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Comparative study of N uptake and distribution in three lines of common bean (*Phaseolus vulgaris* L.) at early pod filling stage

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Summary The uptake and distribution of $^{15}NH_4^+$, $^{15}NO_3^-$ and $^{15}N_2$ was studied in greenhouse-grown beans (*Phaseolus vulgaris* L.) with a commercial cultivar and 2 recombinant inbred backcross lines; ^{15}N was supplied in the nutrient solution at the R3 (50% bloom) stage. Plants were harvested 1, 5 and 10 days after treatment, and were separated into nodules, roots, stems, mature leaflets, immature leaflets, and flowers/fruits. All 3 lines showed rapid increases in the N content of flowers/fruits after the R3 stage. However, the percentage N in these tissues decreased after the R3 stage.

One of the recombinant lines showed a greater uptake of NH_4^+ than the other 2 lines. Rates of $^{15}N_2$ fixation and NO_3^- uptake were similar for all 3 lines. N_2 fixation estimated from total N content showed the 2 recombinant lines with 24 and 34 percent greater activity than the commercial cultivar.

Distribution of ^{15}N at the whole plant level was similar for all 3 lines for a similar N source. $^{15}NO_3^-$ was transported first to leaflets and the label then moved into flowers/fruits. Transport of fixed N_2 was from the nodules to roots, stems and into flowers/fruits; usually less than 10 percent entered the leaflets. This indicates that N_2 fixation furnishes N directly to flowers/fruits with over 50 percent of the fixed N being deposited into flowers/fruits within 5 days after treatment.

Introduction

There has been a growing emphasis on increasing N_2 fixation and yield of grain and forage legumes with the continuing increase in world food demand. Among techniques currently being employed to attain these goals is that of plant breeding and selection of cultivars superior for specific traits. *Phaseolus vulgaris* L., the common bean, is one target of such intense breeding. In addition to selecting superior cultivars, it is necessary to characterize them in order to understand what changes were effected during the breeding program. The three lines of *P. vulgaris* used in this study were a standard commercial cultivar, "Sanilac", and 2 recombinant inbred backcross lines, UW24-21 and UW24-55. Both recombinant lines have backgrounds similar to that of the recurrent parent, "Sanilac", but differ for several traits[9]. The recombinant lines were developed by the inbred backcross line method.[5] Both recombinant lines yield seed and total plant dry matter approximately twice that of "Sanilac". Acetylene reduction (AR) by UW24-21

is approximately 6× that of "Sanilac", whereas AR by UW24-55 is similar to that of "Sanilac".

Our study focused on characterizing the uptake and distribution of N by *Phaseolus vulgaris* by studying 3 lines of common bean. Specific objectives of our study were: (1) to detect any changes in N content or percentage N of the various tissues during early podfill; (2) to determine the distribution, at the whole plant level, of N assimilated by *P. vulgaris* from NH_4^+, NO_3^- or N_2; (3) to compare distribution patterns of assimilated N and rates of N_2 fixation and uptake of NH_4^+ or NO_3^- by UW24-21 and UW24-55 with that of the recurrent parent, "Sanilac".

Materials and methods

Phaseolus vulgaris L. (lines "Sanilac", UW24-21, UW24-55) were grown in 15 cm diam. × 15 cm deep pots containing vermiculite/perlite (1/1) under greenhouse conditions. Seeds, coated with a 4-strain mixture of *Rhizobium phaseoli* (Allen 413-2; CIAT 676; CIAT 75; and Kimberly 5; Nitragin, Milwaukee, WI), were planted 3 per pot, and later seedlings were thinned to one per pot. Plants were fertilized twice weekly with the nutrient solution described by Summerfield *et al.*[10], modified to exclude N. At the R3 (50% bloom) stage (see Lebaron[3]) each line was treated as follows: 9 plants each received 100 ml of 5 m*M* $(^{15}NH_4)_2SO_4$, 7.80 atom % ^{15}N; 9 plants each received 100 ml of 10 m*M* $K^{15}NO_3$, 7.97 atom % ^{15}N; 9 plants each received 100 ml of 5 m*M* $^{15}NH_4NO_3$, 11.70 atom % ^{15}N in NH_4^+. All ^{15}N fertilizers were administered by pouring the solution into the pot. The roots of 9 plants were enclosed in 925 × 200 nm pyrex test tubes fitted with 2 hole #4 rubber stoppers. One hole was fitted with a short piece of 8 mm diam. glass tubing and a vaccine stopper. The rubber stopper was slit along the other hole and sealed around the plant stem with plasticine modeling clay. The air in each tube was evacuated and each tube was filled with an atmosphere containing 20% $^{15}N_2$ (40–50 atom % ^{15}N), 20% O_2 and 60% Ar. $^{15}N_2$ was generated by the procedure outlined by Burris[1]. After 3 h, assays were terminated by exposing $^{15}N_2$-treated plants to a natural atmosphere and leaching NH_4^+- and NO_3^--treated plants 4 times with water and once with nutrient solution.

