Biol Fertil Soils (1997) 24:317–322


ORIGINAL PAPER

**Y. G. M. Galal**

# Dual inoculation with strains of *Bradyrhizobium japonicum* and *Azospirillum brasilense* to improve growth and biological nitrogen fixation of soybean (*Glycine max* L.)

Received: 10 January 1996

**Abstract** The effects of inoculation with *Bradyrhizobium japonicum* and *Azospirillum brasilense* strains on the growth of soybean were evaluated with regard to the estimation of $N_2$ fixation using the $^{15}N$ isotope dilution technique. Inoculation, in general, increased the dry mass of soybean as well as nitrogen content. Dual inoculation with a mixture of *B. japonicum* and *A. brasilense* strains was superior over single inoculation with *B. japonicum*. Nitrogen fixed (Ndfa) varied according to inoculant and soil conditions. Percentages of nitrogen derived from air (% Ndfa) using a non-nodulating isoline were 72% and 76% for *B. japonicum* and *B. japonicum* plus *A. brasilense*, respectively, in non-sterile soil. A similar but higher trend was recorded in sterilized soil, in which the percentages of $N_2$ fixed were 81% and 86% for single and dual inoculation, respectively. The correlation coefficient between $N_2$ fixed and N uptake ($r=0.94$) and dry mass ($r=0.89$) was significant. Application of special bacterial inoculants in agricultural systems of Egypt seems to be a promising technology and could be used for improving soybean growth as well as soil fertility, thus minimizing environmental pollution.

**Key words** Soybean · Isotope dilution · Nitrogen fixation · *Bradyrhizobium japonicum* · Soil sterilization · $^{15}N$ · *Azospirillum brasilense* · Bacterial inoculation

## Introduction

Soybean plants can use nitrogen released from different sources, i.e. mineralized N, soil N, fertilizer N or atmospheric $N_2$ through a symbiotic relationship between *Bradyrhizobium japonicum* strains and soybean plants. Partial supplement of fixed – N to plants may reduce the use of chemical-N fertilizers, and subsequently reduce N losses and environmental pollution.

Inoculation of soybean crops with effective *B. japonicum* strains singly (Galal 1993; Hoque 1993) or in combination with *Azospirillum brasilense* (El-Mokadem et al. 1986; Bashan et al. 1990) was found to be important for improving and maximizing the plant growth and $N_2$ fixation potential of the crop either in soil which lacks indigenous *B. japonicum* (Rennie and Dubetz 1984; Singleton and Tavares 1986) or in those soils high in indigenous *Bradyrhizobium* spp., but less effective than the introduced bacteria (Kucey et al. 1988). However, the exploitation of $N_2$ fixation efficiency depends to a large extent on the host plant constraints affecting it (Mengel 1994).

Amounts of dinitrogen fixed and subsequent growth rate, as influenced by bacterial inoculation, soil sterilization and their interactions, have been reported frequently (Hardarson et al. 1984; Williams-Linera and Ewel 1984; Bergersen et al. 1989; Wolf et al. 1989; Ravuri and Hume 1993). Therefore, much effort has been focused on optimizing dinitrogen fixation to meet nitrogen demand and increase crop yield. In this context, the current study was conducted to evaluate the effect of bacterial inoculation on growth parameters, dry mass yield, N-content and $N_2$ fixation of soybean plants.

Y. G. M. Galal (✉)
Agricultural Department for Soil and Water Research,
Nuclear Research Centre, Atomic Energy Authority,
Abou Zaabl, 13759, Egypt
Fax: (202) 354-0982

## Materials and methods

### Soil characteristics

The soil used was a clay loam. Samples were collected from the 0- to 15-cm horizon of an agricultural field in the area of Shalakan, Egypt, air dried and passed through a 2-mm sieve. Some chemical and physical properties of the soil used are given in Table 1.

### Inoculum strains and seed sources

*B. japonicum* (USDA 110) and *A. brasilense* (Sp 245) with $10^8$ viable cells $ml^{-1}$ were obtained from the Agricultural Microbiology Department, ARC, Giza, Egypt. The nodulating (0102) and non-nodulat-

**Table 1** Physicochemical properties of the experimental soil used (analysed according to FAO Soils Bulletin 1980)

| Determination | Clay loam soil |
|---|---|
| Soil origin | Shalakan, Kaleobeia |
| Clay (%) | 37.6 |
| Silt (%) | 35.6 |
| Sand (%) | 26.8 |
| pH ($H_2O$) | 7.8 |
| Total N (%) | 0.18 |
| Olsen P (mg $kg^{-1}$) | 15.1 |
| K (mg $kg^{-1}$) | 35.0 |
| Organic carbon (%) | 1.17 |
| Extractable N (mg $kg^{-1}$) | 7.0 |
| Soil classification | Vertisol |

ing (0099) isolines of soybean (*Glycine max* cv. D-68) were obtained from Field Crops Research Institute, Agriculture Research Centre, Giza, Egypt.

