1 Evaluation of Symbiotic Nitrogen Fixation by Rhizobial Strains in Common Bean (Phaseolus Vulgaris L.) Using the ^{15}N Isotope Dilution Method

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ABSTRACT

The study was carried out under phytotron conditions to evaluate the symbiotic effectiveness of five strains of Rhizobium leguminosarum bv. phaseoli (CIAT 144, CIAT 166, CIAT 652, CIAT 899 and Sweden 458) on two cultivars of common bean (Phaseolus vulgaris L.) using the ^{15}N isotope dilution method. Nitrogen as $Ca(NO_3)_2$ (5.736 atom% ^{15}N excess) was applied at the rate of 20 mgN/kg soil. The two bean cultivars (Rondina and Stella) differed in their dry matter yield as well as N_2 fixing abilities with the different rhizobial treatments. The per cent of plant N derived from the atmosphere (% Ndfa) averaged 47.6% for Rondina and 54.3% for Stella over all rhizobial strains. The actual amount of N_2 fixed varied between 40 mg/pot and 89 mg/pot depending on the bean cultivar and rhizobial strain. Rondina averaged 53 mg fixed N/pot and Stella 74 mg fixed N/pot.

There were differences in the predictions of symbiotic effectiveness of the rhizobial strains in the bean cultivars using both isotopic and non-isotopic parameters. Strains CIAT 144, CIAT 652 and CIAT 899 were, in decreasing order, the highest N_2 fixers with Stella as were CIAT 652, CIAT 899 and CIAT 144 with Rondina. Using both parameters, strain CIAT 652 seemed to be the best fixer and strain Sweden 458 the worst in the two cultivars.

Key Words: Bean cultivars, N_2 Fixing ability, ^{15}N Isotope dilution, Phaseolus vulgaris L., Rhizobium strains, Symbiotic effectiveness.

INTRODUCTION

In the nitrogen economy of both soils and plants, the contribution from properly managed N_2 fixing systems appears to be the most promising alternative to chemical fertilizer N. Cultivars of common bean (Phaseolus vulgaris L.) have been shown to differ in N_2-fixing ability (1, 2). Effectiveness in N_2 fixation and the competitive ability for nodule formation are among the characters that require examination when selecting Rhizobium strains for use in legume inoculants (3).

Indigenous populations of R. leguminosarum bv. phaseoli are common in most soils in which common beans are grown (4). In such soils there is usually competition between the indigenous and effective inoculant strains for nodulation sites (4,5).

Yield and nodulation responses only occasionally are observed with the suitable cultivars, inoculum strains, carriers, and application rates (6). Failure of applied inoculum to increase seed yield may be due to many causes and interactions (4). Soil pH, nutritional status, and water status control many nodulation responses. Climatic variables such as light (7) and temperature (8), in addition to cultural aspects such as planting density (2), also influence nodulation and N_2 fixation. Using the cultivars "Aurora" and "Kentwood", Rennie and Kemp (9) screened U.S. Commercial and many other R. phaseoli strain isolated from the United Kingdom and Central and South America. They concluded that the choice of strains could significantly alter N_2 fixation and yield.

The objective of this study was to evaluate the N_2-fixing capacity of two common bean cultivars as affected by inoculation with five strains of R. leguminosarum bv. phaseoli using the ^{15}N isotope dilution technique.

MATERIALS AND METHODS

The study was conducted at the Agricultural University of Norway, Aas. Two kg of a sieved (2 mm) Norwegian Gleyic Cambisol (FAO/UNESCO) or Typic Haplumbrepts (S.T./USDA), with sand - 60%, silt- 28%, clay -

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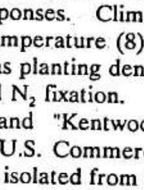

12%, pH - 5.9; organic matter - 1.80%; total N - 0.160%, were placed in plastic pots (top diameter - 18cm; bottom diameter - 13 cm and height - 16 cm). A solution containing $Ca^{15} (NO_3)_2$ with 5.736 atom % ^{15}N excess was uniformly applied at a rate equivalent to 20mg N/kg soil.

