

## Nitrogen Assimilation Traits and Dinitrogen Fixation in Soybean, and Common Bean

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### ABSTRACT

Nitrogen derived from symbiotic and, mineral sources by a legume is determined by the interactions between mineral N supply, plant N demand, and N assimilation traits. These interactions need to be understood to maximize legume N<sub>2</sub> fixation and yield, and to identify plant traits supporting high N<sub>2</sub>-fixation. These interactions were examined in inoculated soybean [*Glycine max* Merr. (L.)] and common bean (*Piureolus vulgaris* L.) by varying N supply (9, 120, and 900 kg N ha<sup>-1</sup>) at two field sites. Nitrogen fixation was measured by <sup>15</sup>N dilution method. Plants were sampled at full bloom (R2), 21 to 25 d from R2, and physiological maturity (R7). Total N of both legumes at R7 was 25% greater with 900 than 9 kg N ha<sup>-1</sup>. With 900 kg N ha<sup>-1</sup>, soybean N accumulation at R7 (271 kg N ha<sup>-1</sup>) was 42% more than common bean but was 22% less at R2 (78 kg N ha<sup>-1</sup>). Applied N had the largest impact on N accumulation rates before R2 and from the second sampling to R7, both periods of minimal N<sub>2</sub> fixation. Rate of N accumulation by common bean (0.19 g N m<sup>-2</sup> d<sup>-1</sup>) was more uniform over the growth cycle than by soybean (0.26 g N m<sup>-2</sup> d<sup>-1</sup>) which peaked (0.58 g N m<sup>-2</sup> d<sup>-1</sup>) between R2 and the second sampling. This peak also coincided with maximum N<sub>2</sub> fixation rate. Our <sup>15</sup>N uptake and extractable soil N data indicate that common bean derived more N from the mineral source than soybean because of more efficient uptake. Greenhouse data indicated greater root weight and uptake of mineral N per unit root weight for common bean than soybean. Maximizing both N<sub>2</sub> fixation and yield might entail timing the mineral N supply during early vegetative and late reproductive phases. The limited N<sub>2</sub> fixation capacity of common bean might be due to N assimilation traits favoring mineral N uptake.

THE N REQUIREMENT OF LEGUMES Can be met by both mineral N assimilation and symbiotic N<sub>2</sub> fixation. The plant N requirement may not be met during early vegetative and later productive phases by N<sub>2</sub> fixation. Symbiotic N<sub>2</sub> fixation begins only after nodule formation, which is preceded by the colonization of the rhizosphere and the infection of legume roots by rhizobia (Hardy *et al.*, 1971). Nitrogen fixation by grain legumes generally reaches a peak at early podfill and then declines during the late reproductive phase (Imsande, 1988; Latimore *et al.*, 1977; Lawn and Brun, 1974a; Thibodeau and Jaworski, 1975). Thus, mineral N may be a critical source of N for grain legumes during both the early vegetative and late reproductive periods. To maximize yield and N<sub>2</sub> fixation by legumes, there is a need to understand the interactions between mineral N supply, N requirements, and N uptake traits.

The interaction between genetic yield potential and environment determines the N requirement of a legume. The contributions of symbiotic and mineral N sources to total plant N are determined by legume N requirement and mineral N supply provided an effective *Rhizobium* symbiosis is ensured. When mineral

N uptake is less than the N requirement, N<sub>2</sub> fixation is promoted. Therefore, N<sub>2</sub> fixation potential can be considered to be equal to the - aggregate of per day deficits in mineral N uptake during the legume growth cycle. The daily N requirement of the legume is dependent on its growth rate and developmental stage. The relative contributions of mineral N and N<sub>2</sub> fixation may also depend on the plant's efficiency of mineral N uptake. Therefore, plant ontogeny, N requirement, and efficiency of mineral N uptake regulate N<sub>2</sub> fixation of effectively modulated legumes. Understanding these characteristics may provide useful diagnostic tools, to identify genotypes with a high capacity to fix dinitrogen.

It has been suggested that both mineral N and fixed N<sub>2</sub> are essential for maximum N accumulation and yield by legumes (Bhangoo and Albritton, 1976; Franco *et al.*, 1979; Harper, 1974), because of the high energy requirement for the development, maintenance, and functioning of the symbiotic system in comparison to mineral N assimilation (Finke *et al.*, 1982; Pate *et al.*, 1979; Ryle *et al.*, 1978, 1979). Imsande (1989), however, reported that N<sub>2</sub> fixation during soybean podfill enhances seed yield compared to urea N assimilation. Other reports indicate that soybean grown in solution culture supplied with mineral N assimilates substantially more N than do modulated plants (Jones *et al.*, 1981; Lathwell and Evans, 1951; Wych and Rains, 1979). Similar results have been reported only rarely from field experiments (Cassman *et al.*, 1981; Thies *et al.*, 1991). Therefore, attaining the genetic yield potential of a legume may require maintaining adequate mineral N throughout the legume's growth.

