Symbiosis, 27 (1999) 251–257
Balaban, Philadelphia/Rehovot

Need for Inoculation of Common Bean (*Phaseolus vulgaris* L.) in Senegal and Assessment of Nitrogen Fixation using ^{15}N Isotope Dilution Technique

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Received May 1, 1999; Accepted September 12, 1999

Abstract

A field experiment was conducted at Bel Air station, in Dakar using ^{15}N isotope dilution technique and the non nodulating soybean (*Glycine max*) variety m129 as reference plant to estimate nitrogen fixation in common bean (*Phaseolus vulgaris*). Dry matter production and nitrogen accumulation were increased in plants inoculated with *Rhizobium* strain ISRA 355 compared to uninoculated plants (+31% and +50%, respectively) and in 20 N-fertilized plants compared to non N fertilized plants (+36% and +39%, respectively). In addition, %Ndfa was increased in common bean inoculated with strain ISRA 355 (+32% higher than non inoculation) or supplied with 20 kg N/ha (+37% higher than non N fertilization). However, the amount of fixed nitrogen was increased by the interaction inoculation × 20 N-fertilization only (+155% over the value averaged across all uninoculated plants). From all treatments, estimates of fixed nitrogen were ranged between 63 to 139 kg N/ha. Beside this estimation, nitrogen fixation was found to be inhibited in common bean supplied with 80 kg N/ha. Thus, 20 kg N/ha should be considered as starting rate of nitrogen fixation in common bean cultivated at Bel Air experimental station.

Keywords: *Glycine max*, isotope dilution, ^{15}N, nitrogen fertilizer, nitrogen fixation, *Phaseolus vulgaris*, reference plant

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Presented at the 8th Congress of the African Association for Biological Nitrogen Fixation, November 23–27, 1998, Cape Town, South Africa

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1. Introduction

In Senegal, common bean (*Phaseolus vulgaris* L.) is cultivated as a cash crop in the north-western zone called Niayes zone. High yields recorded in this zone needed large amount of nitrogen fertilizer supplied at the rate of 300 kg urea/ha. Thus, the underground water is currently nitrate polluted because of the high rate of urea and the nitrate leaching process through the sandy soil (>94% of sand). In these circumstances, biological nitrogen fixation should be an alternative nitrogen source for common bean cultivation in this zone although common bean has been reported to be inferior to other grain legumes in nodulation and N fixation (Graham and Temple, 1984). Indeed variations in amount of fixed nitrogen may be due to either the nitrogen level in the soil or the applied nitrogen fertilizer (Ruschel et al., 1982; Duque et al., 1985; Ssali and Keya, 1986).

This study aims to compare the effect of *Rhizobium* inoculation and nitrogen fertilizer on common bean yields and to estimate the fixed nitrogen using ^{15}N isotope dilution technique.

2. Material and Methods

A field experiment was carried out at Dakar, in Bel-Air station. The soil was a sandy type (94% of sand) containing approximately 10^2 native *Rhizobium*/g counted by infection test method (Brockwell, 1982; Vincent, 1970) using common bean seedlings. The pH of soil was 7.0 with 0.025% N (Bremner, 1965), 40 ppm available P (Olsen et al., 1954). The seeds of common bean variety Bronco and that of non nodulating soybean (*Glycine max*) variety m129 used as non-fixing control plant were surface sterilised by immersion in 95% ethanol for 3 min and 1‰ $HgCl_2$ for 3 min and then washed with sterile water. After washing, seeds were hand sown in a randomized completed factorial design with four replicates. The size of each plot was 1.50 × 2.55 m with 15 cm and 40 cm within and between rows respectively. The main factor was the inoculation: seeds inoculated with peat slurry containing 10^9 *Rhizobium*/g and uninoculated seeds. The *Rhizobium* strain ISRA 355 was used in the inoculum. It was selected at ISRA-MIRCEN laboratory for its high nitrogen fixing potential in association with the variety Bronco. The sub-factor was the nitrogen fertilizer with two rates 0 and 20 kg N/ha. Within each sub-plot, two microplots 0.75 × 0.80 m and 0.60 × 0.80 m were delimitated for common bean and soybean respectively, for the application of ^{15}N-labelled fertilizer solution, $(^{15}NH_4)_2SO_4$ containing 2.185 atom %^{15}N excess. Unlabelled $(NH_4)_2SO_4$ was applied to the remaining plots. To all plots a basal fertilizer was added and consisted of 60 kg P/ha as a triple superphosphate and 120 kg K/ha as KCl.