Soybean seeds were surface sterilized and coated with peat-based inocula as described by Vincent (1970). *A brasilense* was applied as liquid culture in the treatment of dual inoculation.

Treatments and experimental details

Pot experiments were carried out in the greenhouse of the Agriculture Department for Soil and Water Research, Atomic Energy Authority, Egypt. Plastic pots (area 254.6 $cm^2$) were filled with 4 kg soil $pot^{-1}$. The treatments included: (a) uninoculated, non-fertilized soil, (b) uninoculated and fertilized with 40 mg N $kg^{-1}$ soil as $^{15}$N-labelled ammonium sulphate (3.9837% a.e.), (c) inoculated with *B. japonicum* (Rh) plus 20 mg N $kg^{-1}$ soil (AS), and (d) inoculated with both (Rh) and *A. brasilense* (Sp) plus 20 mg N $kg^{-1}$ soil (AS). These treatments were applied to both non-sterilized and sterilized soils. Autoclave soil sterilization was performed to avoid the interference of the indigenous soil bacteria with the introduced inoculants.

Nodulating and non-nodulating soybean seeds were sown at rate of eight seeds $pot^{-1}$ thinned to four seedlings 15 days after emergence. The non-nodulating isoline was grown in a separate series of pots receiving the same treatments as the nodulating isoline. All pots received 100 mg P $kg^{-1}$ soil as triple superphosphate and 50 mg K $kg^{-1}$ soil as potassium sulphate, mixed with the soil before planting as a basal treatment. Normal irrigation practice was followed.

Soybean plants were harvested and separated into shoots and roots, 60 days after sowing. Both shoots and roots were oven dried at 70 °C until constant weight; the dry mass was recorded, then ground and kept for analyses.

Measurements and calculations

Shoot and root samples were digested and total N was determined using the micro-Kjeldahl procedure. The total N and $^{15}N/^{14}N$ ratios were determined as described by Buresh et al. (1982).

Percentages of nitrogen derived from air (% Ndfa), fertilizer (% Ndff), soil (% Ndfs) and $^{15}$N recovery were calculated using the following equations:

$$\%\ \mathrm{Ndfa} = (1 - \mathrm{A\%\ E_f}/\mathrm{A\%\ E_{nf}}) \times 100 \quad (1)$$

where A% $E_f$ is the $^{15}$N% atom excess in the fixing crop and A% $E_{nf}$ is the $^{15}$N% atom excess in the non-fixing crop.

$$\%\ \mathrm{Ndff} = (^{15}\mathrm{N\%\ a.e. in\ plant}/^{15}\mathrm{N\%\ a.e.\ in\ fertilizer}) \times 100 \quad (2)$$

$$\%\ \mathrm{Ndfs} = 100 - \%\ \mathrm{Ndff} \quad (3)$$

$$^{15}\mathrm{NRF}\ = \mathrm{A\%\ Ep} \times \mathrm{TNP}/\mathrm{A\%\ E_f} \times \mathrm{FR} \quad (4)$$

where A% Ep is the $^{15}$N% atom excess of plant, TNP is the total N in plant, A% $E_f$ is the $^{15}$N% atom excess of fertilizer and FR is the rate of applied fertilizer.

Statistical analysis

A randomized complete block design with three replicates was used. The results were statistically analysed using SAS software (SAS Institute 1985). Means were separated by Duncan's multiple range test.

**Table 2** Effect of bacterial inoculation and N addition on dry matter yield (shoots and roots) of nodulating and non-nodulating soybeans with non-sterile and sterilized soils

