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# Sustainable agriculture in the semi-arid tropics through biological nitrogen fixation in grain legumes

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*Key words:* inoculation, legumes, nonnodulation, rhizobia, residual effects, symbiotic $N_2$ fixation

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## Abstract

Sustainable agriculture relies greatly on renewable resources like biologically fixed nitrogen. Biological nitrogen fixation plays an important role in maintaining soil fertility. However, as BNF is dependent upon physical, environmental, nutritional and biological factors, mere inclusion of any $N_2$-fixing plant system does not guarantee increased contributions to the soil N pool. In the SAT where plant stover is also removed to feed animals, most legumes might be expected to deplete soil N. Yet beneficial legume effects in terms of increased yields in succeeding cereal crops have been reported. Such benefits are partly due to N contribution from legumes through BNF and soil N saving effect. In addition, other non-N rotational benefits, for example, improved nutrient availability, improved soil structure, reduced pests and diseases, hormonal effects are also responsible. In this paper we have reviewed the research on the contribution of grain legumes in cropping systems and the factors affecting BNF. Based on the information available, we have suggested ways for exploiting BNF for developing sustainable agriculture in the semi-arid tropics (SAT). A holistic approach involving host-plant, bacteria, environment and proper management practices including need based inoculation for enhancing BNF in the cropping systems in the SAT is suggested.

## Introduction

Sustainable agriculture involves the successful management of agriculture resources to satisfy changing human needs while maintaining or enhancing the environment quality and conserving natural resources (TAC, CGIAR, 1988). Sustainable agriculture relies greatly on renewable resources and on-farm nitrogen contributions are achieved largely through biological nitrogen fixation (BNF). Biological nitrogen fixation helps in maintaining and/or improving soil fertility by using $N_2$ which is in abundance in the atmosphere. Above every hectare of land at sea level, there is 78,000 tones of inert nitrogen gas ($N_2$). Intensive agricultural systems are characteristically expanded nutrient cycles involving the export of crops from a farm and require continued import of nutrients to the farm.

Nitrogen is the most limiting nutrient for increasing crop productivity. Input efficiency of N fertilizer is low (Prasad et al., 1990) and in turn, contributes substantially to environmental pollution. The continued and unabated use of N fertilizers would further deplete stocks of nonrenewable fossil fuels used in fertilizer production.

Annually, BNF is estimated to be around 175 million tones N of which close to 79% is accounted for by terrestrial fixation (Fig. 1). This illustrates the importance of BNF in the context of the global N cycle. The BNF offers an economically attractive and ecologically sound means of reducing external N inputs and improving the quality and quantity of internal resources. In this paper we deal with the BNF systems involving upland grain legume crops grown in the semi-arid tropics (SAT). The SAT are the areas located in the seasonally dry tropical climates, spread over four continents. The mean annual temperature in the SAT is > 18 °C; rainfall exceeds potential evapotranspiration for only 2 to 4.5 months in the dry SAT and for 4.5 to 7 months in the wet/dry SAT (Troll, 1965).

## Contribution of BNF to N balance

Accurate estimation of the amount of $N_2$ fixed by different crops in a particular agro-ecosystem is a prerequisite for assessing and improving the contribution of BNF to a given cropping system. However, as nitrogen fixation is dependent upon physical, environmental, nutritional and biological factors (Chalk, 1991; Nambiar et al., 1988; Peoples and Crasswell, 1992) it can not be assumed that any $N_2$-fixing system will automatically contribute to the N cycle. In general while estimating BNF, plant roots and fallen leaf material are not taken into account which results in underestima-

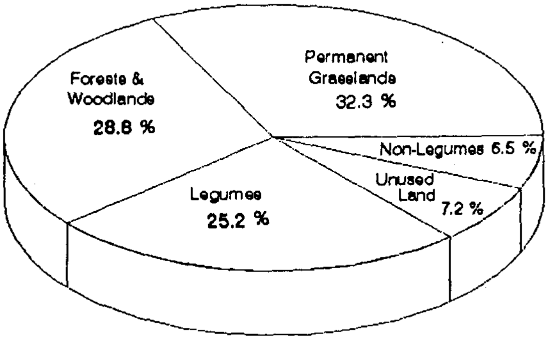

*Fig. 1.* Distribution of 139 million tonnes of $N_2$ estimated to be biologically fixed in various terrestrial systems. Source: Burns and Hardy (1975).

*Table 1.* Examples of estimates of nitrogen fixed by some legumes

| Crop | Nitrogen fixed (kg ha$^{-1}$) |
|---|---|
| Alfalfa | 100–300 |
| Black gram | 119–140 |
| Clover | 100–150 |
| Chickpea | 23–97 |
| Cluster bean | 37–196 |
| Common bean | 3–57 |
| Cowpea | 9–125 |
| Groundnut | 27–206 |
| Lentil | 35–100 |
| Greengram | 50–66 |
| Pigeonpea | 4–200 |
| Rice bean | 32–97 |
| Soybean | 49–450 |
| Peas | 46 |
| Fenugreek | 44 |

Sources: Derived from Wani and Lee (1992) and Peoples and Crasswell (1992).

*Table 2.* Net nitrogen balance for pigeonpea and chickpea cultivars grown at Patancheru and Gwalior (India) respectively

| | Total plant N uptake (kg ha$^{-1}$) | Plant N derived from fixation (kg ha$^{-1}$) | Net N balance (kg ha$^{-1}$)[a] |
|---|---|---|---|
| *Pigeonpea*[b] | | | |
| Prabhat | 69 | 4 | −49 |
| UPAS 120 | 92 | 27 | −39 |
| T 21 | 108 | 43 | −39 |
| BDN 1 | 118 | 53 | −32 |
| Bhedaghat | 101 | 36 | −20 |
| JA 275 | 78 | 13 | −33 |
| Bhandara | 108 | 43 | −22 |
| NP (WR) 15 | 114 | 50 | −27 |
| *Chickpea*[c] | | | |
| Annigeri | 110 | 31 | −77 |
| G 130 | 104 | 26 | −75 |
| ICC 435 | 102 | 29 | −72 |
| ICCC 42 | 88 | 23 | −64 |
| ICCV 6 | 107 | 30 | −76 |
| K 850 | 104 | 40 | −63 |

Source: Derived from Kumar Rao and Dart (1987) and Rupela et al. (pers. commun.).

[a] Net N balance calculated as total plant N uptake – (N derived from BNF + N derived form fertilizer + N added to the soil through plant roots and fallen plant parts).

[b] BNF was estimated by N difference method. N derived from fixation calculated for roots also.

[c] BNF was estimated by $^{15}N$ based A-value method. N derived from fixation calculated for above ground plant parts only.

tion of the quantity of $N_2$ fixed. It is essential that BNF in roots and fallen plant material e.g. leaves should be considered when estimating the amount of $N_2$ fixed by legumes.

Legumes have been an important component of agriculture since ancient times. It is widely believed that legumes improve soil fertility because of their $N_2$-fixing ability. In support of this argument, the reported amounts of $N_2$ fixed by legumes are cited (Table 1). However, in order to assess the role of BNF in the sustainability of different SAT cropping systems not only the amount of nitrogen fixed by the legume component crop in the system is important, but the overall nitrogen balance of the system needs to be considered. The SAT is characterized by a harsh environment with erratic seasonal rainfall and dense human and animal population and it has unique problems in agriculture also. Due to heavy pressure on land for production to feed a large human and animal population, it is a common practice that along with legume grains, plant material is also often taken away from the field for feeding the animals. In such a case only nodulated roots and fallen leaves go back to the soil.