Two cultivars of common bean (Phaseolus vulgaris L.) - Stella and Rondina and five strains of Rhizobium leguminosarum bv. phaseoli were used. The Rhizobium strains were CIAT 144, CIAT 166, CIAT 652 and CIAT 899 (all from the Bean Programme in CIAT, Columbia) and strain 458 from Sweden. Seeds were surface-sterilized using 0.1% acidified mercuric chloride (10) and thoroughly rinsed with demineralized water. The seeds were either surface inoculated with a thick suspension of one of the Rhizobium strains (to ensure approximately 10^7 rhizobia per seed) or left uninoculated as a non-fixing control. The uninoculated control beans were not nodulated.

Five bean seeds were planted per pot and thinned to two healthy plants 10 days after germination. The experimental design was a randomised complete block with three replicates.

The plants were grown in a phytotron at 21-24°C with a 16h light - 8h dark period, light intensity on the average being 12,000 lux and relative humidity 75-80%.

Plants were harvested by cutting at the soil level 55 days after sowing at growth stage R3 (11), dried at 70°C and ground in a Wiley Mill to pass a 60 mesh sieve. Total N was determined by Kjeldahl procedure (12). ^{15}N enrichment was determined with a JASCO N-150 emission spectrometer (13).

The measured 15_N abundances were corrected for natural abundance (0.366) to derive atom % ^{15}N excesses.

The per cent nitrogen derived from the fertilizer (% 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 per cent nitrogen fixed (% Ndfa) was calculated from the ^{15}N isotope dilution using the equation of Fried and Middelboe (14):

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

The amount of nitrogen derived from soil (Ndfs) was found by difference as follows:

$$\text{Ndfs} = \text{Total N Yield} - (\text{Ndff} + \text{Ndfa})$$

The absolute amount of N_2-fixed (mg/pot) was calculated by multiplying the % Ndfa by the total N-yield (less the seed-borne N).

Data were analysed statistically by analysis of variance. Significant differences were assessed by the least significant difference following significant F tests.

RESULTS AND DISCUSSION

Dry matter yield and N content data are presented in Table 1. There was a significant cultivar effect. The Stella cultivar produced significantly (P = 0.05) more dry matter than Rondina. In the two cultivars yields of inoculated beans were greater than uninoculated beans. The significant cultivar x strain interaction in the dry matter yield suggests that the two cultivars did not benefit equally from inoculation with the different rhizobial strains.

There were no overall differneces in N content between the two bean cultivars. However, inoculation resulted in significant differences in shoot N content. Since the two bean cultivars differed in their seed N content (5.6 mg N per Rondina seed and 11.6 mg N per Stella seed), the total N yields were corrected for seed-borne N. Significant differences (P = 0.05) were found in total N yield between the two bean cultivars and with rhizobial treatment. In agreement with dry matter yield data, total N in Stella was higher than in Rondina bean following inoculation. Each of the two bean cultivars inoculated with the different Rhizobium strains had N yield exceeding the uninoculated control, suggesting that a significant amount of N_2 fixation had occurred.

There were no significant differences between the two bean cultivars in the atom % ^{15}N excess, % Ndff, % Ndfa and % Ndfs. Within cultivars, however, atom % ^{15}N excess, % Ndff and % Ndfs differed (P = 0.05) between rhizobial treatments (Table 2). The atom % ^{15}N excess and % Ndff of the inoculated beans were significantly lower than the uninoculated control. The decreases were about 50%, indicating a significant dilution of atom per cent ^{15}N of soil N by atmospheric N_2 in the inoculated beans. The % Ndfa (which did not differ between rhizobial treatments) ranged from 40.6 to 59.1. These results agree with the observation by Rennie and Kemp (9) that beans derive approximately 50% of their plant N from associated symbiotic N_2 fixation.

In the early stages of plant growth, seed-borne N in different species dilute the ^{15}N-enrichment to different extents. In this study the estimates of symbiotic nitrogen fixation were corrected for seed-borne N since the two bean cultivars differed in their seed N. This makes it possible to compare symbiotic nitrogen fixation by the two cultivars using the ^{15}N fertilizer dilution technique. The amount of N_2 fixed differed between the cultivars and between rhizobial treatments.

Table 1: Dry matter yield and N content of two cultivars of *Phaseolus vulgaris* L. as affected by strains of *Rhizobium phaseoli*.