| Treatment | N added (mg $kg^{-1}$ soil) | Plant dry matter yield (g $plant^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Non-sterilized soil | | | | Sterilized soil | | | |
| | | Shoots | Roots | Total | Relative increase | Shoots | Roots | Total | Relative increase |
| Nodulating isoline | | | | | | | | | |
| Uninoculated | 0 | 2.56 d | 0.56 c | 3.12 | – | 2.16 d | 0.46 d | 2.62 | – |
| A sulphate (AS) | 40 | 2.96 c | 0.66 b | 3.62 | 16.02 | 2.70 c | 0.59 c | 3.29 | 25.57 |
| AS+Rh | 20 | 3.30 b | 0.84 a | 4.14 | 32.69 | 3.26 b | 0.70 b | 3.96 | 51.14 |
| AS+Rh+Sp | 20 | 3.57 a | 0.87 a | 4.44 | 42.30 | 3.63 a | 0.80 a | 4.43 | 69.08 |
| Non-nodulating isoline | | | | | | | | | |
| Uninoculated | 0 | 2.35 c | 0.51 c | 2.86 | – | 1.99 d | 0.42 d | 2.41 | – |
| A sulphate (AS) | 40 | 2.70 b | 0.60 b | 3.30 | 15.7 | 2.45 c | 0.53 c | 2.98 | 23.6 |
| AS+Rh | 20 | 2.84 b | 0.72 a | 3.56 | 24.5 | 2.80 b | 0.61 b | 3.41 | 41.5 |
| AS+Rh+Sp | 20 | 3.30 a | 0.80 a | 4.10 | 43.3 | 3.35 a | 0.74 a | 4.09 | 69.7 |
| CV (%) | | 8.01 | 8.32 | | | 5.76 | 10.10 | | |

Means in each column followed by the same letter are not significantly different at $P \leq 0.05$
*AS* ammonium sulphate, *Rh Bradyrhizobium japonicum, Sp Azospirillum brasilense*

## Results and discussion

### Dry mass and N uptake

Tables 2–4 show the effect of inorganic fertilizer and bacterial inoculation on dry mass, N content and N uptake by either nodulating or non-nodulating soybeans. The dry mass, N content and N uptake were markedly increased by the addition of nitrogen fertilizer and inoculation as compared to the uninoculated treatment. This was true with both non-sterile and sterilized soil. The small reduction of dry mass recorded with sterile soil as compared to the non-sterile soil may be attributed to the toxic substances such as Mn released in the sterile soil (Williams-Linera and Ewel 1984). Although the dry mass of the nodulating isoline was not significantly different from those recorded for the non-nodulating isoline, the nodulating soybean showed a greater uptake of nitrogen than the non-nodulating isoline (Table 4). In spite of the absolute values of N uptake in non-sterile soil showing no significant variation from those recorded under sterile conditions, the relative increases were much higher in sterile soil than in non-sterile soil. A similar trend was observed for the non-nodulating isoline. These apparent differences were actually due to the marked difference between the dry mass and N uptake values of the blanco plants (uninoculated) under the two different conditions, i.e. non-sterile and sterilized soils (Tables 2–4).

**Table 3** Effect of bacterial inoculation and N addition on nitrogen content of nodulating and non-nodulating soybeans cultivated in non-sterile and sterilized soils

| Treatment | N added (mg kg$^{-1}$ soil) | Nitrogen content (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Non-sterilized soil | | | | Sterilized soil | | | |
| | | Shoots | Roots | Total | Relative increase | Shoots | Roots | Total | Relative increase |
| Nodulating isoline | | | | | | | | | |
| Uninoculated | 0 | 2.92 c | 1.32 d | 4.24 | – | 3.06 c | 1.39 d | 4.45 | – |
| AS | 40 | 3.15 b | 1.45 c | 4.60 | 8.49 | 3.37 b | 1.53 c | 4.90 | 10.11 |
| AS+Rh | 20 | 3.18 b | 1.55 b | 4.73 | 11.55 | 3.26 b | 1.66 b | 4.92 | 10.56 |
| AS+Rh+Sp | 20 | 3.53 a | 1.73 a | 5.26 | 24.05 | 3.81 a | 1.88 a | 5.69 | 27.86 |
| Non-nodulating isoline | | | | | | | | | |
| Uninoculated | 0 | 2.61 c | 1.22 d | 3.83 | – | 2.70 c | 1.40 d | 4.10 | – |
| AS | 40 | 2.92 b | 1.37 c | 4.29 | 12.0 | 3.11 b | 1.58 c | 4.69 | 14.4 |
| AS+Rh | 20 | 3.10 b | 1.75 a | 4.85 | 26.6 | 3.3 b | 1.87 a | 5.17 | 26.11 |
| AS+Rh+Sp | 20 | 3.58 a | 1.75 a | 5.33 | 39.2 | 3.87 a | 1.89 a | 5.76 | 40.5 |
| CV (%) | | 3.24 | 1.99 | | | 3.07 | 3.24 | | |

