Biological Nitrogen Fixation Through Grain Legumes in Different Cropping Systems of the Semi-Arid Tropics

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Abstract

Biological nitrogen fixation (BNF) by legumes plays an important role in sustaining crop productivity and maintaining fertility of the low-nitrogen (N) containing soils of the semi-arid tropics (SAT). Pigeonpea (Cajanus cajan), chickpea (Cicer arietinum), and groundnut (Arachis hypogaea) are important grain legumes grown in different cropping systems in the SAT. Accurate quantification of BNF by legumes is essential to assess the N contributions from legumes for developing appropriate N-management strategies for the cropping systems. The ^{15}N-based methods are more appropriate for quantification of N_2 fixed by the legumes, compared with other methods such as acetylene reduction assay (ARA) or N-difference method.

The estimates of per cent N derived from atmosphere ($\%N_{dfa}$) by some grain legumes in sole crops ranged from 17 to 85 for chickpea, 22 to 92 for groundnut, and 10 to 88 for pigeonpea. In intercropping situations, the $\%N_{dfa}$ by legumes depends on the species, plant morphology, planting density in the intercrop mixture, and crop management practices. The $\%N_{dfa}$ in intercropped pigeonpea ranged from 65 to 96 depending on soil type. However, there was no benefit through direct transfer of fixed-N from pigeonpea to the associated cereal in intercropping. The grain legumes benefited the subsequent cereal crops, and the increased cereal yields were equivalent to 40 to 60 kg fertilizer N ha^{-1} application. Beneficial effect of these legumes are influenced by the genotype, soil type, crop and soil management practices, and environment. Though the exact mechanisms of such beneficial effects of the legumes are not understood well, the reported mechanisms include BNF (N-saving and N-sparing effects), improved structure and biological activity of soil, improved nutrient supply other than N, reduced diseases, and hormonal effects. Future research includes optimizing BNF contributions of grain legumes to the cropping systems through holistic systems approach through appropriate crop and soil management practices, rhizobial inoculations and screening, and host-plant breeding for high N_2-fixing plants.

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Introduction

One-sixth of the world's population lives in the semi-arid tropics (SAT), including parts of 48 developing countries in Africa, Asia, and Latin America. The SAT region is characterized by unpredictable weather, limited and erratic rainfall, and nutrient-poor soils. Mean annual temperatures in the SAT are > 18°C; and 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). The soils of the SAT are generally low in both total and plant-available N. Crop yields in the SAT are often limited by N-supply. Fertilizers are used only to a limited extent because of low per-capita incomes, limited credit facilities for most farmers, and lack of infrastructures for fertilizer production and distribution. Under such situations the opportunities are greatest for exploitation of the legume's ability to fix atmospheric-N_2 in symbiosis with rhizobia. The successful formation of a functional symbiosis is dependent on many physical, environmental, and biological factors and cannot be assumed to occur as a routine. Nitrogen fixation by legumes is very important in maintaining soil fertility and sustaining land productivity in the SAT (Wani et al. 1994b).

Because the relationship between N_2-fixation and legume growth or production is not always direct and obvious, considerable effort has been put into the development of methods for measuring N_2-fixation (Hardy et al. 1973; Fried and Broeshart 1975; Mariotti et al. 1983; Bergersen et al. 1989). Accurate measurement of N_2-fixation under field conditions allows realistic assessment of legumes' contribution to a system's N-balance and provides a basis for developing sustainable strategies to manage the soil crop systems. Pigeonpea (*Cajanus cajan*), chickpea (*Cicer arietinum*), and groundnut (*Arachis hypogaea*) are the important legumes of SAT, and are grown as components of crop rotations in double cropping, intercropping, and mixed cropping. We discuss the merits of the different techniques for quantifying N_2-fixation and review the importance of symbiotic BNF in the N-economies of the many grain legume-based production systems of the SAT. Finally, strategies will be reviewed that may lead to increased N_2-fixation of the legumes in the system.

Methods of evaluating N_2-fixation of grain legumes

Although there are several methodologies for measuring N_2-fixation in plants, none of them measures N_2-fixed with absolute accuracy. The advantages and disadvantages of different methods available for measuring nitrogen fixation are presented briefly.

Rate of N_2-fixation activity

Acetylene reduction assay

The acetylene reduction assay (ARA) arose from the observations that the N_2-fixing enzyme complex, nitrogenase, catalyzes the reduction of acetylene (C_2H_2) to ethylene (C_2H_4). This method is indirect and has only one time assay of the N_2-fixing activity of the plants, and unless flow-through systems are employed, reliable estimates cannot be obtained (Witty and Minchin 1988). In many legume species, substantial decline in nitrogenase activity occurs after exposure to C_2H_2. This method is qualitative in nature and the ARA greatly underestimated N_2-fixing activity under field conditions in comparison with the estimates from other methods (e.g., Boddey et al. 1984; Kumar Rao and Dart 1987).