At 56 days after sowing, all plants were harvested from the microplots. The harvested plants were separated into different parts. Nitrogen (%N) and atom $\%^{15}N$ excess (%Nae) for individual plant part were determined at the International Atomic Energy Agency (IAEA) laboratory in Seiberdorf, Austria using an automated nitrogen analyser coupled to a mass spectrometer. Nitrogen fixation (%Ndfa) was estimated using the isotope dilution equation (Fried and Middelboe, 1977):

$$\%\text{Ndfa} = \left(1 - \frac{\%^{15}\text{Nae in fixing crop}}{\%^{15}\text{Nae in non fixing crop}}\right)$$

A weighted atom $\%^{15}N$ excess (WAE) for the whole plant was estimated as follows:

$$\text{WAE} = \frac{\text{AE(sh)} \times \text{TN(sh)} + \text{AE(st)} \times \text{TN(st)} + \text{AE(r)} \times \text{TN(r)} + \text{AE(p)} \times \text{TN(p)}}{\text{TN(sh)} + \text{TN(st)} + \text{TN(r)} + \text{TN(p)}}$$

Where AE(sh), AE(st), AE(r) and AE(p) refer to atom excess in shoots, stems, roots, and pods respectively and TN(sh), TN(st), TN(r) and TN(p) designate total nitrogen in shoots, stems, roots and pods.

Then the %Ndfa for the whole plant was:

$$\%\text{Ndfa} = \left(1 - \frac{\text{WAE in fixing crop}}{\text{WAE in non fixing crop}}\right)$$

Data were statistically analysed using the Newman and Keuls test.

3. Results and Discussion

The nitrogen fixed by common bean was calculated using ^{15}N dilution technique with non nodulating m129 soybean cultivar. Although, a suitable reference crop is a prerequisite for a successful use of this technique, it currently is the best available technique for measuring accurately N_2 fixation (Danso et al., 1992). Since a non nodulating common bean cultivar was not so far been identified, we used in our study a non nodulating soybean cultivar as reference plant. Such reference cultivar was used in other experiments with valuable estimates of amount of fixed nitrogen (Hardarson et al., 1984). Because the soil contained 10^2 native *Rhizobium*/g which can nodulate common bean, the non inoculated common bean could not be used as reference: occurrence of N_2 fixation in this reference should make the ^{15}N isotope dilution technique unusable. According to the recommendations of Fried et al. (1983), the different plant

Table 1. ANOVA of total dry matter (total DM), total nitrogen (total N), weighted atom excess (WAE), proportion (%Ndfa) and amount (Ndfa) of fixed nitrogen in field grown common bean (*Phaseolus vulgaris*) inoculated with *Rhizobium* strain ISRA 355 and supplied with N (urea) fertilizer.

Source of variation	ddl	Total DM	Total N	WAE	%Ndfa	Ndfa
Inoculation	1	48.92**	30.04**	6.19*	12.85**	49.82**
N fertilizer	1	43.10**	23.92**	8.77*	8.54*	39.64**
Inoc.×Fertil.	1	4.38	3.81	0.76	2.75	14.10**
CV%		7.8	14.3	56.5	14.4	18.3

*Significant at p = 0.05; **highly significant at p = 0.05.

Table 2. Total dry matter (total DM), total nitrogen (total N), weighted atom excess (WAE) and proportion of fixed nitrogen (%Ndfa) in field grown common bean (*Phaseolus vulgaris*) supplied with N fertilizer or inoculated with *Rhizobium* strain ISRA 355.