Net nitrogen balances calculated for different cultivars of pigeonpea grown at Patancheru, India (Kumar Rao and Dart, 1987) and chickpea grown at Gwalior, India (Rupela et al., pers. commun.) indicated that all studied varieties depleted soil nitrogen (Table 2). In all these cases above ground plant materials were removed from the field. In the case of pigeonpea for computing nitrogen fixation, N in plant roots and fallen plant parts also was accounted for. Different maturity groups of pigeonpea cultivars fixed 4–53 kg N ha$^{-1}$ season$^{-1}$ while depleting 20–49 kg N ha$^{-1}$ from the soil. In the case of chickpea, different cultivars fixed 23–40 kg N ha$^{-1}$ season$^{-1}$ and removed 63–77 kg N ha$^{-1}$ season$^{-1}$ from the soil (Table 2). Groundnut fixed 190 kg N ha$^{-1}$ season$^{-1}$ when pod yields were around 3.5 t ha$^{-1}$ at Patancheru (Nambiar et al., 1986), however, groundnut relied for its 20–40% (47–127 kg N ha$^{-1}$ season$^{-1}$) of the N requirement on soil or from fertilizer (Giller et al., 1987), obviously resulting in a negative N balance. Positive net N balances of up to 136 kg ha$^{-1}$ for several legume crops following seed harvest have been shown by Peoples and Craswell (1992). However,

*Table 3.* Nitrogen balance sheet[a] for different cropping systems for Alfisol, Patancheru, India

| Cropping system[b] by year | | Import (kg ha$^{-1}$)[c](A) | | | | Export (kg ha$^{-1}$)[c] (B) | | Balance (kg ha$^{-1}$)(A)-(B) |
|---|---|---|---|---|---|---|---|---|
| | | Fertilizer | | Leguminous[d] $N_2$-fixation | | Harvest[e] | | |
| 1991 | 1992 | 1991 | 1992 | 1991 | 1992 | 1991 | 1992[f] | |
| S/P | C | 60 | 60 | 0+80 | 0 | 88+68 | 66 | −22 |
| C | S/P | 60 | 60 | 0 | 0+46 | 64 | 93+46 | −37 |
| G/P | C | 18 | 60 | 90+50 | 0 | 108+56 | 72 | −18 |
| C | G/P | 60 | 18 | 0 | 102+82 | 65 | 141+75 | −19 |
| P | C | 18 | 60 | 121 | 0 | 115 | 66 | +18 |

[a] N balance calculated based on main import and export sources of N.
[b] S/P = Sorghum intercropped with pigeonpea, C = castor, G/P = groundnut intercropped with pigeonpea, and P = sole pigeonpea.
[c] Each value within a binomial corresponds to the crop in intercrop.
[d] Including atmosphere-derived N (fixed N) in leguminous roots.
[e] Assumed that groundnut roots were exported by harvest.
[f] N contents in mini-plot grown sorghum, pigeonpea, and groundnut were used to calculate total N in the harvest for 1992.
Source: Lee et al. (1993).

if crop residues were removed from the field then net N balances for groundnut are −27 to −95, for soybean −28 to −104, common bean −28, greengram −24 to −65 and cowpea −25 to −69 kg ha$^{-1}$. Similarly, for soybean grown with different starter N levels after rice which received different fertilization levels, the N balances with seed and stover removed ranged from −12 to −35 kg ha$^{-1}$ in northern Thailand (Jefing et al., 1992). For different cropping systems where pigeonpea and groundnut are grown as intercrops, nitrogen balances were negative (Lee et al., 1993). In the case of sole pigeonpea grown in rotation with sole castor, a positive balance of 18 kg N ha$^{-1}$ during two years crop rotation was observed at Patancheru (Table 3). These results show that legumes also mine the soil N as cereals do. However, total plant N yields from legumes are far higher than the cereal plant N yields. We reach the conclusion that in general, grain legumes, where crop residues are removed, slow the decline of, rather than enhance, the N fertility of the soil in comparison with cereal systems.

## Beneficial effects of legumes

Despite the negative N balances for grain legumes grown in rotation or as intercrops, reported benefits of legumes to succeeding non-legume crops have been observed consistently (Table 4). Improvement in cereal yield following monocropped legumes lie mainly in the 0.5 to 3 t ha$^{-1}$ range, representing around 30 to 350% increase over yields in cereal-cereal cropping sequences (Peoples and Crasswell, 1992). In a long-term crop rotation experiment conducted since 1983 at ICRISAT Center, Patancheru, mean residual effects of legume-based crop rotations over the last ten years were observed on sorghum yield as compared to the yield of sorghum from sorghum + safflower (S+F)-S+F plots (Fig. 2). Such increased cereal yields following legume crops were attributed to the N contribution from legumes in crop rotation (De et al., 1983; Kumar Rao et al., 1983; Nambiar, 1990). This opinion is not held by all (Cook, 1988; Danso and Pappastylianou, 1992; Fyson and Oaks, 1990; Russelle et al., 1987; Wani et al., 1991a, 1994a).

### *Nitrogen effect*

Terms like "N residual effect" (De et al., 1983) and "Fertilizer N replacement value" or N equivalent (Hesterman et al., 1987) are used to describe the role of legumes in crop rotations. They refer to the amount of inorganic N required following a non-legume crop to produce another non-legume crop with an equivalent yield to that obtained following a legume. This comparison provides a quantitative estimate of the amount of N that the legume supplies to the non-legume crop. This concept does not distinguish between BNF and the "N-conserving effect" which results from substitution by legumes of biologically fixed N for soil N. Fertilizer N replacement value (FRV) methodology has been widely used but it overestimates the N contribu-

*Table 4.* Residual effect of preceding legume on cereal yield in terms of fertilizer N equivalents

| Preceding legume | Following cereal | Fertilizer N equivalent (kg ha$^{-1}$) |
|---|---|---|
| Berseem | Maize | 123 |
| Sweet clover | Maize | 83 |
| Winged bean | Maize | 70 |
| Blackgram | Sorghum | 68 |
| Greengram | Sorghum | 68 |
| Greengram (monocrop) | Wheat | 68 |
| Chickpea | Maize | 60–70 |
| Cowpea | Maize | 60 |
| Groundnut | Pearl millet | 60 |
| Cowpea | Pearl millet | 60 |
| Chickpea | Pearl millet | 40 |
| Lentil | Pearl millet | 40 |
| Peas | Pearl millet | 40 |
| Pigeonpea | Wheat | 40 |
| Cowpea (monocrop) | Wheat | 38 |
| Lathyrus | Maize | 36–48 |
| Lablab bean | Maize | 33 |
| Pigeonpea | Pearl millet | 30 |
| Greengram | Pearl millet | 30 |
| Groundnut (monocrop) | Wheat | 28 |
| Pigeonpea | Maize | 20–67 |
| Peas | Maize | 20–32 |
| Lentil | Maize | 18–30 |
| Greengram (intercrop) | Wheat | 16 |
| Cowpea (intecrop) | Wheat | 13 |
| Groundnut (intercrop) | Wheat | 12 |
| Groundnut | Maize | 9–60 |
| Soybean | Maize | 7 |

Source: Derived from Ahlawat et al. (1981), Bandyopadhyay and De (1986), Chandra and Ali (1986), Dakora et al. (1987), De and Goutam (1987), Doughton and MacKenzie (1984), MacCol (1989), Nambiar et al. (1988), Roy Sharma and Singh (1969), and Weil and Samaranayake (1991).

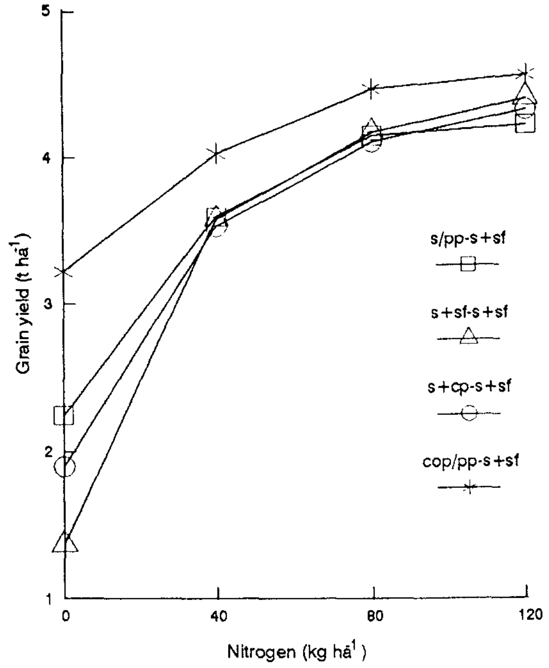

*Fig. 2.* Mean grain yield of sorghum grown in rainy seasons (1983–92) succeeding different cropping system in previous year, ICRISAT, Patancheru. (2 year crop rotation) S - sorghum, PP - pigeonpea, SF - safflower, CP - chickpea, COP - cowpea, / - intercropped, + - sole crop grown during postrainy season. Source: Rego and Burford (1992).

tion of legumes in a crop rotation. The FRV methodology gives variable estimates depending on the test crop used. The N contribution from hairy vetch and big flower vetch was estimated to be 65 and 75 kg N ha$^{-1}$ respectively with maize as test crop and 125 and 135 kg N ha$^{-1}$ using grain sorghum (Blevins et al., 1990). Recently, $^{15}$N methodology has been used to measure the residual effects of legumes to circumvent problems with non-isotopic methods (Danso and Papastylianou, 1992; Senaratne and Hardarson, 1988; Wani et al., 1991a). Based on the estimates obtained via $^{15}$N methodology, Hesterman et al. (1987) argued that the amount of N credited to legumes in a crop rotation in the north-central US may be inflated by as much as 123% due to the use of the FRV method. Based on $^{15}$N methodology it is reported that only 7.3 to 28% of the $^{15}$N in legume crops is taken up by a following grain crop (Ladd et al., 1981, 1983; Vallis, 1983; Wani, unpubl. data). The overestimation is because the FRV method confounds the non-N rotation effect with the N contribution, and this method assumes that use efficiency of fertilizer and legume N is similar.