Legumes differ substantially in their N requirements. While comparisons between soybean and common bean under identical growing conditions are limited (Piha and Munns, 1987; Thies *et al.*, 1991), the data for soybean (George *et al.*, 1988; Cassman *et al.*, 1981; Herridge *et al.*, 1984) and common bean (Rennie and Kemp, 1983; Westerman *et al.*, 1981) indicate that soybean has a greater N requirement than common bean at similar crop durations and yields. This higher N requirement of soybean is primarily due to a greater seed N concentration compared with common bean. Accordingly, soybean meets a major portion of its N requirement through symbiosis in soils of low to moderate N availability (George *et al.*, 1988). Many reports indicate little or no N<sub>2</sub> fixation by common bean (El-Nadi *et al.*, 1972; Graham, 1981) except under low soil N or at high yield (Piha and Munns, 1987; Rennie and Kemp, 1983; Westerman *et al.*, 1981). These contrasting N requirements and N<sub>2</sub> fixation potentials of soybean and common bean provide an opportunity to identify N assimilation traits that may be useful in developing strategies to increase N<sub>2</sub> fixation.

In this paper, we examine the interactions between mineral N supply, plant N demand, N assimilation traits, and N<sub>2</sub> fixation in soybean and common bean. The objectives were to understand these interactions and to identify traits that can be used as tools to select

high  $N_2$ -fixing genotypes. Two elevations and three N regimes were used to manipulate plant ontogeny, yield and N demand, and mineral N supply. In a greenhouse experiment, relationships between root characteristics and mineral N uptake were also examined.

## MATERIALS AND METHODS

### Field Experimental Plan

Determinate cultivars of common bean (*Phaseolus vulgaris* L. cv. Brazil 2, CIAT, Cali, Columbia) and soybean [*Glycine max* (L.) Merr. cv. Clark, USDA, Beltsville, MD] were grown inoculated or left uninoculated with applied N at 9, 120, and 900 kg N ha<sup>-1</sup> at two field sites at 320-m and 1050-m elevations on the island of Maui, Hawaii. The experimental design was a randomized complete block at each site. The treatments were arranged in a split-split plot with legume genotypes assigned to main plots, inoculation to subplots, and N levels to sub-subplots.

### Site Selection and Field Management

Site selection and field management were intended to maximize differences in plant phenological development, yield potential, and N requirement between sites. The two sites used were in an elevational transect at the same latitude (20°52'N) and received similar rainfall and irradiance but differed in mean temperatures. The soil was a Humoxic Tropohumult at the 320-m site and Entic Dystrandept at the 1050-m site. The KCl-extractable N (20-cm depth) at the start of the experiment was 0.05 and 0.10 g N kg soil<sup>-1</sup> at the 320-m and 1050-m sites, respectively.

Soils were amended before the start of the experiment to minimize the differences in plant growth between sites due to differences in soil fertility. Soil pH at the two sites was adjusted between 5.5 and 6.0 by incorporating lime 4 wk before seeding. Nutrients except N were added in amounts suited for maximal fertility. Nutrients added in kg ha<sup>-1</sup> were 600 P, 300 K, 75 Mg, 25 Zn, 3 B, and 2 Mo.

### Plant Culture

The legume genotypes were selected based on previous observations that they had similar crop duration at 91-m elevation at this latitude. This was to minimize differences in their phenological development at higher elevations. The seeds for the inoculated treatments were coated before seeding with peat inoculants containing *Rhizobium leguminosarum* bv. *phaseoli* strains TAL 182, CIAT 632 (TAL 1383) and CIAT 899 (TAL 1797) for common bean and *Bradyrhizobium japonicum* strains USDA 110 (TAL 102), USDA 138 (TAL 377), and USDA 136 (TAL 379) for soybean from the NifTAL Project culture collection. Seeds of Brazil-2 and Clark were sown at the 1050-m site on 8 July 1987 and at the 320-m site on 15 July 1987 in plots 9.5 by 3.0 m. Seeds were sown in rows 60 cm apart to attain a final population of 40 plants m<sup>-2</sup>. Clark nonnodulating isoline (P. Cregan, USDA Nitrogen Fixation Laboratory, Beltsville, MD) was sown in soybean uninoculated plots. Plots were watered to field capacity on the day of seeding and drip irrigated thereafter to maintain tensiometer readings (20-cm depth) at approximately 0.02 MPa.

