5

Factors Affecting Biological Nitrogen Fixation and Residual Effects of Legumes in the Indo-Gangetic Plain

J.V.D.K. Kumar Rao and O.P. Rupela[1]

ABSTRACT

Statistical information suggests a substantial increase in area under irrigation, under rice and wheat, and in use of chemical fertilizers in Asia. However, a slowing down in growth of productivity of rice and wheat has been reported in recent times. In the past, legumes occupied a significant area in the Indo-Gangetic Plain (IGP) but the area has declined due to the more remunerative and relatively more stable cereals. However, legumes still have a potential role in sustaining productivity of rice- and wheat-based systems. Atmospheric nitrogen (N) fixed by legumes in symbiosis with root nodule bacteria potentially meets much of the N demand of the legume and can contribute to the N requirements of subsequent crops. This aspect and the factors affecting biological nitrogen fixation (BNF) by legumes have been reviewed. Data specifically from IGP have been very scanty and substantial information has been drawn, particularly on the factors (temperature, moisture, salinity, host plant, and rhizobia) affecting BNF and residual effects of legumes, from other sources.

Indiscriminate use of nitrogenous fertilizers has resulted in increased mineral-N concentration in soils of IGP, at least in some areas. These concentrations are suppressive for BNF by legumes. Such changes in the micro-environment in soils of IGP will require identification of appropriate legumes and cultivars that yield well and fix adequate N under the changed environments. Experiments to assess the scope of sustaining productivity of rice- and wheat-based cropping systems through increased harnessing of BNF by legumes have been proposed.

In 1996 Asia produced 91% of the world's rice *(Onyza sativa) and* 43% of the world's wheat *(Triticwn aestivum),* amounting to 572 million t rice and 261 million t wheat (FAO 1997). The growthrate of the population in Asia of 3 billion is about 2% per annum, and an ever increasing production (>2.5% per annum) of these staple grains will be needed. This is imposing an inevitable threat to the natural resource base, even in traditionally well-endowed areas, and examples of adverse consequences of continuous cereal cropping are being increasingly documented (Singh and Paroda 1995). A closer

---

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

examination of cropping sequences is needed if productivity of rice and wheat is to be maintained and further increased. In this context, the well-known ameliorative effects of legumes in crop rotations need close attention in relation to the sustainability of rice and wheat production systems. Sustainable agriculture involves the successful management of agricultural resources to satisfy changing human needs while maintaining or enhancing the natural resource base and avoiding environmental degradation (CGIAR/TAC 1988). It relies greatly on renewable resources such as biologically fixed nitrogen. Biological nitrogen fixation (BNF) helps in maintaining and/or improving soil fertility by using nitrogen (N) which is in abundance in the atmosphere. Intensive agricultural systems such as rice-wheat in the Indo-Gangetic Plain (IGP) are characteristically expanded nutrient cycles involving the export of crops from a farm and require continued imports of nutrients to the farm.

Nitrogen is one of the most limiting nutrient for increasing crop productivity. Input efficiency of N fertilizer is low (Prasad et al. 1990; Singh and Paroda 1995; Abrol et al. 1997) and in turn it contributes substantially to environmental pollution. The BNF by legumes offers an economically attractive and ecologically sound means of reducing external N inputs and improving the quality and quantity of internal resources. However, mere inclusion of legumes does not guarantee increased contributions of N and other benefits to the soil/cropping system as legume crop growth and BNF are influenced by a number of physical, environmental, nutritional, and biological factors. In this chapter, factors affecting BNF by legumes and residual effects of legumes on succeeding crops, with particular emphasis on chickpea *(Cicer arietinum),* pigeonpea *(Cajanus cajan),* and groundnut *(Arachis hypogaea)* are reviewed. Although the factors affecting BNF by legumes are largely known, the present review was prepared mainly for the benefit of those interested in improving the beneficial effects of legumes in rice and/or wheat cropping systems. A literature search (1975-97) on these aspects with particular reference to rice-wheat cropping system in IGP gave very little direct information and so we have attempted to extrapolate findings from other systems as well.

CHARACTERIZING RICE-WHEAT AREAS FOR LEGUMES

The increase in world irrigated area during 1961 and 1994 was 116.7 million ha of which 63% was in Asia (i.e., 74 million ha of the total 164 million ha in Asia) (Table 1). This increase is largely the result of canal and tubewell irrigation projects. Rice and/or wheat are preferred cereals on irrigated lands in Asia. It is widely observed that legumes are generally grown as rainfed crops and are rarely irrigated even if this facility is available. Also, whenever a rainfed area receives an irrigation facility legumes are largely replaced by input (mainly fertilizer and water) responsive cereals such as rice, wheat, and maize *(Zea mays).* The area under legumes [pulses + soybean

Table 1: Agricultural area and production and consumption of nitrogenous fertilizers of cereals and legumes.

	Area (million ha)		Total harvest area (million ha)				Nitrogenous fertilizers (million t)	
Year	Total agriculture	Irrigated	Rice	Wheat	Pulses	Soybean	Production	Consumption
1961								
Asia	1052.8	90.2	107.0	61.2	38.8	11.6	1.7	2.1
World	4486.8	138.8	115.5	203.9	63.7	23.8	12.9	11.6
1971								
Asia	1102.6	111.4	122.7	70.0	34.0	9.3	6.4	8.0
World	4610.4	171.1	134.7	213.0	63.1	30.0	38.4	33.5
1981								
Asia	1159.6	133.9	129.4	79.8	34.4	10.3	19.4	21.7
World	4719.8	222.5	145.3	239.2	62.0	50.5	62.3	60.5
1991								
Asia	1265.1	156.7	131.7	87.3	35.7	12.8	32.3	37.9
World	4855.1	245.8	146.7	223.2	68.6	55.0	80.6	75.5
1997								
Asia	1264.5 (1994)[1]	164.2 (1994)	135.2	102.5	39.2	16.2	39.9 (1995)	44.9 (1995)
World	4872.7 (1994)	255.5 (1994)	150.8	229.2	71.8	67.2	86.7 (1995)	78.7 (1995)

[1] Figures in parantheses represent the year.

Source: FAO (1997).

(Glycine max)] and major cereals (rice + wheat) during 1961 to 1997 has increased, both in Asia (by 10%, i.e., 55.4 million ha under legumes and by 41%, i.e., 237.7 million ha under cereals) and the world (by 59%, i.e., 139 million ha under legumes and by 19%, i.e., 380 million ha under major cereals) (Table 1). Therefore, it is interpreted that legumes have been shifted to new lands or cropping systems where these were not grown previously. In 1961/62, Punjab (including present states of Haryana and Himachal Pradesh in India) had 3.4 million ha under irrigation and 2.46 million ha under legumes. In 1990/91 irrigated area increased to 6.6 million ha whereas area under legumes decreased to 0.95 million ha (78%) (Table 2). During the same period, in Madhya Pradesh state of India, the area under grain legumes increased by 16.7% by introduction and spread of a new legume, soybean, on an area of 2.6 million ha (Table 2). Irrigated area in Madhya Pradesh also increased from 1.1 million ha in 1964/65 to 4.3 million ha in 1990/91. Both the states also registered a significant increase in area under both rice (22% in Madhya Pradesh, 5.3 times in Punjab) and wheat (12% in Madhya Pradesh and 1.4 times in Punjab). The extent of increase was greater in Punjab (northwestern IGP) than in Madhya Pradesh (non-IGP area). Bihar (another IGP area) also witnessed a significant reduction (47%) in the area

under pulses during 1964/65 to 1990/91. During the same period the area under pulses in India showed a marginal increase of 0.3 million ha (from 24.2 million ha to 24.5 million ha). This further suggests the trend in legume area shifts from IGP to non-IGP areas in India. A similar scenario appears to be the case for Bangladesh, Nepal, and Pakistan (data presented at a workshop on Legumes in rice-wheat cropping systems of the Indo-Gangetic Plain: Constraints and opportunities, 15-17 Oct. 1997, ICRISAT, Patancheru, India).

Table 2: Changes in area ('000 ha) from 1961/62 to 1991/92 under rice, wheat, pulses and soybean in Punjab[1] and Madhya Pradesh, India.

