Field evaluation of $N_2$-fixation and $N$-utilization by Phaseolus bean varieties determined by $^{15}N$ isotope dilution*

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Summary Differences in $N_2$-fixation by Phaseolus vulgaris bean cultivars were successfully evaluated in the field using $^{15}N$ isotope dilution technique with a non-fixing test crop of a different species (wheat). The Phaseolus cultivars could have been similarly ranked for $N_2$-fixation capacity from either seed yield or total nitrogen yield, but the isotope method provided a direct measure of $N_2$-fixation and made it possible to estimate the proportion of fixed to total nitrogen in the crop and in plant parts.

Amounts of nitrogen fixed varied between 24.59 kg N/ha for the 60-day cultivar Goiano precoce to 64.91 kg N/ha for the 90-day cultivar Carioca. The per cent of plant nitrogen due to fixation was 57-68% for the 90-day cultivars and 37% for Goiano precoce (60-day cultivar).

Fertilizer utilization was 17-30% of a 20 kg N/ha fertilizer application. 100 kg N/ha fertilizer application decreased $N_2$-fixation without suppressing it totally.

Differences in yield between the highest yielding (Carioca) and the lowest (Moruna) 90-day cultivars were also due apparently to varietal differences in efficiency of conversion of nitrogen to economic matter i.e. seed, as well as to differences in capacity of genotypes for $N_2$-fixation.

Introduction

In an earlier paper we drew attention to the possibility of using an isotope dilution technique to screen a series of legume cultivars for $N_2$-fixing ability, or for screening the effectiveness of legume-Rhizobium strain combinations, using a non-legume crop as a reference standard. Such a technique requires the use of a low application of $^{15}N$ labelled fertilizer and subsequent employment of the equation developed by Fried and Mellado, where the proportion of N in the plant, a, derived from the atmosphere will be given by:

$$a = 1 - \frac{\% \ \text{atom excess} \ 15N \ \text{in fixing crop}}{\% \ \text{atom excess} \ 15N \ \text{in test (non-fixing) crop}}$$

We have discussed this procedure in our previous paper in which we employed isotope and classical methods to measure $N_2$-fixation in soybeans, Glycine max using nodulating and non-nodulating isolines. In this pot

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experiment, in which the non-nodulating isolines was the non-fixing crop, it was found that the isotope dilution method was more sensitive while the total-N method suffered from greater variability with correspondingly high standard errors. Deibert et al. have also used successfully the same principle in a field experiment with nodulating and non-nodulating soybean isolines.

For crops other than soybeans, and for associative N$_2$-fixing systems, there is at present no possibility of using non N$_2$-fixing isolines, and it is necessary to select a non-fixing test crop of a different species. In the present experiment we used a dwarf wheat as the non-fixing test crop to evaluate in the field the N$_2$-fixing capacity of a number of Phaseolus bean cultivars when inoculated with a known standard highly effective inoculum. Our choice of dwarf wheat was governed by the fact that the variety chosen has a similar growing period and root depth characteristics to Phaseolus and could initially be assumed to be non-fixing, as apparently only very rare wheat genotypes support N$_2$-fixation by Bacillus polymyxa.

**Methods**

**Cultivars**

A CENA re-selection of the cultivar Carioca, a widely grown landrace variety of brown beans was chosen as the standard variety. Other cultivars were Carioca precoce, a bush type mutant variety developed at CENA from Carioca; Goiano precoce, an early (60 day) variety of brown bean; Costa Rica, a late variety of black bean; Moruna, a bush type brown bean bred at the Instituto Agronômico, Campinas and released 2 years ago.

The non-N$_2$-fixing test crop was Ticena-2, a short straw mutant wheat variety bred at CENA.

**Experimental design**

A randomized block design with 6 replications was used, with 3 x 3 m plots, 50 cm row width, and 10 cm within the row plant spacing for beans. $^{15}$N was given on a subplot of one square m. All cultivars received 0 and 20 kgN/ha, while Carioca precoce and wheat also had an additional treatment of 100 kg N/ha, as a basic check of N-fertilizer response. All bean cultivars were inoculated with a proven effective Rhizobium strain.

**Plot preparation**

The experiment was laid out on a fertile Alfisol in September 1979. After ploughing and cultivation, rows were made and a basic side row dressing of 50 kg K/ha (as KCl) and 150 kg P/ha (as superphosphate) applied. Plots and subplots were then marked and the field sown. Nitrogen was applied 6 days after germination, following thinning of the seedlings (10 plants/m), as ($^{15}$NH$_4$)$_2$SO$_4$ solution (5.085 atom % excess $^{15}$N and 1.235 atom % $^{15}$N respectively) for N$_{29}$ and N$_{100}$ treatments. Unlabelled (NH$_4$)$_2$SO$_4$ was correspondingly given to the remainder of the plots.

**General**

Nodule number and weight were determined on 4 plants/subplot, 35, 60 and 75 days after planting, and nitrogenase activity was evaluated by acetylene reduction (AR) using gas chromatography. Checks were also made periodically of possible AR activity of the wheat. Plants were harvested from the subplot for $^{15}$N analysis, wheat and Goiano precoce beans being harvested 60 days after planting, and other bean varieties 90 days from planting. All plants were grown until full seed development. After harvesting the 1 m$^2$ $^{15}$N-labelled subplots the final yield of seeds was obtained from the rest of...