### Nitrogen and Nitrogen-15 Applications

Fertilizer N levels of 9, 120, and 900 kg N ha<sup>-1</sup> were used to generate a range of mineral N supply and consequent changes in proportions of plant N derived from  $N_2$ -fixation. Total fertilizer N (as ammonium sulfate) for each plot was divided into three equal amounts and applied at

seeding, at full bloom sampling, and again at 21 to 25 d from full bloom sampling. Split application of fertilizer N was employed to maintain mineral N availability throughout plant growth and to minimize the changes in <sup>15</sup>N-abundance in the soil N pool available to the plant. The ammonium sulfate used for the three microplots each of 3.0 by 1.35 m within each whole plot was enriched to provide 30 mg <sup>15</sup>N m<sup>-2</sup> at each application. At each application, aqueous solutions of ammonium sulfate were sprayed on to the soil surface at the rate of 1.4 L m<sup>-2</sup> followed by 0.5 cm of water through overhead irrigation.

### Plant Sampling and Analysis

Plants were sampled at full bloom (R2), at 21 to 25 d from R2, and at physiological maturity (R7). Crop growth stage determinations were based on IBSNAT growth and development scales (International Benchmark Sites Network for Agrotechnology Transfer (IBSNAT) Project, 1984). At each sampling, 10 to 15 random plants were cut at the soil surface from the center three rows of the five-row microplots for fresh weight, dry matter, N content, and <sup>15</sup>N determinations. Plot fresh weight was determined on plants harvested above the soil surface from a 2.4-by 1.8-m area which also included the microplot area. Dry matter content of whole plants was established from the microplot subsample after drying to constant weight at 60 °C for 48 h. The samples at R7 were threshed for seed yield. The dried plant samples were ground and subjected to salicylic acid-thiosulfate modification of Kjeldahl digestion to convert tissue N, including nitrate and nitrite, into ammonium (Bremner and Mulvaney, 1982). Ammonium-N in digest subsamples was measured by the indophenol blue method (Keeney and Nelson, 1982). Kjeldahl digests were steam distilled with 13 M NaOH and the distillates were collected in 0.01 M H<sub>2</sub>SO<sub>4</sub>. Twenty milliliters of ethyl alcohol was distilled between each sample to avoid contamination between samples. For mass spectrometer analysis of <sup>15</sup>N, the distillates were then acidified to a pH of 4.5 to 5.0 and concentrated by evaporation on a hot plate at 60 to 70 °C (Bergersen, 1980, p. 65-110). <sup>15</sup>Nitrogen analysis was carried out by Isotec Inc.<sup>1</sup> (Isotec Inc., Miamisburg, OH).

### Sampling and Extraction of Soil Nitrogen

Soil samples from the <sup>15</sup>N-microplots were collected to a depth of 20 cm (composite of four samples per microplot) at seeding and at all plant sampling dates. Soil suspensions (50 g air dry soil:350 mL 2 M KCl) were agitated for 1 h on a mechanical shaker. Ammonium-N and NO<sub>3</sub>-N were determined from KCl extracts by indophenol blue and cadmium reduction methods, respectively (Keeney and Nelson, 1982). Total extractable N (NH<sub>4</sub>-N + NO<sub>3</sub>-N) is presented on an oven dry (110 °C) soil basis.

### Nitrogen-15 Isotope Calculations

The proportions of plant N derived from soil, fertilizer, and atmosphere, and the fertilizer N uptake efficiency were calculated by equations developed for <sup>15</sup>N dilution techniques (International Atomic Energy Agency, 1983). The atom% <sup>15</sup>N excess was calculated with reference to the natural <sup>15</sup>N abundance of air (0.3663 atom% <sup>15</sup>N). The nonnodulating soybean isoline and the uninoculated common bean served as the non- $N_2$ -fixing reference plants for soybean and common bean, respectively. Because of nodulation in some uninoculated common bean plots at the 320-

<sup>1</sup>Mention of a company or brand name does not constitute an endorsement by authors, University of Hawaii or the granting agencies.

m site,  $^{15}\text{N}$  excess values of only non-contaminated plots were used as reference for common bean at that site.

### Greenhouse Experiment

A determinate common bean cultivar (cv. Calima, CIAT, Cali, Columbia) and one soybean cultivar (cv. Clark) were grown in pots either inoculated or left uninoculated with N levels of 0, 133, and, 400  $\mu\text{g N g}^{-1}$  soil. Soil devoid of soybean or common bean rhizobia was first mixed with 0.8% (w/w) bagasse, wet to field capacity, and incubated for 4 d to immobilize available N. Nutrient amendments added ( $\text{mg kg}^{-1}$  soil) were 200 P, 100 K; 25 Mg, 5 Zn, 1 B, and 0.7 Mo. Five seeds were planted in pots filled with the amended soil (4.2  $\text{kg pot}^{-1}$ ). The pots were arranged in a randomized complete block design: Ten days after seeding, seedlings were thinned to one per pot. Pots were maintained at field capacity throughout the experiment. Plant tops, roots, and nodules were harvested at 33 d after seeding and dry weight and N content were determined.