	Crop				Irrigated area		
Year/State	Rice	Wheat	Pulses	Soybean[2]	Rice	Wheat	Pulses
1961/62[3]					1964/65[4]		
Punjab	446.3	2240.4	2459.0	Nil[2]	377.0	1395.8	518.0 (2251.10)[5]
Madhya Pradesh	4193.8	3176.5	3879.1	7.7	547.2	240.8	72.9 (3914.6)
1991/92[6]					1990/91[7]		
Punjab	2819.9	5419.1	525.4	0.6[2]	2701.8	5014.1	271.0 (931.3)
Madhya Pradesh	5131.5	3547.0	4528.4	2648.8	1019.3	2014.0	586.0 (5008.6)

[1] Includes present states of Haryana and Himachal Pradesh that were formed after 1961/62.

[2] Soybean statistics for Punjab are available for 1971/72 and were not available for previous years. In 1991/92 the crop area was reported for Himachal Pradesh only and it is interpreted that the other states did not have measurable area under soybean.

[3] Government of India (1970).

[4] Government of India (1971).

[5] Values in parentheses refer to total area (irrigated + nonirrigated) under pulses.

[6] Government of India (1993).

[7] Government of India (1994).

In most legumes studied, BNF is suppressed by high levels of mineral N in the soil (Streeter 1988). Soil mineral-N levels of 20-89 mg N kg^{-1} soil have been found to suppress BNF traits by about half in several legumes (Rupela and Johansen 1995b). It is widely believed that N once applied is either used up by the receiving crop or is lost and much of it does not stay in profile. But we recorded increases in soil N level at sowing of chickpea due to the application of increasing N fertilizer levels to the preceding sorghum on a Vertisol (Table 3). The increased soil N levels suppressed BNF by chickpea. It is therefore likely that the N applied to rice and wheat [N-use efficiency is reported to be in the range of 30-35% (Abrol et al. 1997)] is available to the succeeding crops in different soils of the IGP. Mean available soil N level (alkaline permanganate method) measured in farmers' fields growing rice-wheat regularly in Punjab, was 22-224 mg N kg^{-1} soil (Table 4). Thus legumes grown in irrigated rice-wheat areas may face suppressive levels of soil mineral N.

Table 3: Total nitrogen (N) and mineral-N in top 15 cm of a Vertisol at the time of sowing chickpea, 1990-95, ICRISAT, Patancheru, India.

N-application	Total N[1] (mg kg^{-1} soil)			Mineral N (mg kg^{-1} soil)		
to preceding sorghum[2]	Nodulated chickpea	Nonnodulated chickpea	Mean	Nodulated chickpea	Nonnodulated chickpea	Mean
N1	561	517	531	16	14	10
N2	549	527	535	18	15	13
N3	636	544	583	22	23	18
N4	617	583	589	26	27	26
SE	± 12.8 (17.2)[3]		± 4.0	±2.5(1.4)[3]		±2.3

[1] The data for total N are based on four and not five years.

[2] Nodulated and nonnodulated chickpea were subplots and four N levels NI, N2, N3, and N4 were the main plots for five years (1990/91 to 1994/95). No N was applied to chickpea. Preceding sorghum (rainy season) received 0 (NI), 40 (N2), 80 (N3), and 160 (N4) kg N ha^{-1} (in two split) doses in all the five years, except in 1992 when 0 (NI), 80 (N2), 160 (N3), and 320 (N4) kg N ha^{-1} was applied. Twenty kg P ha^{-1} as single super phosphate was applied to chickpea at sowing in 1990/91, 1992/93, and 1994/95. Data are from non replicated demonstration plots (8 m x 7.2 m); year was used as replication for statistical analysis.

[3] SE to compare means within an N-level.

Table 4: Available nitrogen (N) concentration in soil in farmers' rice-wheat fields in Punjab, India.

Available N (mg kg^{-1} soil)[1]		Before sowing	
Mean (n)[2]	Range	of crop (year)	Reference
96 (23)	38-224	NA[3]	Grewal and Kanwar (1967)
67 (7)	22-106	Wheat (1976/77)	Dhillon et al. (1978)
61 (5)	50-67	Rice (NA)	Chand et al. (1984)
65 (20)[4]	36-150	Rice (NA)	Gupta et al. (1988)

[1] Available N concentration by the alkaline permanganate method of Subbiah and Asija (1956). In the references of Dhillon et al. (1978) and Chand et al. (1984), data was available as kg N ha^{-1} in surface (15-20 cm) soil. Concentration of N was calculated assuming that one ha of top 15 cm soil weighs 2242760 kg.

[2] n = number of farmers' fields observed is given in parentheses.

[3] NA = information not available.

[4] Mineral-N concentration by the method of Bremner (1965) for the same samples was 27.3 (range 8-46) mg kg^{-1} soil.

With assured water input, nitrogenous fertilizer use has increased over the years. Global production of nitrogenous fertilizers has increased from

12.9 million t in 1961 to 86.7 million t in 1995 (a 5.7-fold increase over 1961) (Table 1). In 1995, Asia consumed 44.9 million t of nitrogenous fertilizers while it produced only 39.9 million t. Since 1961, Asia has been an importer of at least 5 million t nitrogenous fertilizers annually. Subsidies on agricultural inputs (including fertilizers) available in many countries would have significantly contributed to enhancing their use even by small farmers. In a survey in 1996 it was noted that of the 231 farmers interviewed in Punjab (India), 66% area cultivated by them received higher than the recommended dose of N (120 kg N ha^{-1}) for rice and 37% for wheat (Sidhu et al. 1998). Such an application of more than the recommended dose of nitrogenous fertilizer in intensive cropping of rice-wheat system in the states of Haryana and Punjab has resulted in increased nitrate concentrations in groundwater over a 10-year period (Abrol and Gill 1995). Further, such a high use of N annually seems to be making soils unfit for harnessing BNF by legumes. Alternatively, it may need legume varieties whose BNF system can tolerate high concentration of soil N. Biological nitrogen fixation by legumes is beneficial to the system in several ways. It reduces fertilizer costs, runoff, and leaching. The organic N in legume residues acts as slow release N fertilizer to increase N-use efficiency. Legumes also add organic matter besides N. The latter is particularly important as organic matter content is low in IGP.

FACTORS AFFECTING BNF BY LEGUMES

Biological nitrogen fixation can effectively supply N to a legume provided there is no other factor limiting plant growth except N supply (Bohlool et al. 1992). The interaction of *Rhizobium,* host plant, and environment determines the proportion of legume N derived from the atmosphere. Though the determinants of BNF by legumes have been dealt with in other recent papers (Bohlool et al. 1992; George et al. 1992; Peoples and Craswell 1992), the present coverage will be limited to general principles (discussed in the preceding section) that are relevant to legume production in rice- and/or wheat-based cropping systems.

Rhizobium

Indigenous soil rhizobia may not be as effective as inoculant strains; however, they can compete well with introduced strains for nodule formation. *Rhizobium* inoculation in such a situation can result in no apparent benefit in N_2-fixation (Dowling and Broughton 1986). Further, Thies et al. (1991a) reported that a response to inoculation may not be obtained if the number of native effective soil rhizobia exceeds 50 cells g^{-1} soil.

Soil flooding during the rainy (rice-growing) season adversely affects rhizobial numbers. Kumar Rao et al. (1982) observed that the population of cowpea *(Vigna unguiculata)* group rhizobia was very low (<100 g^{-1} soil) in paddy fields. It appears that continuous cultivation of paddy has an adverse effect on their survival because there were more rhizobia in a field under paddy for two years (i.e., two consecutive rainy seasons) than in one under paddy for 6.5 years. Similarly, an approximately 100-fold decrease in chickpea rhizobial density was observed in flooded soil when rice followed chickpea (Rupela et al. 1987). Such a decline in *Rhizobium* numbers may necessitate regular inoculation of chickpea or pigeonpea or other legumes, when they are grown after rice to ensure establishment of effective symbioses Ladha et al. (1989a) reported survival in high numbers of rhizobia of the aquatic legume *Sesbania rostrata* in flooded rice rhizosphere. Rain splash and flooding often promote stem nodulation by *S. rostrata;* however, stem inoculation is probably beneficial under dry conditions (Ladha et al. 1992). However, there is little information on the status of native *Rhizobium* populations in soils of the rice-wheat cropping systems of IGP. Such information is required to predict likely responses to *Rhizobium* inoculation. Thies et al. (1991b) developed a model that could predict inoculation requirements of legumes for various environments based on native soil rhizobia and soil N mineralization potential. The model input variables can be obtained through soil analysis before planting of the legume.

Average nodulation (rating '3' on a '1' to '5' scale) of chickpea (Rupela 1990) after paddy has been observed in Bangladesh and Myanmar. It seems that some rhizobia can acclimatize to paddy conditions and survive in large numbers after paddy (authors' observation during field visits). Identification of such strains of different rhizobia and their use as inoculants for relevant legumes should help enhance BNF by legume(s) in rice-legume cropping systems.

