral-N tolerant N_2-fixation in legumes - its relevance and prospects for development." Improving Soil Management for Intensive Cropping in the Tropics and Sub-tropics (HUSSIAN, M.S., IMAMUL HUQ, S.M., ANWAR IQBAL, M., KHAN, T.H. Eds.). Bangladesh Agricultural Research Council, Dhaka (1995) 395-402.