### Statistical Analysis

The data from the two field sites were analyzed separately. The *F*-tests for legume-by-N interactions were seldom significant except for data from the greenhouse. Main effects are discussed unless biologically significant interactions were encountered.

## RESULTS AND DISCUSSION

### Total Nitrogen Accumulation

Nitrogen accumulation differed significantly between the legume species at all growth stages in the field (Table 1). Common bean generally accumulated more N in early growth compared to soybean, but soybean accumulated substantially greater N than common bean by R7. The differences in N accumulation between common bean and soybean were generally associated with differences in dry matter (Table 2). During reproductive growth, however, increases in N accumulation were proportionately greater than increases in dry matter for soybean compared to common bean. The average seed yields of common bean and soybean were similar (3033 and 2943  $\text{kg ha}^{-1}$ , respectively), indicating that the increased total N of soybean was primarily due to increased seed N content.

At R2, common bean on average accumulated 37% greater N than soybean at the 320-m site and a similar amount of N as soybean at the 1050-m site. At R7, however, soybean accumulated 70 and 29% greater N than common bean at the 320- and 1050-m sites, respectively. Common bean cultivar used in the green-house accumulated 60% more N than soybean at 33d (Table 3). The average total N of R7 of soybean decreased significantly from 271  $\text{kg N ha}^{-1}$  at the low elevation site to 219  $\text{kg N ha}^{-1}$  at the high elevation site while total N of common bean was similar between the two sites. The data indicate that while, soybean and common bean cultivars maintained differing N requirements throughout growth and across sites, the actual amount of N accumulated by the two was differently influenced by the site environment.

Despite differences in N requirement between legumes or sites, dry matter and total N of both legumes increased significantly when increased mineral N was applied (Tables 1, 2, and 3). Dry matter and total N of field plants at physiological maturity increased on average by 19 and 25%, respectively, with the 900 versus 9  $\text{kg N ha}^{-1}$  treatment. Average increase in plant N due to N application was the greatest at full bloom in the field; 39% increase in plant N with 900 compared to 9  $\text{kg applied N ha}^{-1}$  regime. The response to applied N was even greater in the greenhouse where soil N in the control was immobilized; 185% increase in plant N with 400  $\mu\text{g N g}^{-1}$  soil over no N application after 33 d of growth. The increases in dry matter and total N of soybean in response to applied N were less pronounced at the 1050 m site. This was because of low N requirement and high soil N availability at the 1050-m site as evident from low  $\text{N}_2$  fixation at that site (Table 4 and 5). Plant N concentration increased significantly with increased applied N in both legumes and at both sites (data not shown). The results suggest that N accumulation by these inoculated legumes was generally limited by insufficient mineral N supply.

The data demonstrate that dry matter and N accumulation by inoculated soybean and common bean can be substantially enhanced by mineral N when applied in abundance. Reports on N accumulation by inoculated legumes in the field due to mineral N application

Table 1. Effect of applied N on N accumulation by soybean and common bean grown at 320-m and 1050-m elevations in Hawaii.

N applied†	320-m site			1050-m site		
	Full bloom	Podfill	Maturity‡	Full bloom	Podfill	Maturity
	<b>kg N ha<sup>-1</sup></b>					
	<b>Soybean</b>					
9	58	211	241	55	181	209
120	65	212	252	69	195	229
900	77	233	320	79	201	220
	<b>Common bean</b>					
9	80	126	142	64	126	149
120	83	125	147	67	150	170
900	113	171	189	86	149	192
<b>F-test or LSD (0.05)</b>						
Legume (L)	25	58	51	NS	41	29
Nitrogen (N)	10	26	26	15	14	20
L × N	NS	NS	NS	NS	NS	NS

† Total fertilizer N divided into equal amounts and applied at seeding, and at full bloom (R2) and podfill (21–25 d from R2) sampling times.

‡ Physiological maturity (R7).

Table 2. Effect of applied N on dry matter accumulation by soybean and common bean grown at 320-m and 1050-m elevations in Hawaii.

N applied†	320-m site			1050-m site		
	Full bloom	Podfill	Maturity‡	Full bloom	Podfill	Maturity
	kg ha <sup>-1</sup>					
	Soybean					
9	1411	4814	5971	1550	4466	6628
120	1436	5075	6985	1831	4851	6930
900	1645	5637	8439	1810	4709	6944
	Common bean					
9	2288	4894	5635	1978	3958	6324
120	2488	4836	6210	2237	4497	6572
900	2840	5269	6608	2277	4303	7260
F-test or LSD (0.05)						
Legume (L)	203	NS	718	326	NS	NS
Nitrogen (N)	248	NS	880	NS	316	NS
L × N	NS	NS	NS	NS	NS	NS

† Total fertilizer N divided into equal amounts and applied at seeding, and at full bloom (R2) and podfill (21–25 d from R2) sampling times.