Host legume

The potential BNF capacity of a legume is the aggregate of the per-day deficits in mineral N uptake during the legume growth cycle (George and Singleton 1992). Therefore the higher the N yield potential of a legume for a given growth and soil N supply, the higher would be its proportion and amount of N derived from BNF. Large genotypic variation for BNF traits such as nodule number, nodule mass, and acetylene reductase activity (ARA) has been reported for chickpea (Rupela 1994), groundnut (Nambiar et al. 1988), pigeonpea (Kumar Rao 1990), soybean (Wacek and Brill 1976), and cowpea (Zari et al. 1978). Using ^{15}N isotope-based methods, differences among cultivars have been detected in soybean (Hardarson et al. 1989), groundnut (Giller et al. 1987), mung bean and blackgram (Peoples and Craswell 1992), pigeonpea (J.V.D.K Kumar Rao et al., unpublished), and chickpea (O.P. Rupela, unpublished). However, limited or no efforts have been made to use this variability in breeding for improved BNF in many of these legumes. Intracultivaral variability for nodulation (low, high, and non-nod) was observed in chickpea (Rupela 1994), pigeonpea (Rupela and Johansen 1995a), and groundnut (Venkateswarlu 1997). This is perhaps due to the absence of any natural selection pressure for nodulation or BNF during development of a cultivar allowing the different nodulation types to continue to exist within a cultivar up to release stage. This hypothesis is supported by the fact that during a screening for high nodulating plants at high mineral N in soil, desired plants were observed in 85 out of 90 advanced breeding lines of chickpea (Rupela 1994).

Environment

Temperature

In parts of the tropics the temperature of the surface soil can occasionally reach 65-70°C and at 5 cm depth it is about 50°C (Dudeja and Khurana 1989). The excessive soil temperatures can kill a majority of rhizobia in the surface layers of soil, although some rhizobia can survive for some period at 70° C in dry soil (Marshall 1964). High temperatures can prevent nodulation or can inhibit the activity of N_2-fixation (if nodulation does occur) in legumes. In chickpea, root temperatures of 30°C and above are known to adversely affect the infection and N_2-fixation (Dart et al. 1975; ICRISAT 1983). Chickpea plants exposed to a continuous root temperature of 33°C did not form nodules. Exposure to cycles of favorable and unfavorable temperatures indicated that the nitrogenase activity failed to restart when plant roots were once subjected to 35°C (Dart et al. 1975).

In a glasshouse study at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, root temperature above 30°C resulted in decreased N_2-fixation and poor plant growth. Soil temperature for chickpea roots in pots containing Vertisol with high rhizobial population was maintained by immersing the pots in water baths at 25°C, 30°C, 32°C, and 35°C for eight hours (from 0800 h to 1600 h) a day, for 40 days beginning six days after sowing (ICRISAT 1983). The number of nodules per pot did not differ significantly for the first three temperature regimes, suggesting that the processes involved at the molecular level in the formation of nodules were not affected adversely. However, nodule mass, ARA, and plant growth were significantly reduced at 35°C.

In pigeonpea, it was found that nodulated roots incubated at 26°C gave a higher ARA than nodulated roots incubated at either 20°C or 38°C (Kumar Rao 1990). Eaglesham and Ayanaba (1984) reported the possibility of selecting high temperature tolerant *Rhizobium* isolates that could retain their effectiveness in N_2-fixation in symbiosis with cowpea when the day temperatures were kept above 40°C.

An analysis of the potential areas in IGP for including legumes in rice- and wheat-based cropping systems may indicate the temperature regimes (both favorable and unfavorable) for growing legumes. From such data it would be possible to assess the need for selecting *legume-Rhizobium* symbioses adapted to unfavorable temperatures.

Moisture

Because legumes are generally grown either rainfed or on residual soil water, both water deficit and waterlogging (sometimes) are important factors which influence total legume N derived from fixation. Soil moisture deficiency has a pronounced effect on N_2-fixation because nodule initiation, nodule growth, and nodule activity are all more sensitive to water stress than are general root and shoot metabolism (Gallacher and Sprent 1978; Weisz et al. 1985; Rupela and Saxena 1987; Kirda et al. 1989; Kumar Rao 1990; Nambiar 1990).

Excess soil moisture may also restrict N_2-fixation by reducing the supply of oxygen to nodulated roots or indirectly by reducing availability of photosynthates. Waterlogging in pigeonpea significantly reduced root activity, nodulation, and nitrogenase activity (Matsunaga et al. 1996). It also resulted in root sloughing. With the return of favorable soil moisture conditions (after waterlogging), the surviving plants established new roots that hosted abundant nodules.

Post-rainy season legumes such as chickpea, generally sown on residual soil moisture after the rainy season crop (rice in the IGP), may face water deficit conditions. Rupela and Khurana (1997) reported that soil moisture needed for good nodulation was slightly more than that for good emergence (19%) on a Vertisol. In examining the reason of inoculation failure it was apparent that chickpea had no problem of emergence but failed to form nodules even when an abundant rhizobial population was present. Thus poor nodulation reported on farmers' fields by several researchers may not be due to lack of rhizobia but may be due to sub-optimal soil moisture conditions.

Salinity

Salinity is an emerging constraint to sustained rice and wheat productivity and profitability (FAO 1993). Salinity and sodicity in irrigation water are indeed an increasing problem in Pakistan and in northwestern India (Woodhead et al. 1995). Salinity is a natural phenomenon due to accumulation of salts in the top soil caused by using poor quality (salty) water for irrigation or as a result of poorly-managed irrigation. The pH of sodic soils is usually above 8.5 and can result in reduced availability of phosphorus (P), iron (Fe), zinc (Zn), and manganese (Mn) for plant growth.

Among various crop plants tested, legumes have generally been found to be relatively sensitive to soil salinity (Maas and Hoffman 1977) thus making it more difficult to introduce legumes in saline areas of IGP. However, there is considerable variation in degree of resistance across legume species; for example, *Sesbania* spp. shows a high level of resistance (Keating and Fisher 1985). Legumes such as chickpea and lentil *(Lens culinaris)* grown on residual moisture in a post-rainy season, are particularly prone to salinity damage as salts are progressively concentrated in the soil solution and precipitated towards the soil surface as the soil dries out.

It is generally known that rhizobia can tolerate a higher level of salinity than the host legume (Wilson 1970). The process of root hair infection of legumes is particularly sensitive to salinity stress (Sprent 1984) resulting in reduced nodulation of legumes such as pigeonpea (Subba Rao et al. 1990) and soybean (Singleton and Bohlool 1984). Subba Rao et al. (1990) reported variation among *Rhizobium* strains in their ability to nodulate and fix N_2 with pigeonpea under saline conditions and noted the possibility of selecting effective pigeonpea-*Rhizobium* symbioses for saline soils. Nodulation of groundnut is relatively insensitive to salinity (Sprent 1984).

Insect Damage

Damage to plants by pests and diseases will have deleterious effects on plant growth and thus indirectly on N_2-fixation. Specific damage to root nodules is caused by insects in soil. Two insects, *Sitona* spp. and *Rivellia* spp., are known to damage legume nodules (Gibson 1977). In pigeonpea extensive nodule damage by a Dipteran larva *(Rivellia angulata)* in farmer's fields were reported by Sithanantham et al. (1981). It resulted in significant loss in nodule mass, ARA, and seed yield (Kumar Rao and Sithanantham 1989). Sithanantham and Rupela (1986) reported nodule damage by *Metopina* spp. [subsequently identified as *Metopina ciceri* by Disney (1988)] in chickpea. So far, the activity of this insect is generally seen at locations below 20° N latitude in India. Very little information is available on the occurrence of legume nodule damage by insects and its impact on N_2-fixation with particular reference to IGP. Studies are therefore needed to collect this information and assess its importance with reference to legume inputs of fixed N_2 in rice- and wheat-based cropping systems of IGP.

Nutritional Factors

The *legume-Rhizobium* symbioses impose additional nutritional requirements apart from the minerals needed for the plant growth as a whole.