For each line, 3 plants from each N treatment were harvested at 1, 5 and 10 days after treatment (DAT). Each plant was separated into nodules, roots, stems/petioles, mature leaflets, immature leaflets, and flowers/fruits. Control plants of each line were untreated and harvested together with treated plants. Plant parts were dried at 80°C for 48 h, weighed, ground in a Wiley mill, and samples subjected to Kjeldahl digestion (NO_3^- not recovered) and distillation[2]. Ammonia N contents of the distillates were determined by the Nessler's reaction[2]. Samples then were concentrated, converted to N_2 by hypobromite oxidation and analyzed with a MAT 250 isotope ratio mass spectrometer.

Results

A sufficient number of plants (minimum required for experiment = 39 for each line) of "Sanilac", UW24-21 and UW24-55 reached the R3 (50% bloom) stage by 37, 39 and 41 days after planting, respectively. All three lines showed rapid increases in the N content of flowers/fruits after the R3 stage (Fig. 1). No other tissues showed increases in N content after the R3 stage. By the end of the experiment (10 DAT), the recombinant lines UW24-21 and UW24-55 had N contents of the

flowers/fruits (66.8 and 64.7 mg N, respectively) greater than that of "Sanilac" (52.4 mg N).

The percentage N in the various tissues at 1, 5 and 10 DAT are shown in Table 1. At a given harvest, percent N was similar between different lines. Percent N was highest in the nodules (4.9–5.9%) and less (3.1–4.7%) in the leaflets and flowers/fruits. The roots and stems had the lowest percent N of any tissues (1.3–2.2%). Although there were rapid increases in total N of flowers/fruits (Fig. 1), all three lines showed decreases in percent N in these tissues after the R3 stage (Table 1).

Table 1. Percentage N in *P. vulgaris* plant tissues at 1, 5 and 10 days after R3 stage. Each value is expressed as mean percent N of 13 plants

Line	DAT[1]	Nodules	Roots	Stems	Mature leaflets	Immature leaflets	Flowers fruits
Sanilac	1	5.8	2.0	1.7	3.8	4.5	4.5
	5	5.6	1.8	1.9	3.9	4.1	3.5
	10	5.4	1.6	2.2	3.4	3.6	3.1
UW24-21	1	5.8	1.7	1.6	3.9	4.3	4.7
	5	4.9	1.3	1.6	3.7	3.0	3.3
	10	5.9	1.6	1.9	4.2	4.3	3.0
UW24-55	1	5.8	1.5	1.4	3.6	3.7	4.2
	5	5.4	1.3	1.8	3.5	4.3	3.1
	10	5.1	1.5	1.8	3.2	3.1	3.0
Max. % S.E.[2]		2	3	3	5	5	2

[1] Days after treatment.
[2] Maximum % standard error of the mean.

Because the plants received no fertilizer N, all plant N was derived from the seed or the atmosphere (N_2 fixation). If the average seed N is subtracted from the total plant N, the result gives a conservative estimate of N_2 fixation (Table 2). This analysis indicates that the lines selected previously, UW24-21 and UW24-55, have N_2 fixation rates 24 and 34 percent greater (respectively) than that of the recurrent parent, 'Sanilac".