‡ Physiological maturity (R7).

Table 3. Effect of applied N on N accumulation, N<sub>2</sub> fixation, and root characteristics of inoculated soybean and common bean grown for 33 d in potted soil† in the greenhouse.

Nitrogen applied (ug N g <sup>-1</sup> soil)	Total N‡		N derived from N <sub>2</sub> fixation _____ % _____	Root + nodule weight _____ g pl <sup>-1</sup> _____	Shoot to root ratio§	Mineral N uptake per unit root weight¶ mg N g root <sup>-1</sup>
	Amount	Rate				
	mg N pl <sup>-1</sup>	mg N pl <sup>-1</sup> d <sup>-1</sup>				
				Soybean		
0	121	3.68	92	1.38	2.32	8
133	203	6.16	27	2.46	2.43	68
400	220	6.67	1	1.49	2.89	146
				Common bean		
0	115	3.52	87	1.23	3.72	14
133	304	9.23	8	2.98	3.63	103
400	453	13.72	0	2.71	4.06	168
F-test or LSD (0.05)						
Legume (L)	22	0.7	4	0.24	0.32	8.3
Nitrogen (N)	9	0.3	4	0.19	0.33	10.1
L × N	***	***	**	***	NS	*

† Soil mixed with 0.8% (w/w) bagasse to immobilize available soil N.

‡ Total N in shoot, root and nodules.

§ Shoot weight divided by root + nodule weight.

¶ Mineral N in plant divided by root weight.

Table 4. Effect of applied N on the proportion of plant N derived from N<sub>2</sub> fixation† by soybean and common bean grown at 320-m and 1050-m elevations in Hawaii.

N applied‡	320-m site			1050-m site		
	Full bloom	Podfill	Maturity§	Full bloom	Podfil	Maturity
kg ha <sup>-1</sup>	%					
	Soybean					
9	70	75	69	23	39	32
120	39	55	44	12	28	21
900	24	12	13	-¶	-	-
	Common bean					
9	23	16	16	26	20	18
120	19	12	13	23	19	11
900	-	-	-	0	1	-
F-test or LSD (0.05)						
Legume (L)	18	54	26	NS	7	NS
Nitrogen (N)	15	10	13	6	11	6
L × N	NS	**	*	NS	NS	*

† N<sub>2</sub>-fixation determined by <sup>15</sup>N-isotope dilution method.

‡ Total fertilizer N divided into equal amounts and applied at seeding, and at full bloom (R5) and podfill (21–25 d from R2) sampling times.

§ Physiological maturity (R7).

¶ Negative value.

Table 5. Effect of applied N on  $N_2$  fixation† rates by soybean and common bean grown at 320-m and 1050-m elevations in Hawaii.

N applied‡ kg ha <sup>-1</sup>	320-m site				1050-m site			
	Veg.§	E. rep.¶	L. rep.#	Crop††	Veg.	E. rep.	L. rep.	Crop
	g N m <sup>-2</sup> d <sup>-1</sup>							
	Soybean							
9	0.12	0.52	0.04	0.21	0.02	0.23	—‡‡	0.06
120	0.07	0.40	—	0.13	0.02	0.19	—	0.04
900	0.05	0.04	0.05	0.05	0.00	—	—	—
	Common bean							
9	0.04	0.01	0.03	0.03	0.03	0.04	0.01	0.03
120	0.04	—	0.03	0.03	0.03	0.06	—	0.02
900	0.00	—	0.05	—	0.00	0.01	—	—
F-test or LSD (0.050)								
Legume (L)	0.03	0.11	NS	0.07	NS	0.10	NS	NS
Nitrogen (N)	0.04	0.18	NS	0.05	0.01	0.08	NS	0.01
L × N	NS	NS	NS	NS	NS	NS	NS	NS

†  $N_2$ -fixation determined by <sup>15</sup>N-isotope dilution method.

‡ Total fertilizer N divided into equal amounts and applied at seeding, and at full bloom (R2) and podfill (21–25 d from R2) sampling times.

§ Vegetative period; from seeding to R2.

¶ Early reproductive period; from R2 to podfill sampling.

# Late reproductive period; from podfill sampling to physiological maturity (R7).

†† Crop duration; from seeding to R7.