Growing legumes in rotation does improve mineral N content in soil as compared with the cultivation of non-legume crops. At ICRISAT-Asia Center, Patancheru, India, a long-term rotation experiment is being conducted on a Vertisol since 1983 using two-year crop rotation treatments. The surface soil (0–20 cm) samples collected after harvest of 9th season crop showed in general higher amounts of mineral N contents in the soil from the legume-based cropping system than the non-legume based cropping system (Wani et al., 1994a; Fig. 3). Inclusion of greengram in the crop-

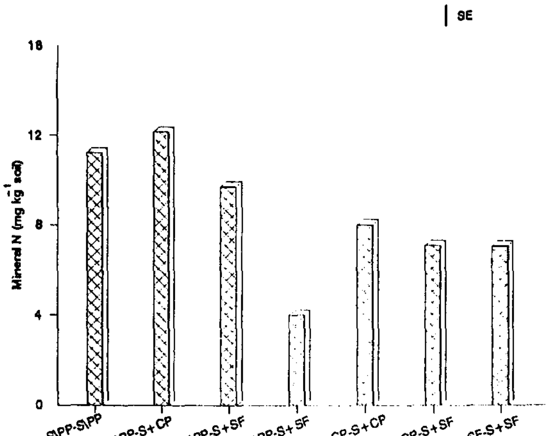

*Fig. 3.* Mineral N content in surface soil samples (0–20 cm) from plots under different cropping systems since last nine years. S - sorghum, PP - pigeonpea, SF - safflower, CP - chickpea, COP- cowpea, / - intercropped, + - sole crop grown during postrainy season.

*Table 5.* Nitrogen mineralization potential ($N_o$), active N fraction, and time (wk) required to mineralize 25 mg N kg$^{-1}$ soil for the soil samples under different cropping systems

| Treatment[a] | $N_o$ (mg kg$^{-1}$ soil) | Active N fraction(%) | Time (wk) to mineralize 25 mg N kg$^{-1}$ soil |
|---|---|---|---|
| S/PP-S/PP | 94.6 ± 15.98 | 13 | 10.3 |
| F+S-F+S | 40.5 ± 8.06 | 9 | 21.4 |
| COP/PP-S+SF | 86.1 ± 19.90 | 17 | 1.5 |
| S/PP-S+CP | 100 ± 10.04 | 16 | 13.8 |
| S/PP-S+SF | 67.3 ± 13.46 | | 10.1 |
| S+SF-S+SF | -[b] | -[b] | -[b] |
| S+CP-S+SF | -[b] | -[b] | -[b] |
| S+CP-S+CP | 56.1 ± 20.98 | | 19.6 |

[a] S = sorghum, PP = pigeonpea, F = fallow during rainy season, COP = cowpea, SF = safflower, CP = chickpea, / = intercropped, +- sole crop grown during postrainy season.

[b] Not estimated as exponential model was not superior over linear model.

Source: Wani et al. (unpubl. data).

ping sequence increased available nitrogen in the soil at harvest to the extent of 12.6% in the non-fertilized control plot (Rao and Singh, 1991). Similarly, a five times higher mineral N content in the soil under an eight year rotation using fababeans as green manure (agro-ecological rotation) was observed than from the soil under continuous barley treatment which was fertilized with 90 kg N ha$^{-1}$ y$^{-1}$ (Wani et al., 1991a).

In addition to mineral N content in the soil from the long-term rotation experiment, N mineralization potential ($N_o$) of the soils under pigeonpea-based cropping system was almost two times higher as compared to the fallow-sorghum treatment (Table 5). The "active N fraction", the quotient of $N_o$ and $N_{total}$ and expressed as percentage, varied between 9–17 % with higher values reported for the soil under pigeonpea-based cropping systems (Wani et al., unpubl. data). Using $N_o$ and k (N mineralization rate constant) values the cropping systems were ranked based on the time required to mineralize 25 mg N kg$^{-1}$ soil. Time required to mineralize a fixed quantity of N was less in the case of cropping systems which contained pigeonpea than the time required in the case of cropping systems which involved chickpea or no legume or which was left fallow for one season (Table 5). Such benefits in terms of increased "mineralizable N ($N_o$)" in the soil were observed even when all the above ground plant parts except fallen leaves were removed. Such increased $N_o$ values at Patancheru were not associated with chickpea which is grown during the post rainy season on residual moisture. Mineralizable soil N ($N_o$) following one cycle of an eight year rotation using fababeans as green manure was about double that following 60 years of a 5-year rotation involving forage and cereal crops but without returning the crop residues to the soil (Wani et al., 1994b).

The analysis of field soil samples collected prior to the start of the experiment in 1983 and later in 1993 showed that, in the case of Fallow+Sorghum (F+S) system, total soil N content was decreased by 72 $\mu g$ g$^{-1}$ soil after ten years. S+CP-S+SF and S+SF-S+SF plots also showed decreased total soil N. The continuous greengram + sorghum maintained the soil N while a substantial increase in total N was observed in S/PP-S+SF and cowpea/pigeonpea-sorghum+safflower (COP/PP-S+SF) systems. (Table 6; Wani et al., 1994a). These results demonstrated that pigeonpea-based cropping systems increased the total soil N substantially during ten years.

Sorghum was grown in the greenhouse using surface soil samples collected from the field plots which were under different cropping systems during the last 9 years. Sorghum grown in the soil from the COP/PP-S+SF plots yielded 63% higher as compared to the sorghum grown in the soil from the S+SF-S+SF plots. In other pigeonpea-based cropping systems, sorghum yielded 36–56% higher than that of sorghum yield from

*Table 6.* Soil total N ($\mu g\ g^{-1}$ soil) in 0–15 cm and 15–30 cm layer under different cropping systems during 1983 and 1993

| | Soil depth | | | |
|---|---|---|---|---|
| | 0–15 cm | | 15–20 cm | |
| Cropping system | 1983 | 1993 | 1983 | 1993 |
| S/PP-S+SF | 559 | 629 | 437 | 480 |
| S+CP-S+SF | 540 | 517 | 407 | 443 |
| C/PP-S+SF | 543 | 645 | 419 | 501 |
| S+SF-S+SF | 537 | 530 | 397 | 438 |
| F+S-F+S | 563 | 491 | 422 | 426 |
| F+CP-F+S | 567 | 507 | 399 | 446 |
| M+S-M+S | 558 | 559 | 422 | 461 |
| | NS | ** | NS | ** |
| | ±18.4 | ±13.2 | ±15.0 | 14.4 |

S - sorghum, PP - pigeonpea, SF - safflower, CP - chickpea, C - cowpea, F - fallow M - mungbean, / - intercrop, + - sequential crop, - – rotation, NS - Not significant.
** $p = \leq 0.01$.
Source: Rego et al. (unpubl. data).

the S+SF-S+SF treatment. In the case of chickpea-based cropping systems sorghum yields were lowered by 18–24.5% over the S+SF-S+SF plot yields (Wani et al., unpubl. results). Using $^{15}N$ methodology it was estimated that 8.4 to 20% of total sorghum plant N in the case of pigeonpea-based cropping systems was derived from the N that was either fixed previously and had accumulated, or the soil N that was made more available due to the presence of pigeonpea in the rotation. This was clear evidence of greater N availability in the case of pigeonpea-based cropping systems over the S+SF-S+SF system. These results were in conformity with the findings of increased $N_o$ potential of these soils reported in Table 5. The A values for the soil from pigeonpea-based cropping system plots were higher by 25.6 to 76.3 mg $pot^{-1}$ (4.5–13.3 kg N $ha^{-1}$ equivalent) than that of the S+SF-S+SF treatment. The fertilizer N replacement values calculated for these treatments using soil from the S+SF-S+SF treatment ranged from 65–161 mg N $pot^{-1}$ (24–28 kg N $ha^{-1}$ equivalent). All these results indicated that increased sorghum yields from the pigeonpea-based cropping systems over the S+SF-S+SF system were partly due to the increased soil N availability and all the benefits can not be explained in terms of the N effects (Wani et al., unpubl. results).

