Pakistan J. Sci. Ind. Res., Vol. 31, No. 4, April 1988

# EVALUATION OF REFERENCE CROPS IN THE STUDY OF NITROGEN FIXATION BY COMMON BEANS

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(Received June 14, 1987; revised March 31, 1988)

Nitrogen fixed by a legume crop can be measured by $^{15}N$ methodology using a nonfixing reference crop. Significantly different estimates of nitrogen fixed can be obtained with different reference crops. In order to evaluate a suitable reference crop for the determination of symbiotically fixed nitrogen by common beans (*Phaseolus vulgaris*), a glasshouse experiment was conducted at Seibersdorf, Austria on the soils from two sites – Seibersdorf and Au. Three reference crops viz. buckwheat (*Fagopyrum esculentum*), uninoculated common beans and sorghum (*Sorghum vulgare*) were used with test crop i.e. inoculated common beans. Results showed that there was no significant difference in estimates of nitrogen fixation in respect to buckwheat and sorghum. Both of these crops had the same percent nitrogen derived from fertilizer and total N content within the limits of significance. Estimates of $N_2$ fixation in respect to uninoculated common beans were smaller and this reference had the same total N content as the inoculated test crop. Its % NdfF was much lower than the other two reference crops showing some substitution from atmosphere.

*Key words:* Isotope dilution technique, $N_2$ fixation, Reference crop.

## INTRODUCTION

$^{15}N$ methodology is considered most suitable for assessing nitrogen fixed by legumes [1]. According to Fried and Broeshart [2], the method involves growing nitrogen fixing and non-fixing (reference) plants in soil fertilized with $^{15}N$ enriched inorganic fertilizers. The available amounts of soil plus fixed nitrogen are determined using the legume crop and the available amount of soil N is determined using the reference crop. The difference between the two amounts is amount of fixed nitrogen. The labelled fertilizer is applied at a low rate to the fixing crop in order to avoid interference with modulation and nitrogen fixation but at a high rate to the reference crop to support its proper growth [1,3].

The accuracy and sensitivity of the method are, however, affected by the suitability of the reference crop selected [4]. Significantly different estimates of $N_2$ fixed can be obtained with different reference crops. Suitable reference crops should take up fertilizer and soil N in the same proportion as do the legume crops. In practice, they also should contain the same percentage N derived from fertilizer when provided the same kind of fertilizer at the same rate.

The object of this pot experiment was to examine the suitability of various reference crops for the determination of symbiotically fixed nitrogen by common beans (*Phaseolus vulgaris*) using $^{15}N$ methodology.

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## MATERIALS AND METHODS

This pot experiment was conducted in 1985 at Seibersdorf, Austria using soils of two locations – Seibersdorf and Au. Each pot was filled with 3 kg of soil. Test crop was inoculated common beans while buckwheat, uninoculated common beans [4,5] and sorghum [1] were three reference crops. 20 kg N/ha as urea containing 10 % $^{15}N$ atom excess was applied to inoculated beans while 100 kg N/ha as urea having 2 % $^{15}N$ atom excess was applied to inoculated beans while 100 kg N/ha as urea having 2 % $^{15}N$ atom excess was applied to each reference. Urea was applied in solution [6] before sowing. All treatments were replicated four times. After germination, the plants were thinned to two plants per pot. The pots were kept in a glasshouse with temperatures controlled at a minimum of $15^0$ (night) and maximum of $30^0$ (day). Freshly prepared inoculum of *Rhizobium phaseoli* was applied in solution to inoculated beans one week after germination. To avoid risk of cross-contamination with rhizobia from inoculated beans to uninoculated beans, pots were not randomized. Treatments on Au soil were exactly the same as on Seibersdorf soil except that sowing on Au soil was done 20 days later. The plants on both soils were harvested at the same time i.e. 70 days after sowing at Seibersdorf soil.

Plants were dried at 70°, weighed, finely ground; and their total N was determined by micro-kjeldahl method. Isotopic enrichment was determined using a Micromass 622 Spectrometer.

Percent nigrogen derived from fertilizer and atmosphere and the amount of $N_2$ fixed were determined following the procedure discussed by Fried and Broeshart [2].