Nitrogen

Significant increases in yield of legumes in response to a basal application of 20-30 kg N ha^{-1} has been reported for several legumes (Tandon 1992). But its effect on nodulation and N_2-fixation has not been reported in those experiments. Reduced nodulation and ARA in chickpea was observed due to the residual effect of N applied to preceding sorghum *(Sorghum bicolor)* (ICRISAT 1994). It seems that the recommendation of the basal N application to legumes was based on yield response and not its effect on BNF; and also perhaps based on experiments conducted in very low-N soils. Soils in the IGP are expected to regularly receive fertilizer N applied to crops preceding legumes and may already be high in N (Table 4). In general high N levels reduce nodulation and N_2-fixation (Table 5). Under such circumstances BNF contribution from the legumes can be improved by managing soil N either through inclusion of appropriate nitrate tolerant high N_2-fixing legume crops or by appropriate management practices. The results in Table 5 also suggest the potential to select appropriate legumes for areas with high soil N without affecting their BNF contribution to the system. For example, 200 kg N ha^{-1} decreased N_2-fixation in groundnut by 19% whereas the reduction in cowpea was 44% (Yoneyama et al. 1990). However, there could be many other overriding factors, e.g., profitability, suitability, and preference for home consumption, that could determine choice of the legume by farmers of the IGP.

Table 5: Effect of nitrogen fertilizer on total N uptake, and the proportion (P-fix) and total amount of N derived from biological nitrogen fixation (BNF).

Crop	Fertilizer N (kg N ha^{-1})	Total N uptake (kg ha^{-1})	P-fix (%)	N from BNF (kg ha^{-1})	Reference
Groundnut	0	196	61	120	Yoneyama et al. (1990)
	100	210	47	99	
	200	243	42	102	
Soybean	0	89	48	43	Yoneyama et al. (1990)
	100	115	24	28	
Cowpea	0	163	77	125	Yoneyama et al. (1990)
	100	138	67	92	
	200	172	33	57	
Chickpea	0	97	81	79	Herridge et al. (1995)
	50	114	59	59	
	100	115	29	25	

A high level of soil mineral N (31.2 µg mineral N g^{-1} soil) at sowing reduced nodulation of chickpea on a Vertisol field by at least 14%, and proportion of fixed N by 63%, compared with that in the control plots (7.3 ug mineral N g^{-1} soil). In a pot trial with Alfisol, application of five levels of fertilizer N up to 200 kg N ha^{-1} equivalent much before sowing was used to simulate a range of soil mineral N concentrations at sowing (Wani et al. 1997). Of the five legume species [pigeonpea, groundnut, cowpea, soybean, and mung bean *(Vigna radiata)]* studied, mean nodule number and nodule mass plant^{-1} in groundnut, soybean, and mung bean were substantially reduced in the presence of a soil mineral N concentration of 31 µg g^{-1} soil compared with a control treatment (no fertilizer) having 23 µg N g^{-1} soil at sowing. Suppression of N_2-fixation was recorded at 43 µg N g^{-1} soil in pigeonpea, and at 66 ug N g^{-1} soil in cowpea. A direct relationship between nitrogenase activity and different soil N pools at sowing and at flowering was observed in pigeonpea, groundnut, cowpea, and soybean (R^2 = 0.56-0.80) but not in mung bean. Based on the available data, it seems that the general recommendation of applying a starter N dose of 20-30 kg ha^{-1} to legumes may not apply to rice-wheat areas.

Other Nutrients

Any factor affecting plant growth is likely to affect N_2-fixation after an effective symbiosis has been established. Nutrition of plants with minerals other than N is one such factor. It is also important because of the additional nutritional requirements of the symbiosis (Robson 1983). In the rice-wheat systems, the nutrient imbalance caused by deficiency of nutrients such as Zn, sulfur(S), Mn, and Fe was perceived as one of the factors responsible for reduced factor productivity of the cereals (FAO 1993). Srivastava et al. (1997) identified boron (B) deficiency [and to some extent molybdenum (Mo) deficiency] as a major cause of flower and pod abortion in chickpea in Chitwan, Nawalparasi, and Makwanpur districts of Nepal (an IGP country). Correcting deficiency of nutrients such as calcium (Ca), cobalt (Co), copper (Cu), Mo, P, and Zn has been shown to increase N_2-fixation. The nutrients Co, Mo, P, Fe, B, and Zn are considered to be directly involved in symbiotic N_2-fixation (O'Hara et al. 1988). In groundnut, fertilization with B, Co, Mo, and Zn in a medium calcareous soil with and without *Rhizobium* inoculation significantly increased nodulation and plant dry matter (Joshi et al. 1987). Application of Co at a rate of 500 mg cobalt nitrate kg"^{1} seed significantly increased pigeonpea grain yield (Raj 1987). Soil application of 0.45 kg Mo ha^{-1} as sodium molybdate significantly increased nodulation and grain yield of pigeonpea (Khurana and Dudeja 1981). In chickpea, soil application of different nutrients, namely, cobalt chloride @ 1 kg ha^{-1} or sodium molybdate @ 1 kg ha^{-1} or zinc sulphate @ 25 kg ha^{-1} were found to increase grain yields compared to control. However, *Rhizobiwn* inoculation along with the application of Co or Mo or Zn was found to increase grain yields significantly over the control (Namdeo and Gupta 1992).

In the real world, it is possible that many physical, chemical, and biological stresses will interact in a single field at the same or different times. Constraints during the legume production phase are very important. Likewise, factors that could influence the survival of rhizobia between cropping seasons may result in subsequent failures of symbiosis. Attempts have been made to estimate the relative importance of different environmental factors using multi-factor models (Woomer et al. 1988). However, it may not always be easy to assign relative importance to various stresses likely to be encountered in the field. But it is certainly important that we should be constantly aware of the complexity of natural environments. In conclusion, stresses such as excessive temperatures and moisture loss from soil can be reduced by improvement of the organic matter content of soils and this will also help to reduce the problems of nutrient availability. Further improvement of the general nutrition of plants for N_2-fixation must rely on better conservation and more efficient use of nutrients within cropping systems. Even then, there will be an inevitable decrease of soil nutrients, as indicated already, and so ultimately, unless we are prepared to accept ever declining yields, these will have to be replenished in the form of organic amendments (e.g., farm-yard manure or crop residues) and inorganic fertilizers.

RESIDUAL EFFECTS OF LEGUMES

Increase in cereal yields following monocropped legumes was 0.5-3 t ha^{-1}, representing 30-350% increase over yields in cereal-cereal cropping sequences

(Peoples and Craswell 1992). The fertilizer N equivalent of the residual effect of preceding legumes [pigeonpea, cowpea, groundnut, mung bean, and black gram *(Vigna mungo)]* on wheat was reported to range from 12 kg ha"^{1} to 68 kg ha^{-1} (Table 6). The fertilizer N replacement value (FRV) or fertilizer N equivalent value refers 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 does not, however, distinguish between BNF and the 'N sparing effect' which results from substitution by legumes of biologically fixed N for soil N. Therefore, FRV methodology overestimates the N contribution of legumes in a crop rotation. The FRV methodology gives variable estimates depending on the test crop used (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 (Senaratne and Hardarson 1988; Danso and Papastylianou 1992). The over estimation by FRV methodology is because it confounds the non-N rotation effect with the N contribution (through BNF or N sparing effect), and also this method assumes that use efficiency of fertilizer and legume N is similar.

Table 6: Grain yield response of rice and wheat to previous legume crops relative to a cereal-cereal cropping sequence.

Crop sequence	Increase in cereal yield (t ha^{-1})	Relative increase in yield^{1} (%)	N-fertilizer equivalence^{2} (kg N ha^{-1})
Legume-rice			
Soybean	0.80	66	NA^{3}
Mung bean	0.20	17	NA
Legume-wheat			
Pigeonpea	0.27	21	NA
Black gram	1.26	98	NA
Mung bean	0.65	51	NA
Cowpea	0.74	58	NA
Groundnut sole)	0.75	23	28
Groundnut (intercrop)	0.34	10	12
Mung bean (sole)	1.60	49	68
Mung bean (intercrop)	0.48	15	16
Cowpea (sole)	1.00	30	38
Cowpea (intercrop)	0.38	11	13

[1] Increase in yield of rice or wheat after legumes over that after cereal.

[2] Amount of inorganic nitrogen required after a non-legume crop to produce a yield of another non-legume equivalent to that produced after a legume.

[3] NA = data not available.

Source: Singh and Verma (1985); Bandyopadhyay and De (1986); Chapman and Myers (1987).

Growing legumes in rotation does improve mineral N content in soil as compared with the cultivation of non-legume crops (Rao and Singh 1991; Wani et al. 1995; Ladha et al. 1996) (Table 7); however, it does not fully explain the beneficial effects of legumes on the following crop. The non-N rotational benefit of the legumes towards yield of subsequent crops has been reported by many researchers (Cook 1988; Danso and Papastylianou 1992; Peoples and Craswell 1992).