Treatments	Total DM (g/pl)	Total N (g/pl)	WAE	Ndfa
N fertilizer (kg N/ha)				
0	11.00 b	0.23 b	0.03 b	83.30 a
20	15.00 a	0.32 a	0.08 a	67.40 b
Inoculation (ISRA 355)				
Non inoculated	11.00 b	0.22 b	0.07 a	65.60 b
Inoculated	14.30 a	0.33 a	0.04 b	85.10 a

For each treatment and in each column, values followed by the same letter do not differ significantly at p = 0.05.

Table 3. Amount of fixed nitrogen (Ndfa) in field grown common bean (*Phaseolus vulgaris*) inoculated with *Rhizobium* strain ISRA 355 and supplied with N fertilizer.

Inoculation (ISRA 355)	N fertilizer (kg N/ha)	Ndfa (g/pl)
Non inoculated	0	0.12 c
	20	0.17 b
Inoculated	0	0.19 b
	20	0.38 a

Values followed by the same letter do not differ significantly at p = 0.05.

parts of the plants were analysed separately for %^{15}Nae to minimize the errors and to use the WAE. This approach was frequently used in a recent past (Danso and Kumarasinghe, 1990; Duc et al., 1988; Jensen, 1986; Vasilas and Ham, 1985).

Analysis of variance was performed on all data recorded from inoculation, nitrogen fertilizer application and interaction between these two treatments (Table 1). Significant effect of both treatments was observed on the dry matter production, the nitrogen yield, the WAE, the proportion (%Ndfa) and amount (Ndfa) of fixed nitrogen. In addition significant effect of the interaction between the treatments was also observed on the amount of fixed nitrogen. Compared to the uninoculated plants, there was a significant increase of dry matter production and nitrogen accumulation in plants inoculated with *Rhizobium* strain ISRA 355: +31% and +50%, respectively. Similarly, plants receiving 20 kg N/ha produced more dry matter (+36%) and accumulated more nitrogen (+39%) than non n-fertilized plants.

Inoculating common bean with *Rhizobium* strain ISRA 355 significantly increased (+32%) the %Ndfa, in comparison to the indigenous rhizobia in the uninoculated treatment (Table 2) whereas the increase due to the supply of nitrogen fertilizer was 37% lower than 0-N treated plants (Table 3). Despite this concern about the %Ndfa, any of these main treatments did not affect the amount of nitrogen which was increased in the inoculated × 20-N treated plants (Table 3): +155% in comparison with value averaged across all uninoculated plants. Although fixed nitrogen estimates of 64 kg N/ha have been reported by Ruschel et al. (1982), our estimates were ranged between 0.12 and 0.38 g N/plant (Table 3) corresponding to 63 and 139 kg N/ha respectively, according to the density of 375,000 plants/ha. Such high values of fixed nitrogen in common bean were observed in field experiments performed in Austria and Chile as part of a FAO/IAEA co-ordinated research programme to investigate the nitrogen fixing potential of several common bean cultivars (Hardarson et al., 1991a). In addition, from our experimental conditions, nodule dry weight in 20-N treated plants (200 mg/plant) was significantly higher than 0-N treated plants. Beside this experience, nitrogen fixation was found to be inhibited with 80 kg N/ha (unpublished data). Similar inhibition was observed with fababean (*Vicia faba* L.) when high rates of N were applied even nitrogen fixation was not completely suppressed (Hardarson et al., 1991b). Thus, compared to nitrogen fixation in fababean, that in common bean is very sensitive to N fertilizer. However, 20 kg N/ha should be considered as starting rate of nitrogen fixation in common bean cultivated at Bel Air experimental station.

Acknowledgements

The authors thank Mr. O. Touré for his valuable technical assistance and

Mrs. M.C. Da Sylva for performing the statistical analysis. They also gratefully acknowledge the assistance of the staff of the Soil Science Unit, IAEA Seibersdorf Laboratory (Austria) for performing the isotopic analyses on plant samples.

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