In the agroecological eight year rotation (which included barley, fababean, barley, fababean, barley undersown to red clover and brome grass, forage, forage, forage) barley grown following fababeans (AER 1) yielded 105% higher than that of the barley grown after continuous barley (CG) for eight years with 90 kg N $ha^{-1}$ $y^{-1}$. Using $^{15}N$ methodology it was estimated that 48.5% (405 mg N $pot^{-1}$) of total barley plant N in the case of the AER 1 treatment was derived from the N source that was not present in the soil from the CG treatment. The presence of legumes in the rotation gave an increased N supplying capacity (A value) of the soils over those in the soil from the CG system (Wani et al., 1991a). These authors concluded that the soil N availability to plants contributed significantly to the higher soil fertility in the legume-based systems. However, increased N availability contributed partly to the increased barley yields from legume-based rotations and other mechanisms than the N effect were also responsible for increased barley yields in these plots (Wani et al., 1991a). Similarly, non-N rotational benefits of the legumes towards yield of subsequent crop have been observed by many researchers (Cook, 1988; Danso and Papastylianou, 1992; Peoples and Craswell, 1992; Weil and Samaranayake, 1991).

### *Non-N rotational effects*

If the benefits of crop legumes in rotations cannot be solely explained in terms of the residual fixed N, then what are the sources of the benefits demonstrated in Table 4? Several factors can be involved, the relative importance of each dictated by site, season, and crop sequences.

Crop rotations increased the availability of nutrients other than N through increased soil microbial activity (Kucey et al., 1988; Ladha et al., 1989; Wani et al., 1991 a, b). A two fold increased microbial biomass C, in the AER soil than in the CG soil was observed. The concentration of microbial N $g^{-1}$ soil; the proportions of soil N, or the proportion of soil $^{15}N$ present as microbial N, and microbial activity as indicated by the respiration rate, were all greater in the agroecosystem than in the CG system (Wani et al. 1991a). These results indicated that higher proportion of soil or fertilizer N was in the labile fraction in the case of AER than in the case of the CG system. Wani et al., (1991b) observed that in an eight year agro-ecological rotation containing fababeans and forage, mycorrhizal colonization of barley roots was increased as compared to a CG system. Further, through positive relationships between levels of mycorrhizal colonization and K, Ca, Mg, Zn, S, and Fe accumulations and barley yields it was inferred that increased mycorrhizae acted as agents to mediate enhanced soil fertility in the rotations over that of a continuous barley system.

Improvements in the soil structure following legumes, mainly improved soil aggregate formation, after three years of alfalfa, clover and hairy vetch mixture (Latif et al., 1992) or with numerous years of a Sod pasture, or hay crop (Olmstad, 1947; Power, 1990; Strickling, 1950) have been observed. Incorporation of legume residues improved soil water-holding (Wani et al., 1994c) and buffering capacity (Buresh and De Datta, 1991).

Ries et al. (1977) suggested that growth promoting substances in legume residues are responsible for the rotation effect. The rotations break the cycles of cereal pests and diseases, and phytotoxic and allelopathic effects of different crop residues (Francis et al., 1986). The effect of crop rotation on pest pressure varies widely, but in general the literature supports Francis and Clegg (1990) who stated that "the greater the differences between crops in a rotation sequence, the better cultural control of pests can be expected". Crop rotation is an effective tool against certain pests, and that efficacy may contribute to the rotation effect, but rotation does not control all pests and diseases. For example, Johanson et al. (1984) reported that black cutworms (*Agrotis ipsilon*) are more of a problem when maize is rotated with either soybean or wheat than when maize is grown continuously. Similarly, Wani et al., (1991b) observed no reduction in the common root rot (*Bipolaris sorokiniana*) of barley grown in rotation plots than the continuous barley plots. On the contrary, marginally higher root rot incidence was recorded from the eight year rotation plots containing fababeans and forages.

## Ways to improve BNF in the SAT

### *Host-related aspects*

### *Host variability for nodulation and nitrogen fixation*

Presence of a large genotypic variability for BNF traits like nodule number, nodule mass and acetylene reduction activity (ARA) per plant has been known since early eighties for chickpea, groundnut and pigeonpea (Nambiar et al., 1988), soybean (Wacek and Brill, 1976), cowpea (Zari et al., 1978), common bean (Graham and Rosas, 1977). Using $^{15}N$ isotope-based methods, differences among cultivars have been detected in soybean (Hardarson et al., 1989; Rennie et al., 1982), common bean (Rennie and Kemp, 1982; Westermann et al., 1981), groundnut (Giller et al., 1987), greengram and blackgram (Sampet and Peoples, unpubl. data cited by Peoples and Crasswell, 1992), pigeonpea (J V D K Kumar Rao, pers. commun.) and chickpea (Rupela et al., unpubl. data). However, efforts to use this variability in breeding for improved BNF has been limited or non existent in many of these legumes. Arunachalam et al. (1984) found that ARA and nodule mass have good predictive value for plant growth and yield related traits in groundnut. After analysis of a six parent diallel cross, Nigam et al. (1985) observed that nonadditive genetic variance for ARA was predominant in groundnut. The groundnut line NC Ac 2821 had the highest general combining ability for ARA, total nitrogen, leaf area and was proposed as a good parent for breeding programs. The crosses made between the high- and low-nodulating chickpea lines to investigate the inheritance of nodulation indicated segregation for nodulation in $F_2$ populations from nonnodulating to nodulating (O P Rupela, unpubl.). These studies thus indicate the complexity of the BNF related traits. Most of the studies reported above for chickpea and groundnut were made in the field. Legumes like pigeonpea offer another difficulty for BNF studies because their nodules are loosely attached to roots and generally fall off during excavation of the field grown plants. It is perhaps due to this reason that there are no reports in pigeonpea so far on studies of the type reported above for groundnut.

Indication of plant to plant variability for nodulation within chickpea cultivars was further investigated. It was observed that not only consistent low- and high-nodulating plants were present within chickpea cultivars (Rupela, 1994), even nonnodulating plants occurred in normal cultivars or land races (Rupela, 1992). Consistent variability for nodulation extent was also subsequently detected within the pigeonpea cultivars. Unlike in chickpea, however, nonnodulating plants in pigeonpea were found in segregating populations at $F_2$ (Rupela and Johansen, 1995). It is perhaps due to the absence of any natural selection pressure for nodulation or BNF during development of a cultivar that the different nodulation types continue to exist within a material up to release stage. This gained strength from the fact that during a screening for high-nodulating plants at high mineral N in soil, we observed

*Table 7.* Different nodulation types of chickpea and pigeonpea plants identified at ICRISAT Center, India

*Chickpea*
- Nonnodulating with native root nodulating bacteria (RNB) (rn6)
- Nonnodulating with IC 59, low nodulating with native RNB
- Low nodulating at low N
- High nodulating at low N
- High nodulating at low N but low nodulating at high N
- High nodulating at high N

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

Parenthesis has the name of the identified gene.
Source: Rupela (1994).

the desired plants in 85 out of 90 advanced breeding lines of chickpea that were studied (Rupela, 1994).

Using appropriate screening procedures several different nodulation types have been identified within several chickpea and pigeonpea cultivars (Table 7) since 1985. Preliminary studies of Venkateswarlu and Katyal (1994) also indicated plant to plant variability within cultivars of groundnut. Intracultivaral variability for nodulation may be present in other legumes also. Obviously the Nod$^-$ (NN) and the low-nodulating (LN) selections are of academic interest and serve as an important reference base in BNF quantification studies. High-nodulating (HN) selections are expected to improve yield in low soil N conditions. In our screening studies the HN selection generally grew better than the NN and LN selections of a given cultivar, but large plot yield trials have been conducted only with the LN and HN chickpea selections of ICC 4948 and ICC 5003. The HN-selection of cultivar ICC 4948 produced 31% more grains than its LN-selection at low soil N(N1) level (Fig. 4). The HN-selection of ICC 4948 yielded better even at high soil N(N2) level. But the LN and HN selections of another cultivar ICC 5003 yielded the same under N1 and N2 levels. In a previous pot trial the root length density of LN-ICC 5003 was 32 m plant$^{-1}$ which was 2-times greater than that of the LN-ICC 4948. Perhaps the cultivar ICC 5003 could scavenge the soil N more efficiently than that of ICC 4948 due to its high root length density and as a result both the HN and LN lines of ICC 5003 yielded similarly.

These studies thus suggest a great scope for enhancing BNF in legumes through host plant selection. Most HN selections yielded higher than the LN selections (Fig. 4 and unpubl. studies). However, it needs to be established in further studies.