Table 7: Some examples of the increased levels of soil nitrate detected following legume growth.

Species	Additional soil nitrate (kg N ha^{-1})[1]	Reference
Chickpea	+ 14	Herridge et al. (1995)
Mung bean	+ 26	Doughton and Mackenzie (1984)
Black gram	+ 38	Doughton and Mackenzie (1984)
Pigeonpea	+ 15	Ladha et al. (1996)
Crotalaria	+ 19	Ladha et al. (1996)
Siratro	+ 26	Ladha et al. (1996)

Calculated as the difference between the levels of soil nitrate after a legume and after a cereal crop or a period of fallow.

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 indicated in Table 6? Several factors may be involved, the relative importance of each dictated by the site, season, and crop sequence. Extra yield from a rotation can result from:

- Increased availability of nutrients other than N such as potassium (K), Ca, magnesium (Mg), Zn, S, and Fe through increased soil microbial activity, deep rooting, and root exudates (Kucey et al. 1988; Ladha et al. 1989b; Wani et al. 1991).
- Improvements in soil structure, mainly soil aggregate formation following legumes, such as after three years of alfalfa *(Medicago sativa),* clover *(Trifolium* sp.), and hairy vetch *(Vicia villosa)* mixture (Latif et al. 1992); or improvements in soil water-holding and buffering capacity with incorporation of legume residues (Buresh and De Datta 1991).
- Growth promoting substances in legume residues (Ries et al. 1977).
- Break in pest cycle. Crop rotations break the cycle of cereal pests and diseases, and phytotoxic and allelopathic effects of different crop residues (Francis et al. 1986). Crop rotation is an effective tool against certain pests, but it does not control all pests and diseases. For example, Johansen 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. Such information on the role of legumes in rice and wheat systems is required.

Factors Affecting Residual Effects of Legumes

Work at ICRISAT indicated that the beneficial residual effect of legumes is influenced not only by the genotype but also the soil type. In pigeonpea, the genotypic differences in nodulation and N_2-fixation could be reflected in the magnitude of the beneficial effect of pigeonpea on a succeeding cereal crop grown on an Alfisol (Table 8). The beneficial effect of ICP 1-6, a medium-maturing and high-nodulating pigeonpea genotype on succeeding sorghum grain yield was equivalent to about 30 kg N ha^{-1} compared to fallow treatment. With ICPL 87, a low-nodulating but high-yielding pigeonpea genotype in multiple harvests, the beneficial effect was less and equivalent to only about 5 kg N ha^{-1} (Kumar Rao 1990). It is therefore important to select genotypes for both high yield and high nodulation characters in the short-duration pigeonpea. ICPL 87 grown in a multiple harvest system on a Vertisol had a residual effect of about 20 kg N ha^{-1} on a sorghum crop grown in the following rainy season, while ICP 1-6 had a residual effect equivalent to about 40 kg N ha^{-1} (Johansen et al. 1990). The mechanism of these beneficial effects of pigeonpea on following crops needs to be elucidated so as to exploit the same to achieve greater yields of following cereals without adversely affecting the sustainability of the system. The beneficial effects of pigeonpea could be due to leaf litter that is added to soil during crop season (Kumar Rao et al. 1983) or deep rooting that might facilitate recycling of nutrients from deeper horizons. The beneficial effect of pigeonpea could be also due to an increased available P pool as a result of P acquisition from insoluble Fe phosphates through root exudates (Ae et al. 1990).

Table 8: Nodulation, acetylene-reducing activity (ARA), and residual effect of pigeonpea genotypes (differing in nodulation), on the following cereal crop grown on an Alfisol at ICRISAT Center, Patancheru, India, rainy season 1987.[1]

	Nodule no. plant^{-1}		Nodule dry mass (mg plant^{-1})		ARA (µM C_2 H_4 plant^{-1})		Residual effect on following cereal
Genotype	A	B	A	B	A	B	(equivalent to kg N ha^{-1})
ICPL 87	16	8	30	38	0.73	0.5	5
ICP 1-6	39	20	51	186	1.24	8.0	30
SE ±	3.8	± 3.7	±3.1	±31.5	±0.163	±1.88	
CV (%)	39	76	21	80	47	125	

[1] Means over irrigation levels; A = 35 days after sowing (DAS); and B = 58 DAS.

The growing season of the legume also affects the residual effect of the legume. For example, rainy season groundnut resulted in 45% more pearl millet *(Pennisetum glaucum)* grain compared to pearl millet following maize (Nambiar et al. 1982). However, if either groundnut or maize were grown in the post-rainy season no residual effect was observed on pearl millet grown in the following rainy season. Although other factors could be involved, it is possible that the observed effect of groundnut was due to leaf fall as a result of foliar diseases in the rainy season, whereas leaf fall due to foliar diseases was minimal during the post-rainy season.

In a field experiment at ICRISAT, Patancheru, a high N_2-fixing (HN) selection from the released chickpea cultivar G 130 (ICC 4948) nodulated 70% higher and had ARA activity 42% higher (mean of two different soil N levels) than its parent at 46 days after sowing. Its yield (a function of several parameters in addition to N acquisition) was only marginally higher (3.2-6.5%) than its parent and its low N_2-fixing selection (Rupela et al. 1995). But the beneficial effect of high N_2-fixing selection was visible in the following sorghum. The yield of sorghum (CSH 6) after the HN selection was higher (by 9.4%) than that grown after the parent line (Fig. 1). Performance of such selections of chickpea in rice-wheat cropping systems is being studied in a collaborative experiment between the Punjab Agricultural University, Ludhiana, India, and ICRISAT.

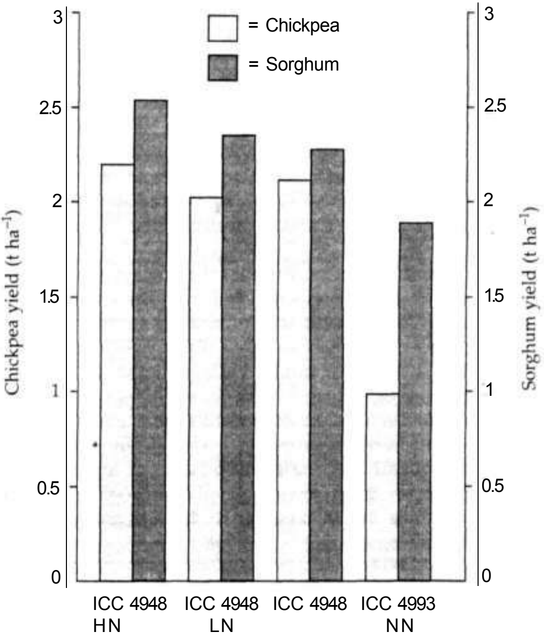

Figure 1: Yield of chickpea lines of different N_2-fixation capacities in post-rainy season 1992/93 and of sorghum grown after these in the rainy season 1993, ICRISAT, Patancheru, India (HN = high N_2-fixing chickpea line; LN = low N_2-fixing chickpea line; NN = nonnodulating chickpea line).

Source: Developed from Rupela et al. (1995).

FUTURE RESEARCH PRIORITIES

There is increasing evidence to suggest that productivity growth of rice-wheat systems in the IGP countries has slowed down. Production driving factors such as fertilizer-responsive high-yielding cultivars of cereals, increased use of fertilizers, and spread of irrigation have reached close to saturation point. Nutrient management was identified as a major issue to understand reasons for decline in rice-wheat yields by a workshop organized by the Rice-Wheat Consortium for IGP in October 1996 that reviewed many long-term soil fertility experiments set up in the region since early 1970s (Abrol et al. 1997). These experiments are focused on N, P, K, micronutrients, and organic fertilizers. Legumes are generally not included as treatments in such experiments (Abrol et al. 1997). We strongly feel that any such experiments should include legumes as one of the treatments.

Few experiments quantifying BNF have used dependable methods (e.g., ^{15}N-based). The experiments studying residual effect of legumes have largely used cereals as non-fixing controls. Non-nodulating lines of some legumes such as groundnut, pigeonpea, chickpea, pea (*Pisum sativum),* and soybean are now available and should separate BNF and non-BNF effects of legumes in relevant cropping systems. Such experiments on legumes and cropping systems of relevance to IGP need to be conducted. Researchers in some Asian countries have expressed inability to use ^{15}N methods because of inaccessibility of analytical facilities. Simple agronomic experiments involving nonnodulating legumes for quantifying BNF and their residual effects need to be examined. And where unavailable, appropriate nonnodulating lines from the legumes of interest need to be developed (Rupela and Johansen 1995a).

