Effects of ^{15}N-nitrate fertilization on yield and dinitrogen fixation in common bean (*Phaseolus vulgaris* L.)

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SUMMARY

The isotope dilution method was used to examine the influence of ^{15}N-nitrate fertilizer on dinitrogen fixation and dry matter production in young common bean (*Phaseolus vulgaris* L.) plant in a phytotron experiment. There were slight variations in dry matter yield with significantly different N concentrations of the bean crop in response to fertilizer nitrogen application. The proportion of total N derived from N_2 fixation in the vegetative plant parts decreased from 43.5 to 4.7 per cent as the rate of fertilizer nitrogen increased from 0 to 150 kg N/ha. However, the degree of inhibition of N_2 fixation by applied nitrate-N was not different at the 75 and 150 kg N/ha rates. Inoculation did not affect the relative contributions of fertilizer and soil nitrogen to total shoot N at the 75 and 150 kg N/ha levels.

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RÉSUMÉ

SAFO, E. Y.: *Les effets de l'application del'azote ^{15}N-nitrate sur le rendement et la fixation d'azote dans l'harricot* (Phaseolus vulgaris *L.*). La méthode de la dilution isotope a été utilisée pour examiner l'effet de l'engrais ^{15}N-nitrate sur la fixation d'azote et la production de matière sèche dans des jeunes plantes d'harricot (*Phaseolus vulgaris* L.) dans une expérience à phytotron. Il y a eu de petites variations de rendements de la matière sèche d'harricot avec les différentes concentrations d'azote appliqué. La proportion du N-total obténue à partir de la fixation de N_2 dans les parties vegetatifs de plante a baissé de 43.5 à 4.7 % avec l'augmentation de taux d'engrais de 0 à 150 kg N/ha. Pourtant, le degré d'inhibition de la fixation de N_2 par le N-nitrate appliqué n'était pas différent aux taux 75 et 150 kg N/ha. L'inoculation n'a pas influencée les contributions relatifs d'azote d'engrais et du sol à la teneur totale en azote des plantes aux niveaux 75 et 150 kg N/ha.

Introduction

Research on dinitrogen fixation has received considerable attention in recent years because of the increasing cost of nitrogen fertilizers. The quantitative and kinetic characteristics of the fixation process in the field have previously been determined only with difficulty (Stewart, 1966). However, the development of the rapid and sensitive acetylene reduction assay provided methodology for the convenient and detailed determination of these fundamental characteristics. This method is nonetheless known to be of little use in obtaining an estimate of N_2 fixation over a growing season (Witty, 1983).

More recent developments in ^{15}N tracer techniques have encouraged their intensive use in N_2 fixation research. These techniques play a major role in assessing the contribution and exploitation of the natural process of biological N_2 fixation in world food production. The isotope dilution procedure, which is based on the differential dilution in the plant of ^{15}N-labelled fertilizer by soil and fixed nitrogen (Fried & Broeshart, 1975; Fried & Middelboe, 1977), appears to offer a potentially-accurate method for assessing seasonal N_2 fixation (Witty, 1983). Estimates obtained with this method vary depending, among other things, on the non-fixing control crop chosen (Wagner & Zapata,

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1982). The availability of the isotope dilution method obviates the need to resort to indirect measurements such as nodule mass, nodule number, nodule placement or leghaemoglobin concentration for evaluation of N_2-fixing activity of the symbiotic system.

It has been estimated by various researchers (Rennie & Kemp, 1983a; Ruschal *et al.*, 1982) that some bean cultivars may obtain between 37 and 68 per cent of their plant N requirements from N_2 fixation and may benefit from 20 - 115 kg N fixed/ha/cycle.

Several factors, such as soil *p*H, levels of applied and soil N, water status of soil, light, temperature, planting density, which limit plant growth may also adversely influence nodulation and N_2 fixation (Antoninew & Sprent, 1978; Graham, 1981; Rennie & Kemp, 1981). An understanding of the effect of different levels of fertilizer nitrogen on nitrogen nutrition and yield of legumes is of particular importance in order to obtain the maximum agronomic benefits from N_2 fixation. This study was undertaken to examine the use of ^{15}N-isotope dilution procedure in the assessment of fertilizer nitrogen effects on dinitrogen fixation in common bean (*Phaseolus vulgaris* L.).

Materials and methods

Three kilograms of a Norwegian Gleyic Cambisol (FAO) (sand - 60 per cent, silt - 28 per cent, clay - 12 per cent; *p*H, 5.9; organic matter, 1.8 per cent; total N, 0.16 per cent, were placed in plastic pots (top diameter - 21 cm, bottom diameter - 15 cm and height - 22 cm). A solution containing $Ca(^{15}NO_3)_2$ with 5.772 atom per cent excess was uniformly applied at rates equivalent to 0, 75 and 150 kg N/ha.