N-solute method

Xylem sap carries N-containing compounds from the roots to the shoots of field-grown legumes originating from (a) nodules as assimilation products of N_2-fixation and (b) soil mineral-N taken up by the roots. Products of N_2-fixation are transported to other parts of the plant in the xylem as ureides or amides, whereas N absorbed from the soil as NO_3 is either transported directly to the shoot or reduced to amides before transportation. Many legumes, primarily those of the tribes Phaseoleae and Desmodieae (e.g., pigeonpea), transport most of their fixed-N in the form of ureides (Peoples et al. 1989a). In most 'ureide exporters' there is little nitrate reductase activity in the roots, and thus the proportion of N in the xylem sap as ureides is directly proportional to the amount of N-fixed. In those legumes that do not transport fixed nitrogen as ureides, it is possible to correlate nitrogen fixation with the proportion of amide-N in the xylem sap only in some species (e.g., *Arachis hypogaea and Cicer arietinum*) where there is little nitrate reductase activity in the roots (Peoples et al., 1987).

The ureide method provides only a short-term measure of symbiotic dependence, and the time delay between decapitating the shoot and vacuum extraction of xylem sap affects the ureide content. This method can be used for comparing treatments for BNF by collecting the sap within 5 min of stem detachment (Peoples et al. 1989b).

Integrated measurements of N_2-fixation

N-difference method

Total N-uptake by a non-fixing reference crop is subtracted from the total N-uptake by a legume, which then gives a measure of the amount of N-fixed in the legume crop. The main assumption is that both the crops exploit similar soil volume with similar rooting and absorb the same amount of soil-N. This can only be verified by using ^{15}N-labeled fertilizer. Comparisons of N_2-fixation calculated by N-difference with estimates by ^{15}N-dilution have often shown good agreement for soils low in N or where recovery of ^{15}N-label is equal in the legume and non-fixing control (Chalk 1985). Compared with the ^{15}N-natural abundance method the N-difference method tended to underestimate the $\%N_{dfa}$ of pigeonpea (Table 1). This is particularly with extra-short-duration and short-duration pigeonpea in both Alfisol and Vertisol fields. With medium duration pigeonpea (grown as sole crop) that are generally intercropped with cereals such as sorghum or millet, the N-difference and ^{15}N-natural-abundance methods gave similar values of $\%N_{dfa}$. Use of the N-difference method is complicated when dealing with intercropped legumes because the intercrop competition may affect the ability of the legume and non-fixing reference crops to access soil-N.

Table 1. Total N-uptake and per cent nitrogen derived from the atmosphere (% N_{dfa}) for pigeonpea genotypes of different maturity groups as determined by the ^{15}N-natural-abundance method and by the N-difference method. (Kumar Rao, J.V.D.K., Johansen, C., Yoneyama, T., Ito, O., Rao, T.P., and Adu-Gyamfi, J.J. unpublished)

	Total N-uptake (kg ha^{-1}) in		% N_{dfa} estimated by			
			$\delta^{15}N$ method		N-difference method[2]	
Genotype/ maturity group[1]	Alfisol (A)	Vertisol (V)	(A)	(V)	(A)	(V)
ICPL 4 (ESD)	51.2	48.7	68.3	72.4	3.7	25.3
ICPL 84023(ESD)	63.6	50.9	64.9	60.2	22.5	28.5
ICPL 87 (SD)	94.6	62.6	65.1	67.4	48.0	41.9
ICPL 151 (SD)	66.3	54.4	74.6	61.6	25.6	33.1
ICP 1-6 (MD)	150.0	96.9	65.7	59.3	77.6	58.7
ICPL 87119 (MD)	153.0	103.4	71.7	60.5	78.0	61.3
ICPL 366 (LD)	191.0	111.5	60.9	83.8	82.4	64.1
T 7 (LD)	212.0	89.4	62.1	87.4	84.1	55.3

1. ESD – extra short-duration; SD short-duration; MD medium-duration; LD—long-duration
2. For ESD and SD pigeonpea, sorghum hybrid CSH 9 (about 120-day duration) was used; IS 17820, a long-duration sorghum (about 180 days) was used as a non-fixing control for MD and LD pigeonpea.