Legumes still remain part of rice-wheat cropping systems (Table 1) even though their area has significantly reduced over the years. On-farm BNF quantification (using ^{15}N or N-difference method) studies to identify/confirm factors that enhance contribution from BNF by legumes, in a cropping system perspective, should be conducted. The major thrust of these studies should be to devise strategies to maximize the net N input of a legume crop in the agro-ecosystem and the net N benefit to the following non-legume crop in rice- and wheat-based cropping systems. Simulation models are probably useful in quantifying the likely benefits of legumes in terms of saving of N fertilizer for the following cereal in rice-wheat systems in the IGP. Therefore, attempts should be made to develop and evaluate the relevant simulation models.

REFERENCES

Abrol, IP., Bronson, K.F., Duxbury, J.M., and Gupta, R.K. 1997. Long-term soil fertility experiments in rice-wheat cropping systems: proceedings of a workshop, 15-18 Oct 1996, Surajkund, Haryana, India. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 23 pp.

Abrol, I.P., and Gill, M.S. 1995. Susta inability of rice-wheat system in India. In: *Sustainahility of rice-wheat production systems in Asia* (R.S. Paroda, T. Woodhead, and R.B. Singh, eds.). New Delhi, India: Oxford & IBH Publishing Co. Pvt. Ltd. pp. 172-183.

Ae, N., Arihara, J., Okada, K., Yoshihara, T. and Johansen, C. 1990. Phosphorus uptake by pigeonpea and its role in cropping systems of the Indian subcontinent. *Science* 248:477-480.

Bandyopadhyay, S.K., and De, R. 1986. Nitrogen relationships and residual effects of intercropping system with legumes. *Journal of Agricultural Science, Cambridge* 107:629-632.

Blevins, R.L., Herbek, J.H., and Fyre, W.W. 1990. Legume cover crops as a nitrogen source for no-till corn and grain sorghum. *Agronomy Journal* 82:769-772.

Bohlool, B.B., Ladha, J.K., Garrity, D.P., and George, T. 1992. Biological nitrogen fixation for sustainable agriculture: A perspective. *Plant and Soil* 141:1-11.

Bremner, J.M. 1965. Inorganic forms of nitrogen. In: *Methods of Soil Analysis* (Black, C.A., ed.). Madison, Wisconsin, USA : American Society of Agronomy, pp. 1179-1237.

Buresh, R.J., and De Datta, S.K. 1991. Nitrogen dynamics and management in rice-legume cropping systems. *Advances in Agronomy* 45:1-59.

CGIAR/TAC (Consultative Group on International Agricultural Research/Technical Advisory Committee). 1988. Sustainable agriculture production: Implications for international agricultural research. Rome, Italy: Technical Advisory Committee Secretariat, Food and Agriculture Organization of the United Nations.

Chand, M., Vig, A.C., Dev, G., and Sidhu, A.S. 1984. Evaluation of fertilizer recommendation based on soil tests for targetting rice yield on cultivators' fields. *Journal of Research of Punjab Agricultural University* 21(l):20-28.

Chapman, A.L., and Myers, R.J.K. 1987. Nitrogen contributed by grain legumes to rice grown in rotation on the Cununurra soils *of* the Ord irrigation area, Western Australia. *Australian Journal of Experimental Agriculture* 24:155-163.

Cook, R.J. 1988. Biological control and holistic plant-health care in agriculture. *American Journal of Alternative Agriculture* 3:51-62.

Danso, S.K.A., and Papastylianou, I. 1992. Evaluation of the nitrogen contribution of legumes to subsequent cereals. *Journal of Agricultural Science, Cambridge* 119:13-18.

Dart, P.J., Islam, R., and Eaglesham, A. 1975. The root nodule symbiosis of chickpea and pigeonpea. In: *International Workshop on Grain legumes,* 13-16 Jan 1975, ICRISAT, India. Patancheru 502 324, Andhra Pradesh, India : International Crops Research Institute for the Semi-Arid Tropics, pp. 63-83.

Dhillon, N.S., Sidhu, A.S., and Dev, G. 1978. Targetting of wheat yields based on soil test values. *Fertilizer Technology* 15:57-58.

Disney, R.H.L. 1988. A remarkable new species of scuttle fly (Diptera: Phoridae) whose larvae infest chickpea root nodules in India. *Journal of Natural History* 22:611-616.

Doughton, J.A., and Mackenzie, J. 1984. Comparative effects of black and green gram (mung beans) and grain sorghum on soil mineral nitrogen and subsequent grain sorghum yields on the eastern Darling Downs. *Australian Journal of Experimental Agriculture and Animal Husbandry* 24:244-249.

Dowling, D.N., and Broughton, W.J. 1986. Competition for nodulation of legumes. *Annual Review of Microbiology* 40:131-157.

Dudeja, S.S., and Khurana, A.L. 1989. Persistence of *Bradyrhizobium* sp. *(Cajanus)* in a sandy loam. *Soil Biology and Biochemistry* 21:709-713.

Eaglesham, A.R.J., and Ayanaba, A. 1984. Tropical stress ecology of rhizobia, root nodulation and legume nitrogen fixation. In: *Current developments in biological nitrogen fixation* (N.S., Subba Rao, ed.). New Delhi, India: Oxford & IBH Publishing Co. Pvt. Ltd. pp. 1-35.

FAO (Food and Agriculture Organization of the United Nations). 1993. Report of the regional expert consultation on the sustainahility of the rice-wheat production system in different agro-ecological settings in Asia, 6-9 July 1993. Regional Office for Asia and the Pacific (RAPA) Report: 1993/20. Bangkok, Thailand: RAPA, FAO. 40 pp.

FAO (Food and Agriculture Organization of the United Nations). 1997. website: http://apps.fao.org.

Francis, C.A., Harwood, R.R., and Parr, J.F. 1986. The potential for regenerative agriculture in the developing world. *American Journal of Alternative Agriculture* 1:65-74.

Gallacher, A.E., and Sprent, J.I. 1978. The effect of different water regimes on growth and nodule development of greenhouse-grown *Vicia faba. Journal of Experimental Botany* 29:413-423.

George, T., Ladha, J.K., Buresh, R.J., and Garrity, D.P. 1992. Managing native and legume fixed nitrogen in low-land rice-based cropping systems. *Plant and Soil* 141:69-91.

George, T., and Singleton, P.W. 1992. Nitrogen assimilation traits and dinitrogen fixation in soybean and common bean. *Agronomy Journal* 84:1020-1028.

Gibson, A.H. 1977. The influence of the environment and managerial practices on the legume *Rhizobium* symbiosis. In: *A treatise on dinitrogen fixation—Section IV: Agronomy and ecology* (R.W.F. Hardy, and A.H. Gibson, eds.) New York, USA: John Wiley and Sons, pp.393-450.

Giller, K.E., Nambiar, P.T.C., Srinivasa Rao, B., Dart., P.J., and Day, J.M. 1987. A comparison of nitrogen fixation in genotypes of groundnut *{Arachis hypogaea* L.) using ^{15}N-isotope dilution. *Biology and Fertility of Soils* 5:23-25.

Government of India. 1970. Estimates of area and production of principal crops in India 1954-55 to 1964-65 (Foodgrain crops). New Delhi, India: Directorate of Economics and Statistics, Ministry of Food, Agriculture, Community Development and Cooperation. 568 pp.

Government of India. 1971. Indian agricultural statistics. New Delhi, India: Directorate of Economics and Statistics, Ministry of Food, Agriculture, Community Development and Cooperation. 110 pp.

Government of India. 1993. Area and production of principal crops in India. New Delhi, India: Directorate of Economics and Statistics, Department of Agriculture and Cooperation, Ministry of Agriculture. 359 pp.

Government of India. 1994. Indian agricultural statistics. New Delhi, India: Directorate of Economics and Statistics, Department of Agriculture and Cooperation, Ministry of Agriculture. 496 pp.

Grewal, J.S., and Kanwar, J.S. 1967. Forms of nitrogen in Punjab soils. *Journal of Research of Punjab Agricultural University* 4:477-480.

Gupta, R.K., Dev, G., and Dhillon, N.S. 1988. Evaluation of different soil test methods of nitrogen for rice growing soils of Punjab. *Journal of Research of Punjab Agricultural University* 25(2):193-200.