## RESULTS AND DISCUSSION

Table 1 and 2 present the results of experiment at Seibersdorf and Au soils respectively. In both soils, nitrogen derived from air (% Ndfa) and amount of $N_2$ fixed in respect to buckwheat and sorghum were the same within the limits of significance. There was no significant difference between total N yield and dry-matter yield of these two controls. Moreover, buckwheat and sorghum derived same amount of N from fertilizer as there was no significant difference in % NdfF of these two controls. Since % NdfF (and hence $^{15}N/^{14}N$ ratios) of buckwheat and sorghum was the same, they took up N from the soil and fertilizer in the same proportion. Since % Ndfa and the amount of $N_2$ fixed were the same (within limits of significance) for these two controls, they can be used as references for quantifying $N_2$ fixed by ***Phaseolus vulgaris*** – although these crops belong to different species.

% Ndfa and the amount of $N_2$ fixed in respect of uninoculated beans were significantly lower than buckwheat and sorghum. Total N content and dry-matter yield of both uninoculated and inoculated beans was the same within the limits of significance. % NdfF of uninoculated beans was less than that of buckwheat or sorghum, as the former obtained its N also from atmosphere. $N_2$ fixation by uninoculated beans plants was also indicated by the presence of some healthy nodules on their roots. This could have been due to rhizobia already present in the soils used or due to cross-contamination between inoculated and uninoculated pots [7]. Fried *et al.* concluded that an appropriate reference crop should be one which does not derive nitrogen from fixation [8]. The extent to which the reference crop derives nitrogen from the atmosphere

Table 1. Estimates of percent nitrogen derived from atmosphere and $N_2$ fixed by common bean (*Phaseolus vulgaris*) on Seibersdorf and Au soils as affected by the reference crop.

| Reference crop | Seibersdorf soil % Ndfa | Seibersdorf soil $N_2$ fixed (mgs/pot) | Au soil % Ndfa | Au soil $N_2$ fixed (mgs/pot) |
|---|---|---|---|---|
| Buckwheat | 50.24 a | 129.38 a | 20.34 a | 46.20 a |
| Uninoculated common bean | 30.08 b | 78.08 b | 13.34 b | 29.73 b |
| Sorghum | 48.56 a | 122.52 a | 19.71 a | 42.99 a |

Means followed by different letters are significantly different at 99 % confidence level.

Table 2. Percent nitrogen derived from fertilizer, % N, total N yield and dry-matter yield of inoculated and uninouculated common bean, buckwheat and sorghum on two soils.

| Crop | Seibersdorf soil % Ndff | % N in plant | Total N yield (mgs/pot) | Dry-matter yield (g/pot) | Au soil % Ndff | % N in plant | Total N yield (mg/pot) | Dry-matter yield (g/pot) |
|---|---|---|---|---|---|---|---|---|
| Inoculated common bean | 2.62 c | 2.51 a | 251.60 b | 10.52 b | 3.71 c | 2.58 a | 221.25 a | 8.65 b |
| Buckwheat | 25.12 a | 1.38 b | 278.32 a | 20.43 a | 19.20 a | 1.38 b | 164.28 b | 12.26 a |
| Uninouclated common bean | 13.84 b | 2.13 a | 256.48 b | 12.66 b | 17.26 b | 2.23 a | 176.14 b | 7.96 b |
| Sorghum | 23.96 a | 1.22 b | 288.50 a | 24.36 a | 19.10 a | 1.29 b | 168.36 b | 13.81 a |

Means followed by different letters are significantly different at 99 % confidence level.

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would lower the estimate of $N_2$ fixed in the fixing crop by that amount. This explains as to why lower estimates of $N_2$ fixed by inoculated beans in respect to uninoculated beans were obtained as compared to buckwheat or sorghum.

As is the case with all legumes which fix $N_2$, % NdfF of inoculated beans was lower as compared to the controls because a decrease in the % $^{15}N$ a.e has been brought about by the nitrogen derived from the atmosphere.

Nitrogen fixation at Au soil was much lower than that at Seibersdorf soil. It was perhaps due to the reason that beans plants on Au soil were still fixing $N_2$ when they were harvested; while plants on Seibersdorf soil were 20 days older and had approached physiological maturity stage [9].

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