^{15}N-isotope dilution method

The test legume and the non-fixing reference crop are grown on a ^{15}N-enriched soil for isotope dilution measurement of N_2-fixation. The ^{15}N-enrichment of the non-fixing reference crop will reflect that of the soil on which it is grown, whereas that of the legume will be reduced in proportion to the amount of atmospheric ($^{14}N_2$) nitrogen fixed. The main assumption of the method is that both the legume and the reference crop take up soil-N (^{14}N:^{15}N) in the same ratio (Fried and Broeshart 1975). The single most important factor affecting the accuracy of this method is finding an appropriate reference crop that matches the legume in its rooting, N-uptake pattern, and duration. As the ^{15}N-enrichment of the available soil-N tends to decline after the ^{15}N-fertilizer is added, and because of the difficulties of mixing the ^{15}N-enriched fertilizer throughout the soil horizons exploited by the plants, any mismatching of reference and test crops will result in absorption of different ^{15}N-enrichment from soil (Witty 1983). These problems are considered in detail by Chalk (1985), Danso (1985), and Witty and Giller (1991).

Natural-^{15}N-abundance method

The N-transformations in soil result in isotopic fractionation resulting in a small increase in the ^{15}N-abundance of soil-N compared with atmospheric-N_2 (Shearer and Kohl 1986). It is possible to carry out isotope dilution measurements of N_2-fixation on such soils without the need to apply ^{15}N-enriched fertilizers. The problems outlined with the use of ^{15}N-enriched fertilizer do not seem to be as great when using the ^{15}N-natural-abundance method because the enrichment of plant-available-N is relatively constant and varies little with depth (Ledgard and Peoples 1988). This method has the added advantage of not requiring the addition of ^{15}N-enriched fertilizer, and thus measurements of N_2-fixation can be made in established experiments or farmers fields where suitable reference plants are present.

Recent reports indicate that the ^{15}N-natural-abundance method is an extremely useful method for measuring N_2-fixation in a wide variety of farming systems (Peoples et al. 1991), but is less likely to be of use in following the fate of fixed N. It is important to note that the accuracy of the technique will depend on the level of natural-^{15}N abundance of the soil. The $\delta^{15}N$ units for most soils are between -2 and +15. Levels of $\delta^{15}N > 6.0$ are preferable, although values as low as 2% might still be useful, depending on the level of Pfix (Unkovich et al. 1994). Soils with very low or variable $\delta^{15}N$ values will be unsuitable for assessing N_2-fixation. Furthermore, the method requires precise mass spectrometer (capable of measuring accurately differences of 0.1%) and meticulous analytical procedures (Weaver and Danso 1994).

Estimates of N_2-fixation by grain legumes

The estimates of N_2-fixed by the food legumes grown in the SAT varied greatly with crop species and location (Table 2). The levels of fixation depend on water supply, inoculation, crop management practices, including application of fertilizer-N and soil-N fertility. In almost all cases, $\%N_{dfa}$ was reduced in the presence of higher combined-N.

Food legumes are not only grown in pure stands but they are often interplanted with other species. Mixed cropping is practiced traditionally in many parts of Africa, Asia, and Latin America. The combination of crops is determined by the length of growing season and environmental adaptation, but usually early- and late-maturing crops are combined to ensure efficient utilization of the resources during the growing season. In tropical regions the legumes such as cowpea, pigeonpea, groundnut, and chickpea are usually intercropped with maize, sorghum, millet, safflower, or rice. The quantity of N_2 fixed by the legume in an intercrop depends on the species, plant morphology and legume density in the intercrop mixture and crop management practices (Table 3). Differences in the competitive abilities of the component crops for soil-N can result in stimulation of N_2-fixation (Rerkasem et al. 1988). However, N_2-fixation by climbing types of common bean was unaffected by intercropping with maize (Graham and Rosas 1978), whereas shading by tall cereal crops can reduce both yield and N_2-fixation of shorter stature legumes such as groundnut (Nambiar et al. 1983).

Beneficial effects of legumes

In intercropping

It is generally assumed that a portion of N_2-fixed by an intercropped legume is made available to the associated nonlegume during the growing season. Decaying roots and nodules are important in this transfer of N, although these organs generally contain only a small fraction of the total plant-N, e.g., 3-40 kg N ha^{-1} (Kumar Rao and Dart 1987; Bergersen et al. 1989). The possibility of exudation of N from living roots should not be ignored (Poth

Table 2. Percentage N_{dfa} of some food legumes grown in tropics (Peoples and Herridge, 1990)

Species	Range
Groundnut	22-92
Pigeonpea	10-88
Chickpea	17-85
Cowpea	8-89
Soybean	0-95

Table 3. Effect of intercropping grain legumes with cereals on crop-N derived from N_2-fixation.