Hardarson, G., Golbs, M., and Danso, S.K.A. 1989. Nitrogen fixation in soybean *(Glycine max* L. Merill) as affected by nodulation patterns. *Soil Biology and Biochemistry* 21:783-787.

Herridge, D.F., Marcellos, H., Felton, W.L., Turner, G.L., and Peoples, M.B. 1995. Chickpea increases soil N-fertility in cereal systems through nitrate sparing and N_2-fixation. *Soil Biology and Biochemistry* 27:545-551.

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). 1983. Biological nitrogen fixation. In: *1CRISAT Annual report 1982.* Patancheru 502 324, Andhra Pradesh, India: ICRISAT. pp. 115-118.

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). 1994. Nitrogen nutrition. In: *Legumes Program, ICRISAT Annual report 1993.* Patancheru 502 324, Andhra Pradesh, India: ICRISAT. pp. 28-30.

Johansen, C, Kumar Rao, J.V.D.K., Rupela, O.P., and Rego, T.J. 1990. Contributions of pigeonpea to the nitrogen economy of different cropping systems. In: *International Symposium on Natural Resources Management for a Sustainable Agriculture,* 6-10 Feb 1990, New Delhi, India. Abstracts, Vol I. New Delhi, India: Indian Society of Agronomy, p. 259.

Johansen, T.B., Turpin, F.T., Schreiber, M.M., and Griffith, D.R. 1984. Effects of crop rotation, tillage, and weed management systems on black cutworm (Lepidoptera: Noctuidae) infestations in corn. *Journal of Economic Entomology* 77:919.

Joshi, P.K., Bhat, D.M., and Kulkarni, J.H. 1987. Groundnut root nodulation as affected by micronutrients application and *Rhizobium* inoculation. *International Journal of Tropical Agriculture* 5:199-202.

Keating, B.A., and Fisher, M.J. 1985. Comparative tolerance of tropical grain legumes to salinity. *Australian Journal of Agricultural Research* 36:373-383.

Khurana, A.L., and Dudeja, S.S. 1981. Field populations of rhizobia and response to inoculation, molybdenum and nitrogen fertilizer in pigeonpea. In: *Proceedings of the International Workshop on Pigeonpeas,* 15-19 Dec 1980, ICRISAT Center, India. Vol. 2. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics, pp. 381-386.

Kirda, C, Danso, S.K.A., and Zapata, F. 1989. Temporal water stress effects on nodulation, nitrogen accumulation and growth of soybean. *Plant and Soil* 120:49-55.

Kucey, R.M.N., Chaiwanakupt, P., Arayangkool, T., Sritwongse, P., Siripaibool, C, Wadisirisuk, P., and Boonkerd, N. 1988. Nitrogen fixation (^{15}N dilution) with soybeans under Thai field conditions. II. Effect of herbicides and water application schedule. *Plant and Soil* 108:87-92.

Kumar Rao, J.V.D.K. 1990. Pigeonpea: Nitrogen fixation. In: *The pigeonpea* (Y.L. Nene, S.D. Hall, and V.K. Sheila, eds.). Wallingford UK.: CAB International, pp. 233-242.

Kumar Rao, J.V.D.K., Dart, P.J., and Sastry, P.V.S.S. 1983. Residual effect of pigeonpea on yield and nitrogen response of maize. *Experimental Agriculture* 19:131-141.

Kumar Rao, J.V.D.K., Dart, P.J., and Usha Kiran, M. 1982. Cowpea-group *Rhizobium* in soils of the semi-arid tropics. In: *Biological nitrogen fixation technology for tropical agriculture:* papers presented at a Workshop, 9-13 Mar 1981, Cali, Colombia (P.H. Graham, and S.C. Harris, eds.). Cali, Colombia: Centro Internacional de Agricultura Tropical. pp. 291-295.

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

Ladha, J.K., Garcia, M., Miyan, S., Padre, A.T., and Watnabe, 1.1989a. Survival of *Azorhizobium caulinodans* in the soil and rhizosphere of wetland rice under *Sesbania rostrata-rice* rotation. *Applied and Environmental Microbiology* 55:454-460.

Ladha, J.K., Kundu, D.K., Coppenolle, M.G.A., Peoples, M.B., Carangal,V.R., and Dart, P.J. 1996. Legume productivity and soil nitrogen dynamics in lowland rice-based cropping systems. *Soil Science Society of America Journal* 60:183-192.

Ladha, J.K., Pareek, R.P., and Becker, M. 1992. Stem-nodulating *legume-Rhizobium* symbiosis and its agronomic use in lowland rice. *Advances in Soil Science* 20:148-192.

Ladha, J.K., Tirol-Padre, A., Runzalan, G.C., Garcia, M., and Watanabe, I. 1989b. Effect of inorganic N and organic fertilizers on nitrogen fixing (acetylene-reduction) activity associated with wetland rice plants. In: *Nitrogen fixation in non-legumes.* (F.A. Skinmer, R.M. Boddey, and I. Fendrik, eds.). Dordrecht, Netherlands: Kluwer Academic Publishers. pp. 263-272.

Latif, M.A., Mehuys, G.R., Mackenzie, A.F., Ali, I., and Faris, M.A. 1992. Effects of legumes on soil physical quality in a maize crop. *Plant and Soil* 140:15-23.

Maas, E.V., and Hoffman, G.J. 1977. Crop salt tolerance—current assessment. *Journal of Irrigation and Drainage Division,* Proceedings of American Society of Civil Engineers 103:115-134.

Marshall, K.C. 1964. Survival of root nodule bacteria in dry soils exposed to high temperatures. *Australian Journal of Agricultural Research* 15:273-281.

Matsunaga, R., Ito, O., Tobita, S., Rao, T.P., and Johansen, C. 1996. Effects of transient waterlogging and nitrogen top dressing on the shoot and root growth of short-duration pigeonpea. In: *Dynamics of roots and nitrogen in cropping systems of the semi-arid tropics* (O. Ito, C. Johansen, J.J. Adu-Gyamfi, K. Katayama, J.V.D.K. Kumar Rao, and T.J. Rego, eds.). Tsukuba, Ibaraki 305, Japan: Japan International Research Center for Agricultural Sciences, pp. 273-283.

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

Nambiar, P.T.C, Rao, M.R., Reddy, M.S., Floyd, C, Dart, P.J., and Willey, R.W. 1982. Nitrogen fixation by groundnut *(Arachis hypogaea)* in intercropped and rotational systems. In: *Biological nitrogen fixation technology for tropical agriculture:* papers presented at a Workshop, 9-13 Mar 1981, Cali, Colombia (P.H. Graham, and S.C. Harris, eds.). Cali, Colombia: Centro International de Agricultura Tropical, pp. 647-652.

Nambiar, P.T.C., Rupela, O.P., and Kumar Rao, J.V.D.K. 1988. Modulation and nitrogen fixation in groundnut *(Arachis hypogaea* L.), chickpea *(Cicer arietinum)* and pigeonpea *(Cajanus cajan* L. Millsp.). In: *Biological nitrogen fixation: Recent developments* (N.S. Subba Rao, ed.). New Delhi, India: Oxford & IBH Publishing Co. Pvt. Ltd. pp. 53-70.

Namdeo, S.L., and Gupta, S.C. 1992. Responses of pulses to microbial inoculants—A review of the work done at Sehore (Madhya Pradesh). In: *National Seminar on Organic Farming.* Jabalpur, Madhya Pradesh, India: Jawaharlal Nehru Krishi Vishwa Vidyalaya. pp. 150-161.

O'Hara, G.W., Boonkerd, N., and Dilworth, M.J. 1988. Mineral constraints to nitrogen fixation. *Plant and Soil* 108:93-110.

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

Prasad, R., Sharma, S.N., Singh, S., and Prasad, M. 1990. Nitrogen management. In: *Soil fertility and fertilizer use.* Vol IV. Nutrient management and supply system for sustaining agriculture in 1990's (Virendra Kumar, G.C. Shrotriya, and S.V. Kaore, eds.). New Delhi, India: Indian Farmers Fertiliser Cooperative Ltd. pp. 41-51.

Raj, A.S. 1987. Cobalt nutrition of pigeonpea and peanut in relation to growth and yield. *Journal of Plant Nutrition* 10:2137-2145.

Rao, C.C.S., and Singh, K.D. 1991. Effect of residual mung bean *(Vigna radiata* L. Wilczek) after maize-wheat sequence on soil available nitrogen, phosphorus and potassium. *Legume Research* 14:125-130.