The uptake of ^{15}N as NH_4^+, NO_3^-, NH_4NO_3, and N_2 is shown in Table 3. UW24-55 showed rates of NH_4^+ uptake similar to that of "Sanilac", and each showed lower rates than that of UW24-21. Uptake rates of NO_3^- were similar for all three lines. Rates of $^{15}N_2$ fixation were similar, although "Sanilac" was slightly more active than the other two lines. However, due to the large maximum % S.E., no significant difference appears in the rates of N_2 fixation. Rates of $^{15}N_2$ fixation

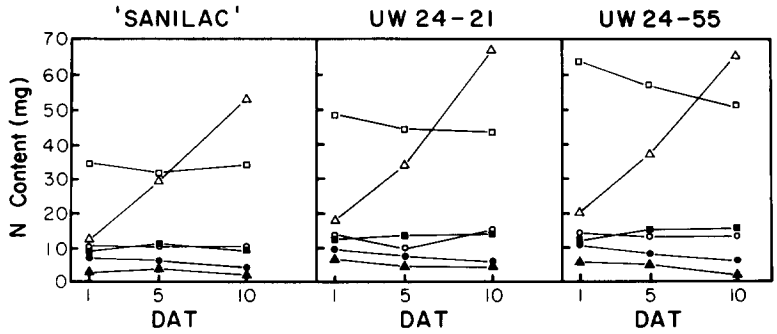

Fig. 1. N content in *P. vulgaris* tissues at 1, 5 and 10 days after R3 stage. Each value is expressed as mean mg N of 13 plants. • Nodules; ○ roots; ▪ stems; □ mature leaflets; ▲ immature leaflets; △ flowers and fruits. DAT = Days After Treatment.

reported here for "Sanilac" and UW24-55 agree with acetylene reduction (AR) rates reported by Rosas[9] (when a conversion factor of 3:1 is used). However, the $^{15}N_2$ fixation rate reported here for UW24-21 is much lower than the AR rate reported by Rosas[9].

Figure 2 shows the distribution of ^{15}N at the whole plant level for the different N treatments. By 10 DAT UW24-55 transported less NH_4^+ (from $(NH_4)_2SO_4$) to flowers/fruits and more to mature leaflets than did the other two lines. Otherwise, NH_4^+ distribution was similar between the lines when taken up either from $(NH_4)_2SO_4$ or NH_4NO_3. Distribution of ^{15}N from NO_3^- was similar for all three lines with ^{15}N first appearing in largest amounts in the leaflets, then moving into the flowers/fruits. However, at 10 DAT, only 50–55 percent of the NO_3^-

Table 2. N_2 fixation rates of *P. vulgaris* lines determined by total N content. Each value represents the mean of 3 plants

Line	DAT[1]	Total N fixed mg/plant	Age (days)	mg N fixed/ day/plant	Mean of nine plants
Sanilac	1	71.6[2]	38	1.88	
	5	87.0	42	2.07	2.07
	10	106.5	47	2.27	
UW24-21	1	99.4	40	2.49	
	5	104.1	44	2.37	2.58
	10	140.6	49	2.87	
UW24-55	1	117.9	42	2.81	
	5	124.6	46	2.71	2.77
	10	142.9	51	2.80	

[1] Days after treatment.
[2] Total Plant N – mean original seed N (Sanilac, 6.76: UW24-21, 7.35: UW24-55, 6.53 mg N).