‡‡ Negative value.

have been variable ranging from no response to substantial increases in plant N (Bhangoo and Albritton, 1976; Cassman *et al.*, 1981; Diebert *et al.*, 1979; Harper, 1974; Lawn and Brun, 1974b; Piha and Munns, 1987; Weber, 1966; Westerman *et al.*, 1981). A review by Imsande (1989) indicates that soybean plants grown with excess of mineral N may accumulate less total N than well nodulated plants. Our data, however, indicate the contrary. The amount of mineral N applied or the application regimen used in some experiments may have been inadequate to maintain sufficient N in the soil solution to meet plant N assimilation potential. High levels of N in the root zone can increase yield through more favorable partitioning of dry matter to shoots than to roots compared to plants that rely on symbiotic  $N_2$  fixation (Cassman *et al.*, 1980; Tolley-Henry and Raper 1986; Vessey and Layzell, 1987). The increased shoot to root ratios at the higher N level observed for both soybean and common bean in the greenhouse (Table 3) also support the above suggestion.

Increased plant N from applied N was due primarily to increased rate of N accumulation by both legumes (Tables 3 and 6) although N application also delayed maturity slightly (2–5 d). Excluding soybean at the 1050-m site, the average relative increase in the rate of N accumulation during the crop cycle was 25% with 900 kg N ha<sup>-1</sup> compared to 9 kg N ha<sup>-1</sup>. However, the same response to applied N was not observed at all growth phases. The relative increase in N accumulation rate with increasing N applied was generally greatest during the vegetative (seeding to R2) and late reproductive periods (second sampling to R7). Applied N did not increase the rate of N accumulation of soybean during the early reproductive period (R2 to second sampling), but did increase the N accumulation rate of common bean during this period. The average increase in N accumulation rate during vegetative period at the highest N level was 37% in the field and 185% in the greenhouse compared to the lowest N level. During the late reproductive period,

soybean at the 320-m site and common bean at the 1050-m site, both receiving 900 kg N ha<sup>-1</sup>, assimilated N at about twice the rate of plants supplied with only 9 kg N ha<sup>-1</sup>. The fact that rates of N accumulation generally increased with applied N relatively more during vegetative and late reproductive periods, when  $N_2$  fixation was limited (Tables 5 and 6), indicates that growth during these stages was particularly limited without significant additions of mineral N.

Reduced response of soybean and common bean to mineral N application during the early reproductive phase was probably not due to decreased ability to take up mineral N, rather to an insufficient mineral N supply. It has been reported that soybean and other legumes do not assimilate mineral N efficiently during most of reproductive phase (Imsande, 1986; Imsande, 1989; Imsande and Edwards, 1988). Therefore, legumes may be N deficient during podfill regardless of mineral N availability (Imsande, 1986). Afza *et al.* (1987) reported that soybean can maintain mineral N uptake during the late reproductive phase. Our results show that soybean and common bean cultivars used in this study were capable of assimilating mineral N throughout reproductive growth. During the early reproductive phase, soybean supplied with 900 kg N ha<sup>-1</sup> assimilated N at a rate similar to plants supplied with 9 kg N ha<sup>-1</sup> which derived most of its N from  $N_2$  fixation (Tables 5 and 6). Therefore, mineral N availability during the reproductive phase, particularly in the late reproductive growth was critical to attain maximum N accumulation in these legume cultivars.

### Pattern of Nitrogen Accumulation

Soybean and common bean followed distinct patterns of N accumulation over time. The N assimilation patterns are clearer when N accumulation and growth periods are expressed on a relative basis (Fig. 1). Since the relative durations and N assimilation patterns of each legume were not different between the sites, combined data for the two sites are illustrated. On

Table 6. Effect of applied nitrogen on the rate of N accumulation by soybean and common bean grown at 320-m and 1050-m elevations in Hawaii.

N applied† kg ha <sup>-1</sup>	320-m site				1050-m site			
	Veg.‡	E. rep.§	L. rep.¶	Crop#	Veg.	E. rep.	L. rep.	Crop
	g N m <sup>-2</sup> d <sup>-1</sup>							
	Soybean							
9	0.17	0.67	0.15	0.31	0.11	0.50	0.07	0.18
120	0.19	0.64	0.16	0.30	0.14	0.50	0.08	0.19
900	0.22	0.68	0.34	0.38	0.15	0.49	0.04	0.18
	Common bean							
9	0.19	0.22	0.16	0.20	0.12	0.28	0.11	0.15
120	0.20	0.20	0.19	0.20	0.12	0.38	0.10	0.17
900	0.28	0.28	0.14	0.25	0.16	0.29	0.20	0.19
<i>F-test or LSD (0.05)</i>								
Legume (L)	NS	0.19	NS	0.06	NS	0.11	0.07	NS
Nitrogen (N)	0.03	NS	NS	0.04	0.03	NS	NS	NS
L × N	NS	NS	*	NS	NS	NS	*	NS

† Total fertilizer N divided in to equal amounts and applied at seeding, and at full bloom (R2) and podfill (21–25 d from R2) sampling times.

‡ Vegetative period; from seeding to R2.

§ Early reproductive period; from R2 to podfill sampling.