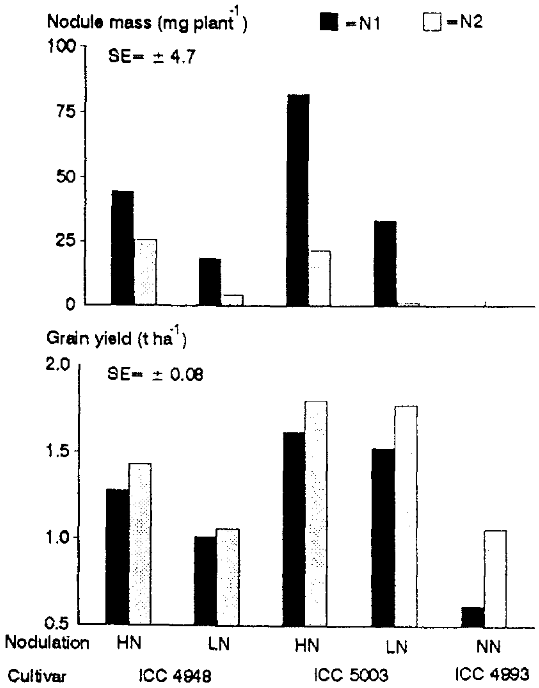

*Fig. 4.* Nodule mass at 45 days after sowing and grain yield of chickpea cultivars of different nodulation ratings (HN = high nodulating, LN = low nodulating, NN = nonnodulating; grown at two mineral N levels in soil low N (N1, about 10 mg kg$^{-1}$ soil) and high N (N2, about 20 mg kg$^{-1}$ soil); postrainy season, 1991/92, Vertisol, ICRISAT. Both N-levels and nodulation were significantly different ($p = 0.05$) for the above parameters. Their interactions were also significantly different for nodule mass. Source: Rupela, (unpubl.).

*Breeding for increased BNF and nitrate tolerance*

Soybean cv. Dunadja from Romania showed no reduction in N$_2$-fixation with application of 100 kg N ha$^{-1}$ while in all the other seven cultivars N$_2$ fixation was substantially reduced (Hardarson et al., 1989). Similarly soybean cultivars of Korean origin with higher N$_2$-fixation than the commercial cultivars grown in Australia have been identified and used as donor parents in a breeding program in Australia (Betts and Herridge, 1987; Peoples and Herridge, 1990). Plant mutagenesis has been used to generate NO$_3$ tolerant N$_2$-fixing phenotypes e.g. nitrate-tolerant symbiont in soybean (Carroll et al., 1985). Extreme super nodulating mutants of soybean and *Phaseolus vulgaris* produced significantly lower biomass and/or grain yield than their parent lines (Buttery et al., 1990; Hansen et

*Table 8.* Response of chickpea, pigeonpea and groundnut to fertilizer nitrogen in experiments on farmers' fields in India

| State | Chickpea<br>No. of trials | <br>kg grain $kg^{-1}$ N | Pigeonpea<br>No. of trials | <br>kg grain $kg^{-1}$ N | Groundnut<br>No. of trials | <br>kg grain $kg^{-1}$ N |
|---|---|---|---|---|---|---|
| Andhra Pradesh | 47 | 16.5 | 56 | 17.5 | 258 | 18.0 |
| Bihar | 77 | 17.0 | –[a] | – | 25 | 25.0 |
| Gujarat | – | – | 159 | 15.5 | – | – |
| Haryana | 88 | 12.0 | – | – | – | – |
| Himachal Pradesh | 50 | 11.0 | – | – | – | – |
| Karnataka | 275 | 11.0 | 104 | 8.0 | 310 | 14.5 |
| Madhya Pradesh | 624 | 19.0 | 15 | 10.5 | – | – |
| Maharashtra | 351 | 8.5 | – | – | 495 | 12.0 |
| Orissa | 71 | 8.5 | 39 | 19.0 | – | – |
| Punjab | 113 | 10.5 | – | – | 62 | 17.0 |
| Rajasthan | 267 | 18.0 | 159 | 13.0 | 38 | 12.5 |
| Tamil Nadu | – | – | – | – | 384 | 14.0 |
| Uttar Pradesh | 408 | 21.5 | – | – | 14 | 12.0 |
| Average | | 15.4 | | 14.2 | | 14.4 |

[a] Not conducted.

Source: Tandon (1992).

al., 1989; Wu and Harper, 1991). Species differ considerably in their symbiotic tolerance to mineral N and when sufficient natural variation already exists (Betts and Herridge, 1987; Hardarson et al., 1984) it may not be necessary to resort to mutagenesis procedures for breeding purposes (Gibson and Harper, 1985).

### *Management practices*

#### *Nitrogen*

Most of the legumes cannot derive 100% of their N requirement through BNF. In the tropics where legume residues are not returned to the soil, most legumes deplete the soil N (Table 2 and 3). In the long run, such systems cannot be sustainable. Further, large numbers of on-farm experiments in India showed that legumes responded markedly to fertilizer N; such responses are expected as legumes have a high N requirement. The SAT soils are poor in N, $N_2$-fixation mechanisms do not become functional from day one and all the legume requirement cannot be met from BNF. Significant responses to 20–30 kg N $ha^{-1}$ as starter have been observed under good growth conditions (Table 8). At application rates of 20 kg N $ha^{-1}$, overall response rate (grain $kg^{-1}$ N) was 14.2 in pigeonpea, 14.4 in groundnut and 15.4 in chickpea all under non-irrigated conditions (Table 8). Responses of such high magnitude point that to achieve increased legumes productivity along with increased BNF and maintaining the soil fertility, we need to adopt need-based mineral N application to legumes. Soil mineral N status at the time of sowing of the legume crop must be taken into account before deciding on the need and rate of N fertilizer application.

In general, high soil nitrogen levels, applied or residual, reduces nodulation and $N_2$ fixation (Tables 9 and 10). To improve BNF contribution from the legumes under such circumstances soil N must be managed through inclusion of appropriate nitrate tolerant high $N_2$-fixing legume crop or genotype of a given crop as mentioned earlier and/or appropriate cropping and management practices. It has been observed that application of 200 kg N $ha^{-1}$ decreased $N_2$ fixation by groundnut only by 18% (from 120–102 kg $ha^{-1}$) whereas in cowpea by 54% (from 125 to 57 kg $ha^{-1}$) (Yoneyama et al., 1990). These results suggest that there exists a potential to select appropriate legume crops or cultivars of a given legume for specific areas with high soil N contents without decreasing their BNF contribution to the system.

#### *Intercropping*

Legumes are generally grown as intercrops with cereals or other non-legumes in the SAT (Willey, 1979) and application of N to the cereal crop reduced $N_2$ fixation by the component legume crop (Nambiar et

*Table 9.* Nitrogen concentrations in root environment where approximately 50% reduction in $N_2$– fixation was recorded

| Suppressive concentration (in reference)[a] | ppm equiv. | BNF as | Crop | Plant culture | Reference |
|---|---|---|---|---|---|
| 1.43 m*M* | 20 | Nodule no. | Chickpea | Pot | Rawsthorne et al. (1985) |
| 6 m*M* | 84 | Nod mass, ARA | Soybean | Pot | Buttery and Dirks (1987) |
| 2 mol $m^{-3}$ | 28 | $^{15}N$ | Chickpea, Fababean | Pot | Peoples et al. (1987) |
| 5 m*M* | 70 | ARA | Chickpea | Pot | Sawhney et al. (1989) |
| 200 kg $ha^{-1}$ | 89 | ARA | Soybean | Field | Wu and Harper (1991) |
| 112 kg $ha^{-1}$ | 50 | Nod mass | Pigeonpea, Soybean | Field | Buttery et al. (1988) |
| 3 m*M* | 42 | Nod mass, ARA | Common bean | Pot | Buttery et al. (1990) |
| 112 kg $ha^{-1}$ | 50 | Nod mass | Common bean | Field | Buttery et al. (1990) |
| 10 m*M* | 140 | Nod mass, ARA | Fababean | Field | Buttery and Gibson (1990) |
| 5 m*M* | 70 | Nod mass | Soybean | Pot | Cho and Harper (1991) |

[a] In all cases, except for Rawsthorne et al. (1985), the listed nitrate concentration was the lowest level used in different trials.

al., 1983; Ofori and Stern, 1987). Similarly, shading by associated cereals reduced BNF in the component legumes (Nambiar et al., 1983; Wahua and Miller, 1978). Strip cropping of the cereals and legumes can overcome both these problems and improve the systems productivity without reducing BNF contributions in the system from the associated legumes. Indeterminate legumes fix more N than determinate types in intercropping (Fugita et al., 1992). Nitrogen fixation in climbing bean (Francis, 1986; Graham and Rosas, 1978), cowpea (Ofori et al., 1987) and Siratro (Ogata et al., 1986) was unaffected by intercropping with cereals. In cases where strip cropping is not possible, climbing type legumes can be used.