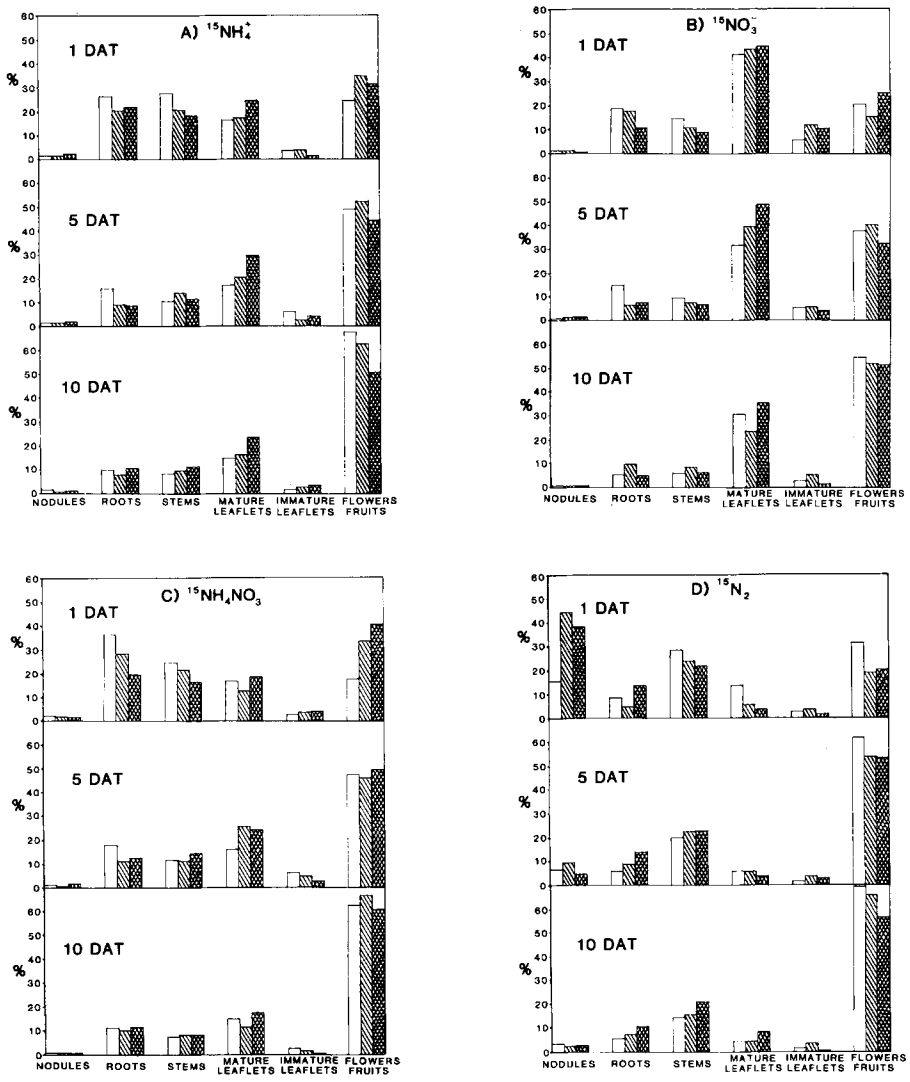

Fig. 2. Distribution of ^{15}N in *P. vulgaris*, assimilated from A) $^{15}NH_4^+$; B) $^{15}NO_3^-$; C) $^{15}NH_4NO_3$; D) $^{15}N_2$. Each value represents the mean of 3 plants, expressed as percentage of total N assimilated. DAT = Days After Treatment. □ "Sanilac"; ▨ UW24-21; ■ UW24-55.

assimilated was in flowers/fruits, as compared to 55–70 percent for NH_4^+ and N_2.

Transport of fixed N_2 was from nodules to roots, stems, and into flowers/fruits with usually less than 10 percent entering leaflets (the only exception is 13.5% for "Sanilac" at 1 DAT). By 5 DAT, over 50%

Table 3. N uptake from $(^{15}NH_4)_2SO_4$, $K^{15}NO_3$, $^{15}NH_4NO_3$, and $^{15}N_2$ by *P. vulgaris*. Each value represents the mean of 3 plants exposed to ^{15}N for 3 h, expressed as mg N/plant

Line	DAT[1]	$^{15}NH_4^+$	$^{15}NO_3^-$	$^{15}NH_4NO_3$	$^{15}N_2$
Sanilac	1	2.94	1.30	1.59	0.078
	5	4.94	2.01	2.46	0.052
	10	4.31	2.25	2.65	0.059
	$\bar{x}$	4.06	1.85	2.23	0.063
UW24-21	1	4.68	1.49	2.49	0.048
	5	4.65	2.15	2.57	0.054
	10	6.89	2.67	3.25	0.036
	$\bar{x}$	5.41	2.10	2.77	0.046
UW24-55	1	3.86	1.97	2.28	0.036
	5	3.77	2.12	2.28	0.043
	10	4.99	2.70	3.05	0.067
	$\bar{x}$	4.21	2.26	2.54	0.049
Max. % S.E.[2]		10	14	14	50

$\bar{x}$ = Mean of 9 plants.
[1] Days After Treatment.
[2] Maximum % standard error of the mean.

of the N fixed from N_2 had been deposited in flowers/fruits in all three lines. These high percentages of distribution were not observed with any other N source.