¶ Late reproductive period; from podfill sampling to physiological maturity (R7).

# Crop duration; from seeding to R7.

average, 50% of the total N accumulation by common bean occurred during vegetative growth, a period which accounted for 56% of its total crop duration, indicating that growth phase duration and N accumulation were nearly proportional. This proportional relationship between N accumulation and growth periods of common bean continued through R7. In contrast, only 28% of the total N of the soybean cultivar was assimilated during vegetative growth, a period that accounted for 44% of its total crop duration. The early reproductive period of soybean was the period of most rapid N accumulation when 57% of the total N was assimilated in 25% of its total crop duration.

The distinct patterns of N accumulation by the soy-

bean and common bean cultivars help to explain the differences in N<sub>2</sub>-fixation of the two. Data in Tables 3, 4, and 5 indicate that N derived from N<sub>2</sub> fixation by common bean was substantially lower than soybean in almost all instances. There seems to be an association between the rate of N<sub>2</sub> fixation and the late of N accumulation (Tables 5 and 6), especially in the soybean cultivar. Rapid N accumulation during the early reproductive period of soybean was also the time when N<sub>2</sub> fixation reached a maximum in the field. Nitrogen fixation reached a maximum rate of 0.52 kg N ha<sup>-1</sup> d<sup>-1</sup> at the 320-m site supplying 78% of the N requirement during the early reproductive phase. High rate of N<sub>2</sub>-fixation during the early reproductive

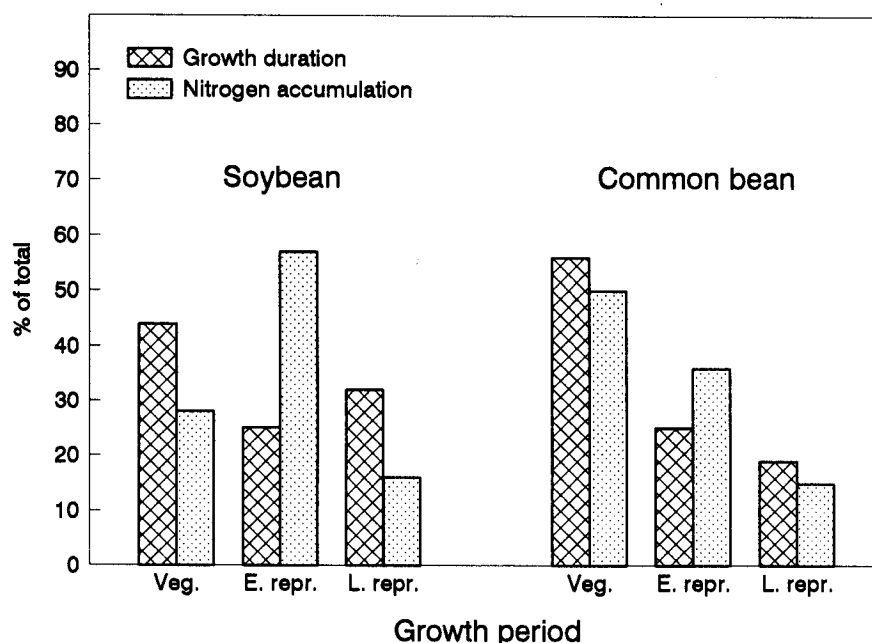


Fig. 1. Relative N accumulation and growth periods of soybean and common bean across three N levels and two elevations. Veg. = Vegetative period (seeding to R2); E. repr. = Early reproductive period (21–25 d period from R2); L. repr. = Late reproductive period (end of E. repr. to R7).

**Table 7. Effect of applied N on mineral nitrogen uptake efficiency† of soybean and common bean grown at 320-m and 1050-m elevations in Hawaii.**

N applied‡ kg ha <sup>-1</sup>	320-m site			1050-m site		
	Full bloom	Podfill	Maturity§	Full bloom	Podfill	Maturity
	%					
	Soybean					
9	5	12	26	8	50	45
120	12	26	37	9	43	49
900	11	21	21	9	18	16
	Common bean					
9	12	22	24	17	40	50
120	16	26	29	15	37	51
900	21	20	15	15	17	13
F-test or LSD (0.05)						
Legume (L)	4	NS	NS	7	NS	NS
Nitrogen (N)	3	3	7	NS	7	5
L × N	NS	**	NS	NS	NS	NS

† Nitrogen uptake efficiency determined by <sup>15</sup>N isotope and is the percent of applied fertilizer nitrogen assimilated by the plant.

‡ Total fertilizer N divided into equal amounts and applied at seeding, and at full bloom (R2) and podfill (21–25 d from R2) sampling times.

§ Physiological maturity (R7).

phase of soybean has been reported (Zapata *et al.*, 1987). The period of greater N demand by soybean, therefore, occurred at a time when the symbiotic system was fully established. In contrast, common bean accumulated more than half its total N during vegetative growth when the symbiotic system was still being established.