Means in each column followed by the same letter are not significantly different at $P \leq 0.05$
*AS* ammonium sulphate, *Rh Bradyrhizobium japonicum, Sp Azospirillum brasilense*

**Table 4** Effect of bacterial inoculation and N addition on nitrogen uptake by nodulating and non-nodulating soybeans in non-sterile and sterilized soils

| Treatment | N added (mg kg$^{-1}$ soil) | Plant N uptake (mg plant$^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Non-sterilized soil | | | | Sterilized soil | | | |
| | | Shoots | Roots | Total | Relative increase | Shoots | Roots | Total | Relative increase |
| Nodulating isoline | | | | | | | | | |
| Uninoculated | 0 | 74.7 d | 7.4 d | 82.1 | – | 66.1 d | 6.4 d | 72.5 | – |
| AS | 40 | 93.2 c | 9.6 c | 102.8 | 25.14 | 90.9 c | 8.9 c | 99.8 | 37.71 |
| AS+Rh | 20 | 104.9 b | 13.0 b | 117.9 | 43.51 | 106.3 b | 11.7 b | 118.0 | 62.54 |
| AS+Rh+Sp | 20 | 126.0 a | 14.9 a | 140.9 | 71.51 | 138.3 a | 15.0 a | 153.3 | 111.31 |
| Non-nodulating isoline | | | | | | | | | |
| Uninoculated | 0 | 61.4 d | 6.25 d | 67.65 | – | 53.7 d | 5.9 d | 59.6 | – |
| AS | 40 | 78.8 c | 8.22 c | 87.02 | 28.6 | 76.3 c | 8.4 c | 84.7 | 42.1 |
| AS+Rh | 20 | 88.1 b | 12.61 b | 100.71 | 48.8 | 92.5 b | 11.4 b | 103.9 | 74.3 |
| AS+Rh+Sp | 20 | 118.1 a | 14.0 a | 132.1 | 95.3 | 129.6 a | 14.0 a | 143.6 | 140.9 |
| CV (%) | | 5.99 | 8.10 | | | 6.93 | 11.93 | | |

Means in each column followed by the same letter are not significantly different at $P \leq 0.05$
*AS* ammonium sulphate, *Rh Bradyrhizobium japonicum, Sp Azospirillum brasilense*

**Table 5** Nitrogen derived from soil (Ndfs) and fertilizer (Ndff) by nodulating and non-nodulating soybeans as affected by bacterial inoculation under non-sterile and sterilized soils

| Treatment | N added (mg kg$^{-1}$ soil) | Nitrogen derived from soil (Ndfs) | | | | | | | | Nitrogen derived from fertilizer (Ndff) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nodulating isoline | | | | Non-nodulating isoline | | | | Nodulating isoline | | | | Non-nodulating isoline | | | |
| | | Shoots | | Roots | | Shoots | | Roots | | Shoots | | Roots | | Shoots | | Roots | |
| | | % | mg | % | mg | % | mg | % | mg | % | mg | % | mg | % | mg | % | mg |
| Non-sterilized soil | | | | | | | | | | | | | | | | | |
| AS | 40 | 65.1 a | 60.64 a | 62.8 a | 6.03 c | 73.6 b | 57.99 c | 65.3 c | 5.37 c | 23.3 a | 21.76 a | 33.4 a | 3.2 a | 26.4 a | 20.8 b | 34.7 a | 2.85 b |
| AS+Rh | 20 | 36.3 b | 38.06 b | 59.5 b | 7.73 b | 74.3 b | 65.46 b | 75.0 b | 9.46 b | 12.6 b | 13.17 b | 19.8 b | 2.75 b | 25.7 a | 22.64 b | 25.0 b | 3.15 a |
| AS+Rh+Sp | 20 | 32.5 c | 40.95 b | 64.9 a | 9.66 a | 76.4 a | 90.23 a | 80.4 a | 11.25 a | 10.0 c | 12.65 b | 15.8 c | 2.36 b | 23.6 b | 27.87 a | 19.6 c | 2.74 b |
| CV (%) | | 0.5 | 12.23 | 0.2 | 6.58 | | | | | 1.8 | 13.12 | 0.4 | 6.8 | | | | |
| Sterilized soil | | | | | | | | | | | | | | | | | |
| AS | 40 | 76.9 a | 69.89 a | 76.1 b | 6.77 c | 81.4 b | 62.11 c | 77.5 b | 6.51 c | 17.6 a | 15.99 a | 22.1 a | 1.97 a | 18.6 a | 14.19 b | 22.5 a | 1.89 b |
| AS+Rh | 20 | 18.4 b | 19.59 c | 81.4 a | 9.52 b | 80.3 b | 74.28 b | 85.5 a | 9.75 b | 4.5 b | 4.80 b | 13.8 b | 1.61 b | 19.7 a | 18.22 a | 14.5 b | 1.65 b |
| AS+Rh+Sp | 20 | 19.0 b | 26.23 b | 77.5 b | 11.63 a | 86.3 a | 111.84 a | 84.2 a | 11.79 a | 3.0 c | 4.16 b | 14.6 b | 2.18 a | 13.7 b | 17.75 a | 15.8 b | 2.21 a |
| CV (%) | | 2.5 | 1.40 | 0.5 | 9.07 | | | | | 11.7 | 14.25 | 1.6 | 9.69 | | | | |