Seeds of the common bean (*Phaseolus vulgaris* cv. Jolanco) were surface sterilized using 0.1 per cent acidified mercuric chloride and either surface inoculated with a thick suspension of *Rhizobium phaseoli* strain 458 (from Sweden), or left uninoculated as a non-fixing control. Five bean seeds were planted per pot. The experimental design was a randomized block with four replications.

The plants were thinned to two 10 days after germination and were grown in a phytotron at 21 - 24 °C with a 16-h light and 8-h dark period, light intensity on the average being 12,000 lux and relative humidity 75 - 80 per cent.

Plants were harvested by cutting at the soil level 55 days after sowing at growth stage R3 (LeBaron, 1974), dried at 70 °C and ground in a Wiley mill to pass a 60 mesh screen. Total N was determined by Kjeldahl procedure (Bremner & Mulvaney, 1982). ^{15}N enrichment was determined with a JASCO N-150 emission spectrometer.

Since the natural environment has an ^{15}N abundance of 0.37 per cent (0.3663 per cent), the measured ^{15}N-abundances were corrected for natural abundance, 0.37, to get ^{15}N excesses for calculation of relevant ^{15}N-indices.

The percent nitrogen derived from the fertilizer (percent NdfF) was calculated by:

$$\%\ \text{NdfF} = \frac{\text{Atom \% }^{15}\text{N excess in plant material}}{\text{Atom \% }^{15}\text{N excess in fertilizer}} \times 100$$

The percent nitrogen fixed (percent Ndfa) was calculated from the ^{15}N isotope dilution using the equation:

$$\%\ \text{Ndfa} = 1 - \frac{\text{Atom \% }^{15}\text{N excess (fixing system)}}{\text{Atom \% }^{15}\text{N excess (non-fixing system)}} \times 100$$

Since for a nodulating crop, total N yield = N yield from fertilizer + N yield from soil + N yield from fixation, the amount of N derived by nodulating crop from soil (Ndfs) was calculated as follows:

$$\text{Ndfs} = \text{Total N yield} - (\text{NdfF} + \text{Ndfa})$$

The A-value is defined mathematically as:

$$\text{A-value} = \frac{(100\ -\ \%\text{NdfF})}{\%\ \text{NdfF}} \times \text{Rate fert. N applied (kg N/ha)}$$

The percent fertilizer use efficiency (percent FUE) was calculated from the equation:

$$\%\ \text{FUE} = \frac{\%\ \text{NdfF} \times \text{Total N in plant (kg N/ha)}}{\text{Rate fert. N applied (kg N/ha)}} \times 100$$

Results and discussion

Dry matter yield and N content

Data in Table 1 indicate that the dry matter yield of inoculated beans were not significantly different from the uninoculated non-fixing control plants at the various levels of fertilizer nitrogen application. Increasing rates of fertilizer also did not affect the yields.

TABLE 1

Yield and Nitrogen Content of Phaseolus vulgaris *L. as Affected by* ^{15}*N-nitrate Fertilization and Inoculation with* Rhizobium phaseoli

Fertilizer N rate (kg/N/ha)	*Dry matter yield (g/pot)*		*Nitrogen content (g/100 g DM)*		*Total nitrogen (mg/pot)*	
	Inoculated	*Uninoculated*	*Inoculated*	*Uninoculated*	*Inoculated*	*Uninoculated*
0	2.65	2.55	2.46	2.07	65.2	52.8
75	3.28	2.87	3.62	4.11	118.7	117.9
150	3.23	2.83	4.58	4.63	147.9	131.0
LSD (P=0.05)	Dry matter yield		N content		Total N	
	NS		0.44		16.5	

NS - Not significant

In both inoculated and uninoculated plants, there were significant increases in nitrogen content with increasing rate of nitrate application. The lack of significant differences in dry matter yield and the significantly different N concentration of the bean crop resulted in differences in total uptake of N in response to the fertilizer application. Inoculation increased total N of the beans.

Isotope derived data

Table 2 shows the isotope derived data : atom percent ^{15}N excess, percent nitrogen derived from fertilizer percent (NdfF), percent nitrogen derived from soil (percent Ndfs), percent nitrogen fixed (percent Ndfa), A-value and fertilizer use efficiency (FUE).