### Efficiency of Mineral Nitrogen Uptake

The apparent differences in N<sub>2</sub> fixation between the soybean and the common bean cultivars may also have been associated with differences in mineral N uptake characteristics. With a given level of soil N availability, the relative importance of mineral N and N<sub>2</sub> fixation may be determined by the mineral N uptake efficiency of the legume, defined here as “N uptake per unit N in the soil.” The lower amount and proportion of N<sub>2</sub> fixed by the common bean cultivars indicate their greater reliance on mineral N uptake compared to soybean. Nitrogen-15 isotope analysis indicated that the mineral N uptake efficiency (<sup>15</sup>N fertilizer was used as a proxy to measure crop uptake efficiency of mineral N) of common bean was two times greater than soybean at R2 in the field (Table 7). The greenhouse data also support this observation. At all N levels, a greater amount of mineral N was assimilated by the common bean cultivar than soybean at 33 d in the greenhouse (Table 3). Compared to soybean, more of the early N requirement of common bean was met by mineral N uptake. Consistent with the greater reliance on mineral N by common bean, KCl-extractable soil N was significantly lower in common bean plots than soybean plots at R2 (Tables 8), and when 9 kg N ha<sup>-1</sup> was applied, KCl-extractable N remained lower than soybean plots up to the second sampling time. Since N mineralization would be the same in both soybean and common bean plots within a site, lower extractable N in common bean plots is due to greater plant mineral N uptake.

Root growth and partitioning of dry matter and N between shoots and roots measured at 33 d in the greenhouse (Table 3) may help explain the greater

**Table 8. Effect of applied N on KCl-extracted soil N (NO<sub>3</sub> + NH<sub>4</sub>) during growth of soybean and common bean grown at 320-m and 1050-m elevations in Hawaii.**

N applied†	320-m site			1050-m site		
	Full bloom	Pod-fill	Matur-ity†	Full bloom	Pod-fill	Matur-ity
kg ha <sup>-1</sup>	g N kg soil <sup>-1</sup>					
	Soybean					
9	0.017	0.017	0.015	0.032	0.021	0.016
120	0.027	0.022	0.015	0.036	0.023	0.015
900	0.078	0.075	0.145	0.124	0.235	0.196
	Common bean					
9	0.010	0.012	0.016	0.024	0.016	0.016
120	0.016	0.015	0.013	0.036	0.030	0.024
900	0.024	0.100	0.179	0.115	0.180	0.251
F-test or LSD (0.05)						
Legume (L)	0.020	NS	NS	NS	NS	0.018
Nitrogen (N)	0.013	0.011	0.008	0.019	0.019	0.019
L × N	**	*	**	NS	*	*

† Total fertilizer N divided into equal amounts and applied at seeding, and at full bloom (R2) and podfill (21–25 d from R2) sampling times.

‡ Physiological maturity (R7)

mineral N uptake efficiency of common bean compared to soybean. Common bean not only had 61% greater root biomass than soybean, but also maintained more shoot weight per unit root weight. Further, N accumulated per unit root weight was higher for common bean than soybean. The data indicate that root characteristics of common bean favor greater mineral N uptake compared to soybean.

### CONCLUSIONS

Attaining maximum N accumulation by common bean and soybean in the field required greater mineral N levels applied at more frequent intervals than has been reported in other experiments. The data presented here show that when the proportion of plant N derived from N<sub>2</sub> fixation is substantial, total N accumulation is less than the maximum attainable with adequate supply of mineral N. Total N and yield was the least when N<sub>2</sub> fixation was the greatest in both

soybean and common bean. The pattern of N accumulation in soybean was characterized by low accumulation rate during vegetative growth, followed by rapid accumulation during the early reproductive period. Nitrogen accumulation by common bean was more evenly apportioned over the growth cycle than soybean. Nitrogen accumulation by soybean followed a pattern similar to the ontogeny of the symbiotic  $N_2$ -fixing system; the period of maximum N accumulation coincided with the period of peak  $N_2$  fixation. Common bean, instead relied more on soil derived-N due to greater mineral N uptake efficiency than soybean, especially during the vegetative growth. The greater mineral N uptake by common bean might be due to its greater root biomass, shoot to root ratio, and N uptake per unit root biomass. Maximizing both  $N_2$  fixation and yield might entail timing the mineral N supply during vegetative and late reproductive phases. The limited  $N_2$  fixation capacity of common bean compared to other legumes might be due not only to lower N requirements but to N assimilation traits favoring mineral N uptake. Enhancing  $N_2$  fixation by common bean might require strategies to decrease mineral N uptake efficiency and delay the growth stage where rapid N accumulation occurs.

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