R. phaseoli Strain	Dry Matter Yield (g/pot)		N Content (g/100g DM)		Total N** (mg/pot)	
	Rondina	Stella	Rondina	Stella	Rondina	Stella
Control	3.27	4.45	1.06	0.98	23.5	20.6
Sweden 458	3.99	5.01	2.50	2.52	88.8	103.1
CIAT 144	4.97	6.25	2.94	2.78	134.9	150.1
CIAT 166	4.08	6.79	2.82	2.14	103.9	121.7
CIAT 652	5.02	6.69	2.82	2.67	129.8	155.6
CIAT 899	4.16	6.55	2.78	2.63	104.3	149.4
LSD (P = 0.05)						
Cultivar	0.33		NS*		14.25	
Strain	0.57		0.47		24.68	
Cultivar x Strain	0.80		NS		NS	

*NS - Not significant

** - Corrected for seed-borne N of 11.2 mg N/2 Rondina seeds and 23.2 mg N/2 Stella seeds.

Table 2: Isotope Derived data for two cultivars of *Phaseolus vulgaris* L. as affected by inoculation with five strains of *Rhizobium phaseoli*.

R. phaseoli Strain	Atom % ^{15}N Excess		% Ndff		% Ndfa		% Ndfs		N_2-Foxed (mg/pot)	
	R	S	R	S	R	S	R	S	R	S
Control	0.674	0.763	11.76	13.31	-	-	88.24	86.69	-	-
Sweden 458	0.374	0.384	6.52	6.69	44.5	49.7	48.95	43.64	40	51
CIAT 144	0.392	0.312	6.83	5.44	41.8	59.1	51.33	35.49	56	89
CIAT 166	0.400	0.353	6.98	6.16	40.6	53.7	52.42	40.14	42	65
CIAT 652	0.315	0.345	5.50	6.01	53.2	54.8	41.27	39.22	69	85
CIAT 899	0.283	0.349	4.94	6.08	58.0	54.3	37.09	39.65	60	81
LSD (P = 0.05)										
Cultivar	NS		NS		NS		NS		14	
Strain	0.11		1.96		NS		11.14		22	
Cultivar x Strain	NS		NS		NS		NS		NS	

R = Rondina S = Stella NS = Not Significant

Stella bean cultivar demonstrated much higher N_2 fixation with all rhizobial strains.

In an attempt to evaluate N_2 fixation by the five rhizobial strains, both isotopic (% ^{15}N a.e., % Ndff and N_2 fixed) and non-isotopic parameters (dry matter yield and total N differences) were used. Since all treatments received equal fertilizer of the same ^{15}N enrichment, it is not surprising that both % Ndff and % ^{15}N a.e. gave similar ranking of effectiveness of the five strains. The two parameters are known to be inversely related to N_2 fixing ability.

As observed by Hardarson et al. (15), there are differences in the predictions of symbiotic effectiveness of the strains of Rhizobium in the bean cultivars by the methods used. Such differences are not unexpected since according to Rennie and Kemp (9) even rhizobial strains originating from the same source often do not fix identical amounts of N_2. Rennie and Kemp (9) presumed that deleterious genetic mutations during prolonged storage may alter their N_2-fixing ability. The differences could also be due to plant-rhizobial interactions. For instance % Ndff and N_2 fixed estimates (Table 2) showed strains CIAT 144 and CIAT 652 to be the best in N_2 fixing ability in Stella cultivar. However, % Ndff and N_2 fixed estimates gave strains CIAT 652 and CIAT 899 to be the best fixers in Rondina cultivar. Total N and dry matter yield estimates showed CIAT 144 and CIAT 852 to be among the best fixers in both Rondina and Stella cultivars. Using both isotopic and non-isotopic parameters strain 652 seemed to be the best fixer and strain Sweden 458 the worst in the two bean cultivars.

This attempt at evaluating the strains of Rhizobium support the suggestion by Hardarson et al. (15) that when selecting rhizobial strains for inoculant production, critical screening should be done using both isotopic and non-isotopic parameters and in different crop varieties.

CONCLUSION

The study showed that the two bean cultivars (Rondina and Stella) differed in their dry matter yield and N_2 fixing abilities with the different rhizobial treatments. Using both isotopic and non-isotopic parameters, predictions of symbiotic effectiveness of the rhizobial strains in the bean cultivars were made and discussed. The quantity of N_2 fixed varied between 40 mg/pot and 89 mg/pot depending on the bean cultivar and rhizobial strain.

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