Species	Location	Intercrop ratio Legume: Cereal	N_2-fixed		Ref.
			P	Amount (kg N ha^{-1})	
Rice bean	Thailand	100:0	0.36	49	1
		25:75	0.86	41	
Cowpea	Australia	100:0	0.69	87	2
		71:29	0.66	59	
	India	100:0	0.54	64	3
		66:33	0.58	48	
	Hawaii	100:0	0.30	18	4
		75:25	0.34	10	
	Nigeria	100:0	0.79	118	5
		62:38	0.59	72	
Pigeonpea	India	100:0	0.88	88	6
		100:100	0.96	75	
	India	100:0	0.63	150	7
		100:100	0.86	165	

1. Rerkasm et al. (1988), 2. Ofori et al. (1987), 3. Patra et al. (1986), 4. van Kessel and Roskoski (1988), 5. Eaglesham et al. (1981) 6. Kumar Rao et al. (1987), 7. Tobita et al. (1994).

et al. 1986). Evidence of N-transfer from legume to cereal has been obtained in intercropping studies (Eaglesham et al. 1981; Bandopadhyay and De 1986; and Patra et al. 1986), including genotypic differences in the extent of 5-39% of legume fixed-N transferred to cereal in intercropping (Senaratne and Ratnasinghe 1993), although not confirmed in other investigations (Kumar Rao et al. 1987; Ofori et al. 1987; Rerkasem and Rerkasem 1988; van Kessel and Roskoski 1988). This suggests that direct transfer of N from legume to nonlegume component crop during the season may be minimal (Chalk 1996 in this proceedings).

On cereals following legumes

The total amount of N in a legume crop comes either from N_2-fixation or from uptake of mineral-N from the soil. In food legumes, total-N is partitioned into either the harvested seed or the vegetative parts that generally remain as crop residues. When the quantities of N involved in plant growth, in N_2-fixation and in the seed are calculated for food legume crops it is apparent that the net N-balance is often low and in some cases negative. Positive net N-balances of up to 136 kg ha^{-1} for several legumes following seed harvest have been reported (Table 4). However, Wani et al. (1994b) recalculated the N-balances from these data assuming that crop residues are removed from the field as per the common practice in the SAT, and showed negative net N-balances (kg ha^{-1}) from -27 to -95 for groundnut, -28 to -104 for soybean, -28 for the common bean, -24 to -65 for greengram, and -25 to -69 for cowpea.

Despite the variable N-balances the benefits reported in tropical crop legumes to subsequent cereal crops are consistent and substantial (Wani and Lee 1992) and may persist for several seasons (MacColl 1989), regardless whether or not the legume was grown in monoculture or was intercropped. Responses to previously intercropped legumes are more modest (Table 5) than the corresponding monocrop. When the contribution of the legume was quantified as fertilizer-N equivalent, as much as 68 kg N ha^{-1} was required in the cereal-cereal sequence to achieve similar yield improvements.

The overall benefits of legumes are not fully explained when only their BNF effects are considered. The other likely benefits include increased availability of nutrients other than N, improved soil structure, enhanced level of growth-promoting substances, and reduced pest and disease incidence (Wani et al. 1994b). The extent of these benefits are dictated by site, season, and crop sequence.

Improving the contribution of legumes in cropping systems

Although legumes have the ability to fix N_2, it cannot be assumed that the inclusion of any legume in a cropping system will ensure significant contributions to the N cycle. As is evident from published reports most legumes deplete soil-N when plant material is removed from the field (Wani et al. 1994 a,b). To derive maximum benefits from legumes, we must take a holistic approach and understand the entire BNF and N-cycling system.

Table 4. Net N-balance for grain legumes following seed harvest.

Species	Location	Seed yield (N kg ha^{-1})^{1}	Total crop (N kg ha^{-1})^{2}	NHI^{3}	N_2 fixed^{4} P	N_2 fixed^{4} Amount (kg N ha^{-1})	Net N balance^{5}	Ref.^{6}
Groundnut	Thailand	116	245	0.41	0.61	150	+34	7
Pigeonpea	India E	39	72	0.54	0.10	7	-32	8
	India M	49	120	0.41	0.46	55	+6	
	India L	28	134	0.21	0.51	69	+41	
Cowpea	Australia	80	125	0.64	0.69	87	+7	9
	Ghana	65	226	0.29	0.89	201	+136	10
Greengram	Australia	89	177	0.50	0.63	112	+23	11

1. N removed in seed, SN
2. Total N at maturity, TN
3. Nitrogen harvest index = SN/TN
4. Quantity of N_2 fixed, Nf = TN x P
5. Net contribution of legume residue N to soil = Nf-SN
6. 7. Suwanarit et al. (1986); 8. Kumar Rao and Dart (1987); 9. Ofori et al. (1987); 10. Dakora et al. (1989); 11.