Discussion

Phaseolus vulgaris shows a distinct relationship between total N uptake and seasonal AR or seed yield[12]. Because of this relationship, three common bean lines used in our study ("Sanilac", UW24-21, UW24-55) were selected to represent different combinations of AR and seed yields. All treatments in our study were administered to plants in the R3 (50% bloom) stage when *P. vulgaris* has a maximal rate of AR or N_2 fixation[8,11,12].

All three lines displayed an increase in N content in flowers/fruits after the R3 stage, which was to be expected. Ohyama[7] showed a similar increase in seed N for soybeans during pod filling, while the total N content of the pod shells remained unchanged. This may suggest that the increase in the N content of flowers/fruits reported here is due to an increase in seed N.

Common beans are similar to other nodulating legumes for percent N in the various tissues. The percent N in nodules is the highest of all plant tissues, with lower percent N in leaflets and flowers/fruits. The

roots and stems have the lowest percent N of all tissues. The percent N in nodules, roots and reproductive parts of common bean are the same as those reported for soybeans[7], although common bean has higher percent N in stems and leaflets. Our study shows a noticeable decrease in percent N for flowers/fruits of common bean during pod development. This may indicate greater N loading into fruits during very early pod development and relatively less N loading during mid and late pod development. This is in contrast to soybeans where percentage N remains almost constant[7].

The $^{15}N_2$ fixation rates reported here for "Sanilac" and UW24-55 correspond very closely with AR values determined at the same physiological stage, under similar conditions in a previous study[9]. However, we did not find significant differences in $^{15}N_2$ fixation rates between the three lines. Under similar conditions UW24-21 was shown to have significantly higher AR rates than "Sanilac" or UW24-55.[9] It must be noted, however, that a one time assay (AR or $^{15}N_2$) is not satisfactory as a method to rank cultivars or lines[8]. The total N fixed per plant (Table 2) was more closely related to yields under similar conditions[9] than were $^{15}N_2$ fixation rates determined at the R3 stage.

The uptake and distribution of NO^-_3 by *P. vulgaris* in our study is similar to that reported for soybeans[7] and peas[4]. NO^-_3 is transported first to leaflets and then is assimilated and transported to flowers/fruits. Lewis and Pate[4] suggest leaves as the principal tissue of uptake and assimilation of NO^-_3 in peas. Ohyama[7] has shown this to be the case in soybeans, as does our study with common beans. Distribution of fixed N_2 in *P. vulgaris* shows direct assimilation into flowers/fruits with very little entering leaflets during early pod fill. This same pattern has been reported for soybeans; however, McNeil and LaRue[6] showed a much greater amount of ^{15}N from $^{15}N_2$ entering leaflets of soybeans from 1-14 days after treatment (approximately 40–50%). Our results suggest that in common beans, N_2 fixation at and beyond the R3 stage furnishes N directly to reproductive parts (flowers/fruits). Uptake of other forms of N results in large percentages of assimilated N entering vegetative parts (leaflets) during pod fill.

Rennie and Kemp[8] reported average total N_2 fixation at 46–135 mg N/plant by anthesis (R1) and 140–435 mg N/plant by maturity (R9) for various cultivars of beans. Rates reported here for UW24-21 and UW24-55 at R3 stage (Table 2) show these two lines to be very similar in N_2 fixation activity to the vigorously fixing cultivars used in the Rennie and Kemp[8] study.

In conclusion, N_2 fixation in *Phaseolus vulgaris* furnishes N directly to flowers and fruits during early pod fill, and there did not appear to

be any major differences in distribution patterns among lines. The use of a one time assay as a method of ranking N_2 fixation in lines of *P. vulgaris* does not appear to be satisfactory. The recombinant lines of UW24-21 and UW24-55 show increased N_2 fixation over the recurrent parent (when measured by total N accumulation) which indicates that N_2 fixation can be improved through plant breeding techniques.

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