Ries, S.K., Sweeley, V.W.C.C., and Leavitt, R.R. 1977. Tricontinol: A new naturally occurring growth regulator. *Science* 195:1339-1341.

Robson, A.D. 1983. Mineral nutration. In: *Nitrogen fixation.* Vol III. Legumes (W.J. Broughton, ed.). Oxford, UK: Clarendon Press, pp. 36-55.

Rupela, O.P., 1990. A visual rating system for nodulation of chickpea. *International Chickpea Newsletter* 22:22-25.

Rupela, O.P. 1994. Screening for intracultivaral variability for nodulation of chickpea and pigeonpea. In: *Linking biological nitrogen fixation research in Asia: Report of a meeting of the Asia Working Group on Biological Nitrogen Fixation in Legumes* (O.P. Rupela, J.V.D.K. Kumar Rao, C. Johansen, and S.P. Wani, eds.) Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics, pp. 75-83.

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

Rupela, O.P., and Johansen, C. 1995b. High mineral-N tolerant N_2-fixation in legumes - Its relevance and prospects for development. In: *Improving soil management for intensive cropping in the tropics and sub-tropics:* proceedings of the Inter-Congress Conference of Commission IV, International Society of Soil Science, 1-3 Dec 1992, Dhaka, Bangladesh (M.S. Hussain, S.M. Imamul Huq, M. Anwar Iqbal, and T.H. Khan, eds.). Dhaka, Bangladesh: Bangladesh Agricultural Research Council, pp. 395-402.

Rupela, O.P., and Khurana, A.L. 1997. Limitations and potentials in improving biological nitrogen fixation of pulses—A critical assessment. In: *Recent advances in pulses research* (A.N. Asthana, and Masood Ali, eds.). Kanpur, India: Indian Society of Pulses Research and Development, Indian Institute of Pulses Research, pp. 525-542.

Rupela, O.P., and Saxena, M.C. 1987. Nodulation and nitrogen fixation in chickpea. In: *The chickpea* (M.C. Saxena, and K.B. Singh, eds.). Wallingford, Oxfordshire, UK: CAB International, pp. 191-206.

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

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

Senaratne, R., and Hardarson, G. 1988. Estimation of residual N effect of faba bean and pea on two succeeding cereals using ^{15}N methodology. *Plant and Soil* 110:81-91.

Sidhu, B.S., Rupela, O.P., Beri, V., and Joshi, P.K. 1998. Sustainability implications of burning rice- and wheat-straw in Punjab. *Economic and Political Weekly.* 33(39): A163-168.

Singh, R.B., and Paroda, R.S. 1995. Sustainability and productivity of rice-wheat systems in the Asia-Pacific region: Research and technology development needs. In: *Sustainability of rice-wheat production systems in Asia* (R.S. Paroda, T. Woodhead, and R.B. Singh, eds.) New Delhi, India: Oxford & IBH Publishing Co. Pvt. Ltd. pp. 1-36.

Singh, S.B., and Verma, B.S. 1985. Effect of kharif grain legumes on nitrogen economy in succeeding crop of wheat. *Indian Journal of Agronomy* 30:397-400.

Singleton, P.W., and Bohlool, B.B. 1984. Effect of salinity on nodule formation by soybean. *Plant Physiology* 74:72-76.

Sithanantham, S., Kumar Rao, J.V.D.K., Reed, W., and Dart, P.J. 1981. Studies on nodule damage in pigeonpea. In: *Proceedings of the International Workshop on Pigeonpeas,* 15-19 Dec 1980. ICRISAT Center, India. Vol 2. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics, pp. 409-415.

Sithanantham, S., and Rupela, O.P. 1986. Insect damage to chickpea root nodules. *International Chickpea Newsletter* 15:12-13.

Sprent, J.I. 1984. Effects of drought and salinity on heterotrophic nitrogen fixing bacteria and on infection of legumes by rhizobia. In: *Advances in nitrogen fixation research* (C. Veeger, and W.E. Newton, eds.) The Hague, Netherlands. Martinus Nijhoff/Dr W. Junk. pp. 295-302.

Srivastava, S.P., Yadav, C.R., Rego, T.J., Johansen, C, and Saxena, N.P. 1997. Diagnosis and alleviation of boron deficiency causing flower and pod abortion in chickpea *(Cicer arietinum* L.) in Nepal. In: *Boron in soils and plants* (R.W. Bell, and B. Rerkasem, eds. Dordrecht, Netherlands: Kluwer Academic Publishers, pp. 95-99.

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

Subba Rao, G.V., Johansen, C, Kumar Rao, J.V.D.K., and Jana, M.K. 1990. Response of the pigeonpea-*Rhizobium* symbiosis to salinity stress: Variation among *Rhizobium* strains in symbiotic ability. *Biology and Fertility of Soils* 9:49-53.

Subbiah, B.V., and Asija, G.L. 1956. A rapid procedure for assessment of available nitrogen in soils. Current Science 25:259-260.

Tandon, H.L.S. 1992. Fertilizers and their integration with organic and biofertilizers. In: *Fertilizers, organic manures, recylable wastes, and biofertilizers* (H.L.S. Tandon, ed.) New Delhi, India: Fertilizer Development and Consultation Organization, pp. 12-36.

Thies, J.E., Singleton, P.W., and Bohlool, B.B. 1991a. Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. *Applied and Environmental Microbiology* 57:19-28.

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

Venkateswarlu, B. 1997. Development of high nitrogen-fixing selections in groundnut—Strategy and progress. In: *Extending nitrogen fixation research to farmers' fields:* proceedings of an International Workshop on Managing Legme Nitrogen Fixation in the Cropping Systems of Asia, 20-24 Aug 1996, ICRISAT, India (O.P. Rupela, C. Johansen, and D.F. Herridge, eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics, pp. 289-296.

Wacek, T.J., and Brill, W.J. 1976. Simple, rapid assay for screening nitrogen-fixing ability in soybean. *Crop Science* 16:519-523.

Wani, S.P., Mc Gill, W.B., and Tewari, J.P. 1991. Mycorrhizal and common root infection, and nutrient accumulation in barley grown on Breton loam using N from biological fixation or fertilizer. *Biology and Fertility of Soils* 12:46-54.

Wani, S.P., Rupela, O.P., and Lee, K.K. 1995. Sustainable agriculture in the semi-arid tropics through biological nitrogen fixation in grain legumes. *Plant and Soil* 174:29-49.

Wani, S.P., Rupela, O.P., and Lee, K.K. 1997. Soil mineral nitrogen concentration and its influence on biological nitrogen fixation of grain legumes. In: *Extending nitrogen fixation research to farmers' fields:* proceedings *of* an International Workshop on Managing Legume Nitrogen Fixation in the Cropping Systems of Asia, 20-24 Aug 1996, ICRISAT, India (O.P. Rupela, C. Johaasen, and D.F. Herridge, eds.). Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics, pp. 183-198.

Weisz, P.R., Denison, R.F., and Sinclair, T.R. 1985. Response to drought stress of nitrogen fixation (acetylene reduction) rates by field-grown soybeaas. *Plant Physiology* 78:523-530.

Wilson, J.R., 1970. Response to salinity in *Glycine.* VI. Some effects of a range of short-term salt stresses on the growth, nodulation and nitrogen fixation of *Glycine wightii* (formerly *javanica). Australian Journal of Agricultural Research* 21:571-582.

Woodhead, T., Huke, R., and Huke, *E.* 1995. Areas, locations and ongoing collaborative research for the rice-wheat systems of Asia. In: *Sustainability of rice-wheat production systems in Asia* (R.S. Paroda, T. Woodhead, and R.B. Singh, eds.). New Delhi, India: Oxford & IBH Publishing Co. Pvt. Ltd. pp. 68-96.

Woomer, P., Singleton, P.W., and Bohlool, B.B. 1988. Ecological indicators of native rhizobia in tropical soils. *Applied and Environmental Microbiology* 54:1112-1116.

Yoneyama, T., Nambiar, P.T.C., Lee, K.K., Srinivasa Rao, B., and Williams, J.H. 1990. Nitrogen accumulation in three legumes and two cereals with emphasis on estimation of N_2-fixation in the legumes by the natural ^{15}N abundance techniques. *Biology and Fertility of Soils* 9:25-30.

Zari, K.W., Miller, J.C. Jr., Weaver, R.W., and Barnes, L.W. 1978. Intraspecific variability for nitrogen fixation in southern pea *(Vigna unguiculata). Journal of American Society of Horticulture Science* 103:806-808.