### *Tillage*

Nodulation and $N_2$ fixation in soybean grown in subtropical Australia were substantially improved under no tillage with N balance of 80 kg N $ha^{-1}$, compared with the cultivated system with 30 kg N $ha^{-1}$ N balance. Increased $N_2$ fixation resulted mainly from the higher proportion of plant N derived from fixation since yields were unaffected by tillage practice (Peoples and Crasswell, 1992). Clean cultivation accelerates the oxidation of organic matter in soils and generally results in higher $NO_3$ in the profile (George et al., 1992; Thomas et al., 1973) which would affect BNF in legumes.

### *Land form*

Greengram, pigeonpea and soybean grown on broad bed and furrows (BBF) on Vertisol improved nodulation than when grown on a flat surface. However, improved nitrogenase activity on BBF was recorded with greengram and pigeonpea only (Wani and Potdar, unpubl. data). However, in Vertisols, chickpeas sown on flat beds nodulated better than those sown on ridges with the same sowing density (Rupela and Saxena, 1987). As the ridged fields had greater evaporation losses due to increased surface area, this may be important when moisture is limiting.

### *Deep sowing*

Deep sowing of groundnut results in the development of an elongated hypocotyl, poor rooting, poor nodulation and nitrogen fixation, notably in spanish types. Virginia types have considerable nitrogenase activity even when sown deep because of their ability to nodulate on the hypocotyl (Nambiar et al., 1988). Farmers tend to sow chickpea at a sufficient depth to ensure good crop stand as it is generally grown on residual moisture. Deep sown chickpea crops in heavy black soils suffer a substantial reduction in nodulation and $N_2$ fixation. In lighter soils chickpea have been found to nodulate at depth (Rupela et al., 1985).

### *Other nutrients*

It should be realized however, that poor $N_2$ fixation can be due to poor plant growth resulting from pests, diseases, and nutrient deficiencies. Addition of P stimulated pigeonpea nodulation in both an Alfisol and a Vertisol (Kumar Rao and Dart, 1981). In Karnataka, India, trials on farmer's fields with pigeonpea showed increased nodulation due to application of diammonium phosphate (DAP) alone than to the inoculation with

*Table 10.* Effect of soil mineral N and N fertilizers on crop N productivity and the proportion (P) and amount of crop N derived from $N_2$ fixatic

| Species | Location | Level | | Total crop N (kg N ha$^{-1}$) | $N_2$ fixed | | Reference |
|---|---|---|---|---|---|---|---|
| | | Soil mineral N (kg N ha$^{-1}$) | Fertilizer N (kg N ha$^{-1}$) | | P | Amount (kg N ha$^{-1}$ crop$^{-1}$) | |
| Groundnut | India | – | 0 | 196 | 0.61 | 120 | Yoneyama et al. (1990; |
| | | | 100 | 210 | 0.47 | 99 | |
| | | | 200 | 243 | 0.42 | 102 | |
| Chickpea | Australia | 10(to 120 cm) | | 114 | 0.85 | 97 | Doughton et al. (1993) |
| | | 326 | | 194 | 0.17 | 33 | |
| | | | 0 | 97 | 0.81 | 79 | |
| | | | 50 | 114 | 0.59 | 59 | Herridge et al. (1994 |
| | | | 100 | 115 | 0.29 | 25 | |
| Soybean | Australia | 70(to 120 cm) | | 230 | 0.34 | 78 | Herridge et al. (1990) |
| | | 260 | | 265 | 0.06 | 16 | |
| | India | – | 0$^a$ | 63 | 0.29 | 18 | Yoneyama et al. (1990) |
| | | | 100 | 108 | 0.26 | 28 | |
| | | - | 0$^b$ | 89 | 0.48 | 43 | |
| | | | 100 | 115 | 0.24 | 28 | |
| | Malaysia | - | 40 at sowing | 169 | 0.68 | 115 | Norhayati et al. (1988) |
| | | day 45+ further or | 20 as nitrate | | | | |
| | | | 20 as urea | 200 | 0.15 | 30 | |
| Common bean | Kenya | - | 10 | 149 | 0.39 | 58 | Ssali and Keya (1986) |
| | | | 100 | 158 | 0.10 | 16 | |
| Cowpea | Kenya | - | 20 | 116 | 0.53 | 62 | Ssali and Keya (1984) |
| | | | 100 | 137 | 0.08 | 11 | |
| | India | - | 0 | 163 | 0.77 | 125 | Yoneyama et al. (1990) |
| | | | 100 | 138 | 0.67 | 92 | |
| | | | 200 | 172 | 0.33 | 57 | |

[a] Uninoculated.
[b] Inoculated .

*Rhizobium* alone (Chinmulgund and Hegde, 1987). Cassman et al. (1981) found that field-grown soybean had a higher P requirement when it was dependent on BNF for its N supply as compared to the mineral N dependency. Based on the results from 140 on-farm demonstration plots with soybean in Uganda it was observed that on an average 300 kg ha$^{-1}$ yield increase was obtained with 40 kg $P_2O_5$ ha$^{-1}$ application and further increase of 300 kg ha$^{-1}$ was obtained through inoculation with *Rhizobium* (Keyser and Li, 1992).

In groundnut, fertilization with B, Co, Mo and Zn in a medium calcareous soil, with and without *Rhizobium* inoculation significantly increased nodulation, percentage of effective nodules and plant dry matter (Joshi et al., 1987). It has been reported that Fe deficiency specifically limits nodule development in groundnut grown in the calcareous soils of Thailand (O'Hara et al., 1988). Soil acidity along with Mn and Al toxicities can also restrict $N_2$ fixation in groundnut. Excess Mn was detrimental to plant growth per se rather than to nodulation, but nitrogenase activity was more affected by Al than plant growth (Nambiar and Anjaiah, 1989a). Application of Co at a rate of 500 mg cobalt nitrate kg$^{-1}$ seed significantly increased grain yield of pigeonpea (Raj, 1987), soil application of 0.45 kg Mo ha$^{-1}$ as sodium molybdate significantly increased nodulation and grain yield of pigeonpea

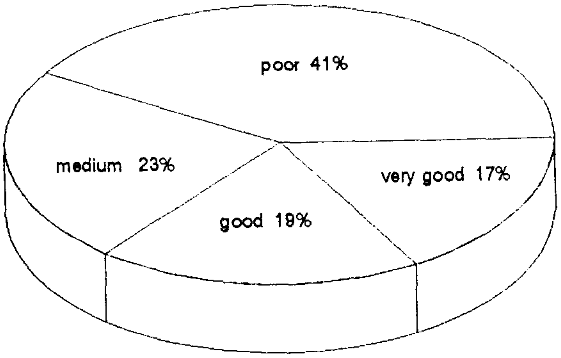

*Fig. 5.* Nodulation status of chickpea based on 314 fields. (AICPIP data cited by Tauro and Khurana, 1986).

(Khurana and Dudeja, 1981). Soil application of 1 kg cobalt chloride, 1 kg sodium molybdate ha$^{-1}$ and 25 kg $ZnSO_4$ ha$^{-1}$ increased chickpea grain yield by 10, 7 and 4% respectively over the control. Inoculation with *Rhizobium* increased chickpea yield by 26% over the non-inoculated control however, inoculation along with Co, Mo and Zn application increased yield by 41, 39 and 28% respectively over the control (Namdeo and Gupta, 1992).

*Insects*

Extensive nodule damage to pigeonpea by a Dipteran larva, *Rivellia angulata* was reported in farmers' fields (Sithanantham et al., 1981). The extent of nodule damage was greater in pigeonpea grown in Vertisols (up to 86%) as compared to 20% in Alfisols (Nambiar et al., 1988). Nambiar et al. (1990) reported reduced nodule damage by 50% due to inoculation of pigeonpea with engineered *Bradyrhizobium* carrying an insecticide gene (*Bacillus thuringiensis* subsp. israelensis) in the presence of *Rivellia angulata* larvae under greenhouse conditions. These results suggest the potential benefits from planned introduction of engineered *Bradyrhizobia* carrying insecticide genes into natural environments. Another possible solution is to select pigeonpea genotypes that can resist or tolerate attack by nodule damaging insects. Soil application of a single dose of insecticide (aldrin) prevented nodule damage up to 45 DAS however, during later stages insect damage could not be controlled (Kumar Rao and Sithanantham, 1989).

*Use of inoculants*

Much of the applied research efforts in studying BNF have gone into identifying efficient strains of bacteria as inoculants. Before inoculation with appropriate strains to be used, it needs to be determined whether inoculation is needed?