Table 5. Residual effect of legumes grown as sole and intercrop with a cereal on a following cereal yield in terms of fertilizer-N equivalents.

Preceding legume	Following cereal	Fertilizer-N equivalent (kg ha^{-1})	Ref
Pigeonpea (sole)	Maize	40	1
Pigeonpea/sorghum	Maize	20 +	
Groundnut	Wheat	28	2
Groundnut/maize	Wheat	12	
Greengram	Wheat	68	
Greengram/maize	Wheat	16	
Cowpea	Wheat	38	
Cowpea/maize	Wheat	13	

1. Kumar Rao et al. (1983); 2. Bandyopadhyay and De (1986)

Host-plant improvement

Variability exists in legumes for the amount of N_2 fixed and for the proportion of plant-N derived from BNF. We need to identify legumes and genotypes that yield more, and derive a large part of their N-requirement from fixation. For example, compared with chickpea, pigeonpea returned a large amount of fixed-N to the soil through nodulated roots and fallen leaves. Similarly, there is a need to identify genotypes that can fix well under adverse soil conditions, such as high soil-N, soil acidity and alkalinity, Al and Mn toxicity, waterlogging, high- and low-soil temperature. The natural occurrence of non-nodulating plants within chickpea genotypes indicate a need to ensure that their proportion in the released genotypes does not increase. Most plant breeding and testing work is done on research stations where soil-mineral-N is invariably higher than that in farmers' fields. Nonnodulating and low-nodulating plants are therefore not detected during selection and testing of improved genotypes. This has been demonstrated in chickpea and pigeonpea (Rupela 1994) and may also be true for other legumes. To avoid this, appropriate procedures must be adopted in breeding and testing programs. Herridge and Danso (1995) in a recent review suggested methods for concurrently measuring N_2-fixation and assessing heritability and repeatability of N_2-fixation for breeding and selection programs with particular emphasis on common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*).

Improved crop management

Appropriate crop and soil management practices should be followed to ensure maximum BNF contribution by legumes. For example, high mineral-N in soil that reduces BNF can be managed either by immobilization of the soil-N through addition of organic material with a high C/N ratio or through reduced tillage. In intercropping situations in which application of fertilizer-N is essential for obtaining high cereal yields, an appropriate form of fertilizer, e.g., slow-releasing formulations or organic-N, should be used. Also, suitable methods of fertilizer application, e.g., placement of fertilizer in cereal crop rows rather than broadcasting and mixing in soil, must be followed (Wani et al. 1994b). Appropriate amendments with nutrients other than N that might limit legume growth—and in turn BNF—should be applied.

Rhizobial inoculation

Under field conditions, response to rhizobial inoculation in traditional legume-growing areas has not been consistent. Situations that need inoculation should be identified and efforts must be focused on such areas. Research for selection of efficient strains and identification of specific host-bacteria combinations must continue. The important constraints limiting the exploitation of inoculation technology are: (a) poor quality of the inoculants; (b) lack of knowledge about inoculation technology among extension personnel and farmers; (c) ineffective inoculant delivery systems; and (d) lack of appropriate policy support by governments that would favor use of inoculants by farmers.

Conclusion

Legumes have an important role as intercrops and sequential crops in sustaining the productivity of different cropping systems in the SAT. A holistic system approach is a must for maximizing the benefits from BNF in legumes. High N_2-fixing legumes and cultivars should be selected for inclusion in the cropping systems. Appropriate soil and water management practices supporting good plant growth, for e.g., sowing on ridges or broad beds for protecting from waterlogging, using scoops for light textured soils to increase water storage, need to be followed. To ensure good nodulation and N_2-fixation by legumes in cropping systems, farmers must (a) use appropriate crop management practices, such as application of phosphatic fertilizers or other deficient plant nutrients; (b) control pests and diseases that may affect plant canopy and in turn photosynthate supply to roots; (c) practice N-management in soil (e.g., use of slow releasing formulations, applying N to cereals only by placement, use of organic sources); and (d) use need-based inoculations with good quality rhizobial inoculants. If returned to the soil, plant residues would help in increasing the soil organic matter content, and thereby increase the soil fertility. Through such an approach, benefits from legumes BNF can be maximized for improving or sustaining productivity of SAT cropping systems.

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