There were significant increases in the atom percent ^{15}N excess and percent NdfF for both inoculated and uninoculated beans with fertilizer N-rates. This was a possible indication of decreasing N_2 fixation with fertilizer N-rates. The atom percent ^{15}N excess and the percent NdfF values were lower at all fertilizer levels in the inoculated than uninoculated control beans, again suggesting that N_2 fixation was occurring.

The percent Ndfs decreased with increasing rate of fertilizer N for both inoculated and uninoculated beans. The A-value increased with fertilizer rate. The percent FUE decreased with fertilizer rate in both inoculated and uninoculated beans.

The percent nitrogen in the plant derived from the atmosphere was calculated using the uninoculated bean at identical fertilizer levels as the non-fixing control (Fried & Middelboe, 1977). The contribution of dinitrogen fixation to total plant nitrogen decreased from 43.5 to 4.7 per cent as the rate of fertilizer nitrogen increased from 0 to 150 kg N/ha (Table 2). Similar results were reported by Rennie (1979). He found that plant N derived from the atmosphere decreased from 33 to 0 per cent as fertilizer N application increased from 0 to 320 kg N/ha. Data here indicate, however, that with the short-growth period of the experiment the degree of inhibition of dinitrogen fixation by applied nitrate-N was not different at the 75 and 150 kg N/ha rates. It is difficult to make generalizations on the effect of fertilizer N on dinitrogen fixation in this experiment

TABLE 2

Isotope Derived Data for Phaseolus vulgaris *as Affected by* ^{15}*N-nitrate Fertilization and Inoculation with* Rhizobium phaseoli

Fertilizer N rate (kg N/ha)	*Atom % ^{15}N excess*		*% NdfF*		*% Ndfa*		*% Ndfs***		*A-value*		*% FUE*	
	Inoc.	*Uninoc.*	*Inoc.*	*Uninoc.*	*Inoc.*	*Uninoc.*	*Inoc.*	*Uninoc.*	*Inoc.*	*Uninoc.*	*Inoc.*	*Uninoc.*
0	0.044	0.078	-	-	43.5	-	56.50	-	-	-	-	-
75	2.343	2.465	40.58	42.71	4.9	-	54.52	57.29	110	101	47.5	48.9
150	3.030	3.179	52.49	55.07	4.7	-	42.81	44.93	136	123	38.5	35.7
LSD (P=0.05)	0.196		3.09		15.9		9.27		15		4.9	

** Calculated by difference

since it is probable that if the plant had been allowed to grow to full maturity, the pattern of fractional uptake of nitrogen in the plant, derived from soil-N, fertilizer-N and from N_2-fixation, would have been different.

Table 3 shows that using the isotope dilution technique, it is possible to partition quantitatively total nitrogen yield in shoots into contributions from fertilizer (Nf), soil (Ns) and N_2 fixation (Na). Using the principle of ^{15}N isotope dilution (Rennie, Rennie & Fried, 1978), these absolute amounts were calculated by multiplying the fractions - NdfF, Ndfs and Ndfa, obtained from ^{15}N-yield data (Table 2) by corresponding total N-yields (Table 1). Even though these are relative contributions, they are useful in the assessment of the fixation process under different growth conditions, especially under varied N fertilizer rates with or without inoculation. Such data may also provide an indication of the time of depletion of fertilizer and soil nitrogen and the onset of dinitrogen fixation. The amount of N_2 fixed were not significantly different for the 75 and 150 kg N/ha fertilizer treatments. However, the quantity of N_2 fixed under the no fertilizer treatment was significantly different from the 75 and 150 kgN/ha rates. These results are identical to that observed by Rennie & Kemp (1983b) who examined the N_2 fixation in six cultivars of *Phaseolus vulgaris* at two levels of applied N. They found that the amount of N_2 fixed varied with cultivar but not with rate of applied fertilizer N. Inoculation with *Rhizobium* did not affect the contributions of fertilizer and soil N to total N in shoots at both the 75 and 150 kg N/ha fertilizer levels. From the results of this study it may be concluded that if due precautions are taken to ensure acceptable isotopic dilution, the potential of the technique is obvious.

TABLE 3

Absolute Amounts of N Derived from Fertilizer (Nf), Soil (Ns) and N_2 Fixation (Na), mg/pot

Fertilizer N rate (kg N/ha)	*Nf*		*Ns*		*Na*	
	Inoc.	*Uninoc.*	*Inoc.*	*Uninoc.*	*Inoc.*	*Uninoc.*
0	-	-	37	-	28	-
75	48	50	65	67	6	-
150	78	72	63	59	7	-
LSD (P=0.05)	9		9		14	

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