*Need for inoculation*

The most important point is do we need inoculation of the legumes in a region where these crops have been grown over long periods? Development of an inoculation industry in many countries has been largely motivated by the desire to introduce legume species to new areas (Burton, 1982). Most cultivated tropical soils are assumed to have relatively large populations ($> 100$ g$^{-1}$ dry soil) of rhizobia capable of nodulating the legumes grown in such soils (Nambiar et al., 1988). However, surveys of farmers' grain and fodder legume crops have shown poor nodulation in large areas and good nodulation only in a few pockets (Fig. 5) (IARI, 1980; Kabi and Poi, 1988; Kulkarni and Joshi, 1988; Tauro and Khurana, 1986). In a survey of farmers' chickpea fields around Gwalior, Madhya Pradesh (M.P.), 39% fields had $< 100$ rhizobia g$^{-1}$ soil, 17% had $10^2$–$10^3$ and 44% fields had a population $> 10^3$ (Rupela et al., 1987). In a similar survey conducted for 43–47 villages from each of the three districts of Madhya Pradesh, India for nodulation of pigeonpea, black gram, green gram and lentil showed poor nodulation (0–10 nodules plant$^{-1}$) in 64 to 100% of the surveyed area (Namdeo and Gupta, 1992). The need to inoculate the legumes grown on cultivated soils must be assessed by considering the interacting factors between the soil, the host plant and *Rhizobium*.

Presence of nodules on plant roots does not necessarily mean that sufficient $N_2$ is being fixed for maximum benefit to the host plant. In groundnut or pigeonpea nodulation occurs naturally at most locations due to the cross-species promiscuity of the cowpea rhizobia. However, the ability to fix high amounts of N (efficiency) is governed by the symbiotic capability between *Rhizobium* and the host plant. Hence, it may be necessary to introduce superior (more competitive and efficient) strains of *Rhizobium* to ensure adequate $N_2$ fixation for maximum growth and yield of the host plant. In a survey of groundnut crops grown in farmers' fields in southern India, 52 out of 95 fields showed inadequate nodulation with less than 10 per cent ARA of that which can be obtained under reasonable field conditions (Nambiar et al., 1982). Although, adequate nodulation was observed in some parts, ineffective nodules exceeded the number of effective nodules. Field surveys have shown that proportion of ineffective strains was as high as 40% in chickpea, 53% in green gram and 63% in groundnut (Tauro and Khurana, 1986). In another study 94% strains of rhizobia were observed ineffective in groundnut (Kulkarni and Joshi, 1988). Poor nodulation in farmers' fields could be due to several factors e.g. inadequate soil moisture, lack of appropriate rhizobia in soil, deficiency or toxicity of a particular nutrient, unfavorable conditions like prolonged water logging, unfavorable pH, abundance of bacterial predators, pests and disease attack, etc.

Using the network approach NifTAL initiated Worldwide Rhizobial Ecology Network (WREN) and conducted standardized inoculation trials with extensive environmental data. Thies et al. (1991) developed a mathematical model using native rhizobia numbers (estimated by most probable number method) and soil mineral N data as inputs to predict the inoculation responses at different sites. This approach accounted for 83% of the variation observed due to inoculation. These models have been incorporated into an interactive computer program called "RESPONSE" which reduces the need for costly, site-specific field inoculation trials to determine the need for inoculation with *Rhizobium*. This remains a valid approach to determine the need for inoculation in most of the cases. However, Nambiar (1985) reported significant yield increases from Cameroon, India, and China in the case of groundnut due to inoculation with NC 92 strain from the soils having large populations of native rhizobia. These results indicate that a simulation model using most probable number (MPN) data and mineral N data can not provide reliable answers in all the cases and there is a need to fine-tune the model.

### *Competitive and effective strains*

In soils lacking rhizobia nodulating a particular legume, inoculation with efficient strains increased yields (Nambiar et al., 1988). In soils which contain established native *Rhizobium* populations, the introduced strains should be competitive and efficient. The degree of establishment and persistence of an inoculant strain generally decreased with increase in population density of the native rhizobia (ICRISAT, 1981). However, some inoculant strains have succeeded in forming more nodules even in the presence of active indigenous competing rhizobia eg. NC 92 on groundnut (Nambiar et al., 1984). Little is known of the factors controlling competitiveness but host cultivar, soil properties, soil microflora, environmental factors and the nature of the competing strains influence the success of inoculant strains in nodule formation (Alexander, 1982). The success of the strain NC 92 in terms of nodule formation increased with repeated inoculation (Table 11). Higher inoculum rate of $10^6$–$10^8$ cells per seed at the initial inoculation helped in early establishment (Nambiar et al., 1984). Strains of vesicular arbuscular mycorrhizae (VAM) significantly influenced nodule formation by bradyrhizobia strains. In the absence of any VAM, when mixtures of NC 92 and NC 43.3 were inoculated, strain NC 92 occupied more nodules (89%) than strain NC 43.3 (34%). In the presence of *Acaulospora laevis*, 86% nodules in the NC 92 + NC 43.3 mixture were formed by NC 92, but the presence of *Glomus fasiculatus* reduced the competitive ability of strain NC 92 (49% NC 92 nodules) (Nambiar and Anjaiah, 1989b). Field trials with soybean have demonstrated that to achieve nodule occupancy of greater than 50%, inoculant rhizobia/bradyrhizobia must be applied at a rate at least 1,000 times greater than the estimated number of indigenous bradyrhizobia in soil (Weaver and Frederick, 1974). Competition between inoculated and native *Rhizobium* strains and response to inoculation was less pronounced in the presence of soil mineral N than under conditions where such N was immobilized and made unavailable (Somasegaran and Bohlool, 1990). Use of massive inoculation rates can overcome competition from indigenous strains (Kapusta and Rouwenhorst, 1973), but such a delivery system is not yet economical and practical.

In many rice-growing areas, legumes are grown after paddy, using residual moisture. In such fields, less than 100 cowpea group rhizobia $g^{-1}$ soil were observed and continuous cultivation of paddy had an adverse effect on *Rhizobium* survival. Under such conditions inoculation with effective strains showed significant responses in chickpea and pigeonpea (Nambiar et al., 1988).

### *Factors affecting performance of inoculant strains*

Crop responses to inoculation with biofertilisers are not as visible as those with fertilizer N. Being biological agents, these are subjected to a range of hostile environments and their survival and efficiency is governed by several factors. Generally, there is a decline in the rhizobial population on seeds but conventional wisdom is that multiplication should occur as the rhizosphere forms, so that accelerated germination can also assist in ensuring an adequate population. The seed coat of a dicot is often carried on the top of the cotyledons into

*Table 11.* Persistence of inoculum strain NC92 over two seasons on groundnut

| Season | | % nodules formed on groundnut plants | |
|---|---|---|---|
| 1st | 2nd | 72 days after sowing | 116 days after sowing |
| Uninoculated | Uninoculated | 9 (5)[a] | 11 (8) |
| Uninoculated | Inoculated | 31 (27) | 27 (25) |
| Inoculated | Uninoculated | 28 (25) | 42 (32) |
| Inoculated | Inoculated | 39 (41) | 75 (54) |
| SE | | ± 2.5 | ± 5.4 |

[a] Data analysed after arsine transformation: original means in parenthesis.
Source: Nambiar (1985).

the open air, so that only a part of the inoculum may be left to multiply within the rhizosphere. In the case of crops grown on residual moisture, such as chickpea, the inoculated rhizobia cannot move downwards with the growing root from the top soil where inoculated, resulting in poor nodulation. Secondly, deep sowing results in a good crop stand but affects nodulation adversely (Nambiar et al., 1988).

Carrier-based inoculants are usually coated on seeds for the introduction of bacterial strains into the soil. However, alternative inoculation methods are necessary where seed treatment with fungicides and insecticides is needed or where seed of crops such as groundnut and soybean can be damaged when inoculated with an adhesive. In addition, use of superphosphate as the P source can be harmful for *Rhizobium* because of contact with the acidic fertilizer. Often the soils themselves are acidic and lime coating of seed has been a popular measure for additional protection. The normal carrier-based inocula can be successfully applied separately from the seed (Bonnier, 1960; Burton, 1982). While all methods of inoculation were successful under favorable conditions, "liquid" and "solid" methods were superior to seed inoculation under adverse conditions (Brockwell et al., 1980). Increased groundnut yields were obtained when inoculation was done by applying a slurry of peat-based inoculum in the seed furrow (Table 12). At ICRISAT, a bullock-drawn seed drill commonly used by farmers has been modified for simultaneous *Rhizobium* application in the seed furrow (Nambiar, 1985).

Soil properties can also affect the survival of inoculated rhizobia. For example, out of 11 locations tested for response of groundnut cv Robut 33–1, inoculation with strain NC 92 failed to increase yields at two locations, namely Tirupathi and Kadiri, India (AICORPO, 1983). Subsequent analysis of soil samples from Tirupathi revealed a high (150 mg $kg^{-1}$) available manganese content (Nambiar, 1985). Manganese and aluminum can be toxic to symbiotic $N_2$ fixation even if they are not at a level high enough to affect plant growth (Franco, 1977). Soil acidity and alkalinity can also pose problems for symbiotic $N_2$ fixation. For such problem areas, specific strains with the ability to overcome such adverse conditions need to be selected as inoculants. Significant differences were observed among pigeonpea rhizobial strains for their ability to nodule and fix $N_2$ under saline conditions (Subba Rao et al., 1990).

### *Yield response to inoculation*

The field performance of inoculation is variable. Not many on-farm data are available on the impact of inoculation on grain yields. In 12 trials with chickpea, inoculated plots gave on an average 116 kg $ha^{-1}$ more grain as compared to non-inoculated plots. In another set of field demonstrations, inoculation resulted in grain yield increase in the range of 112–227 kg $ha^{-1}$ (Chandra and Ali, 1986). The results of 1500 demonstrations on farmers' fields with pigeonpea conducted in Gulbarga district of Karnataka State in India showed 100% increase in yield (1035 vs. 516 kg $ha^{-1}$) due to balanced use of DAP and *Rhizobium* inoculation (Chinmulgund and Hegde, 1987). On research stations in 16 trials inoculation of chickpea with *Rhizobium* increased grain yield by 342 kg $ha^{-1}$ (range 30–610). Significant improvement in chickpea grain yield was reported from 7 out of 16 locations (Subba Rao, 1976) and 6 out of 12 locations (Subba Rao and Tilak, 1977), predominantly in central and northern India with yields varying from −14 to 30% compared to the control plots yield. Increase in grain yield of the pigeonpea inoculated with effective *Rhizobium* ranged from 19 to 68% over non-inoculated controls (Nambiar et al., 1988). In groundnut, inoculation responses varied from

*Table 12.* Effect of fungicide and method of inoculation on nodulation by strain NC92[a] on groundnut

| Treatment | Method of inoculation and % nodules formed by strain NC 92[a] | | |
|---|---|---|---|
| | Liquid | Seed | Uninoculated |
| Untreated | 30 (27) | 22 (20) | 4 (2) |
| Captan | 28 (23) | 7 (4) | 3 (1) |
| Thiram | 25 (18) | 6 (4) | 7 (2) |
| Dithane | 19 (10) | 14 (9) | 7 (3) |
| Bavistin | 24 (16) | 14 98) | 10 (3) |
| Mean | 25 (19) | 13 99) | 6 (2) |

SE mean for comparing inoculation means within a fungicide treatment is ± 5.5.

[a] Nodules typed by ELISA 60 days after sowing. Data analysed after arcsine transformation: original means in parenthesis.
Source: Nambiar (1985).

decreased yields to significantly increased yields over non-inoculated controls (Kulkarni and Joshi, 1988; Nambiar et al., 1988; Subba Rao, 1976). Over 228 inoculation trials were conducted under the International Network of Legumes Inoculation Trials (INLIT) by cooperating scientists in 28 countries over the years. In approximately 52% of the cases, inoculation resulted in significant yield increases (Davis et al., 1985). In summary, yield responses to inoculation were site specific, depending on location, species, fertility, and other factors.

Sometimes, legumes yields are not increased by inoculation but N concentration in grains or plant parts is increased over N concentration in non-inoculated control plants. In cases where both types of responses are not observed, it might simply result in a saving of soil N which might be useful for the succeeding crop.

## Conclusion and future areas of research

Biological nitrogen fixation plays an important role in sustaining productivity of the soils in the SAT. Legumes fix substantial amounts of nitrogen (Table 1) through the BNF process and play an important role in the N cycle. However, mere inclusion of legumes in the cropping systems in the SAT will not ensure N contributions to the system through BNF. The important issue is how best we can exploit BNF technology for developing sustainable cropping systems in the SAT?

Until now considerable effort in BNF research has gone in the area of selection of efficient bacterial strains for using as inoculants. For realizing the maximum benefits from BNF we must take a holistic approach (Bantilan et al., 1994; Wani et al., 1994a). There is need to understand the BNF system which includes host, bacterium and environment and ensure that all the partners involved work in harmony to deliver maximum benefit. There is a need to accurately quantify $N_2$ fixation by legumes in a system after taking into account the $N_2$ fixed in the roots and fallen plant parts. Such information will help us to identify the systems which really maintain or improve the soil N status. Host controlled factors play an important role in regulating BNF but have not received its due share by researchers. We need to identify type of legume and also genotype of a given legume which yields more and also derive larger part of its N requirement from fixation in a particular cropping system. For example, we need to identify crops and genotypes of legumes which can fix more $N_2$ under sole cropping and intercropping situations without being affected by high mineral N contents in soil. There is a need to identify host genotypes which can fix well under adverse soil conditions like soil acidity, Al and Mn toxicity, alkalinity, water logging, etc.

At ICRISAT nonnodulating lines of chickpea, pigeonpea and groundnut have been developed from the existing cultivars and/or segregating populations. Natural occurrence of nonnodulating plants ranged from 120 to 490 per million plants and efforts are required to see that occurrence of such plants do not increase. Most of the breeding and testing work is done at the research stations where mineral N contents are far higher than observed on the farmers fields. There is every likelyhood that low- or nonnodulating plants may not be identified as they will grow normally using soil N. To avoid this, appropriate checks during breeding and testing for discarding low-nodulating plants must be built in the breeding programs.

Along with the selection of appropriate host plant and genotypes there is need to provide optimum management practices to ensure maximum contribution from the BNF. Through appropriate management practices soil N should be manipulated in intercropped situations for example appropriate form of fertilizer like slow releasing formulations, organic N and suitable method of application for example placement between cereal rows rather than broadcasting and mixing in the soil must be worked out. Appropriate amendments with nutrients other than N which might limit the plant growth and BNF should be done.

Suitable land management practices which can improve water storage capacity of soils or which can drain excess water away from the plant depending on the situation need to be used to harness maximum benefits from BNF. Efforts for selection of efficient strains of bacteria to use as inoculants and identification of specific host-bacteria combinations must go on. Situations which need inoculation should be identified and efforts for success to inoculation in such areas must be concentrated.

For increasing crop yields through biofertilizers, the following strategy is suggested. Most important constraints to effective exploitation of BNF technology in the SAT are:

- the quality of the inoculants
- lack of knowledge about inoculation technology for the extension personnel and the farmers.
- effective inoculant delivery system
- formulation of the policy to exploit BNF successfully.

The history of inoculant manufacture and of many strain collections is full of examples of organisms which look like rhizobia but are not! Contaminated cultures contribute to the problems which placed the inoculant industry of Australia in peril in the early 1950s. Many inocula of poor quality were sold and the losses at sowings of new legumes into poor soils were enormous (Thompson, 1982). This was repeated in India during the late 1970s and early 1980s. Several rhizobial inocula from the Indian manufacturers were examined at ICRISAT (Thompson, 1982) for their infectivity tests. Irrespective of private or public institution origin, the majority failed to pass the published standards (ISI, 1977). There must be strict quality control mandatory on all biofertilizer producers irrespective of their status as private/public or government organization.

For success of biofertilizers in the SAT concerted efforts right from production, demonstration to distribution will be required. The next step is convincing and educating the farmers regarding the benefits of these inoculants. The pricing of the biofertilizers must be controlled if private agencies are involved, otherwise if farmers don't see the significant effects in term of economic yields, they may not be interested in using the biofertilizers. There is a need to demonstrate the benefits from BNF technology in terms of maintenance or improvement of soil fertility through long-term experiments. At this stage the policy issue arises that biofertilizers should be used or considered as an insurance for harnessing BNF to its maximum potential taking systems approach. As discussed earlier the nonnodulating or LN plants look similar in appearance to well nodulated plants in chickpea but this is at the cost of soil or fertilizer N. We must take the view that in the end we may derive benefit in terms of maintaining or improving the productivity of our soils. We should not be disappointed by not seeing the direct benefits in terms of increased legume yields in some cases. A holistic approach to improve production of legumes is needed and we must ensure that all the constraints for good plant growth other than N nutrition are alleviated and suitable management practices are provided for better performance of BNF technology.

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