Means in each column followed by the same letter are not significantly different at $P \leq 0.05$
*AS* ammonium sulphate, *Rh Bradyrhizobium japonicum*, *Sp Azospirillum brasilense*

Regarding the type of inoculation, the dual inoculation with *B. japonicum* and *A. brasilense* was superior to the single inoculation with *B. japonicum* only, giving better results for dry mass, N percent and N uptake than the uninoculated treatment and/or ammonium sulphate treatment. The enhancement of N uptake in the case of dual inoculation may be attributed to a stimulating effect of hormones excreted by *Azospirillum* on both nodulation and nutrient uptake (Bashan et al. 1990). This was examined and proved visually in the current study, where the harvested inoculated plants reflected a good nodulation on the main and lateral roots. This confirms previous results obtained by the author and coworkers (Soliman et al. 1995), who found that nodule number and dry weight were increased significantly by dual inoculation (*B. japonicum* + *A. brasilense*) of soybean.

Soil nitrogen (Ndfs) was the major source of N uptake by both nodulating and non-nodulating soybeans fertilized with 40 mg N kg$^{-1}$ soil as ammonium sulphate with non-sterile soil, whereas the non-nodulating isoline tended to derive more soil N than the nodulating isoline (Table 5). A similar trend was observed in sterilized soil, and a smaller increase in values of soil N uptake by shoots and roots of both nodulating and non-nodulating isolines was detected as compared to non-sterile soil. Dual inoculation enhanced the soil N uptake by the whole plant (shoot + root) as compared to single inoculation with either sterilized or non-sterile soil.

With respect to nitrogen derived from fertilizer (Ndff), no significant difference was noticed between nodulating and non-nodulating soybeans fertilized with 40 mg N kg$^{-1}$ soil with either sterilized or non-sterile soil. Generally, the inoculation had reduced the dependence of nodulating soybean on N fertilizer as compared to the non-nodulating isoline. This reduction in Ndff was more pronounced under sterilized soil. Therefore, the $^{15}N$ recovery by nodulating soybean was somewhat less in inoculation treatments than in the uninoculated treatment (ammonium sulphate treatment) under sterilized soil (Table 6). This may be attributed to the competitiveness of microorganisms under these conditions, which makes the host more dependable on Ndfa than other sources. Similar findings were reported previously (Rennie 1984; Kucey et al. 1989). The opposite was observed in non-sterile soil, where the $^{15}N$ recovery had increased by inoculation as reported previously (Galal 1993).

## Dinitrogen fixation

The estimated values of fixed nitrogen based on the isotope dilution concept (Table 7) reflected the superiority of dual inoculation over single inoculation in both non-sterile and sterilized soil. The percentages of $N_2$ fixed (% Ndfa) were approximately 81%, 86% and 72%, 77% for single and dual inoculation with sterilized and non-sterile soil, respectively. These percentages were close to those estimated by Wada et al. (1986). The superiority of dual inoculation is by virtue of the promotion of microbial activ-

**Table 6** Effect of bacterial inoculation and soil condition on $^{15}N$ recovery by nodulating soybean fertilized with different rates of labelled ammonium sulphate

| Treatment | N added (mg $kg^{-1}$ soil) | Recovery of $^{15}N$ fertilizer[a] (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Non-sterilized soil | | | Sterilized soil | | |
| | | Shoots | Roots | Total | Shoots | Roots | Total |
| AS | 40 | 54.4 b | 8.0 b | 62.4 | 39.97 a | 4.92 c | 44.89 |
| AS+Rh | 20 | 65.85 a | 12.85 a | 78.7 | 24.0 b | 8.05 b | 32.05 |
| AS+Rh+Sp | 20 | 63.25 a | 11.80 a | 75.05 | 20.8 c | 10.9 a | 31.7 |
| CV (%) | | 13.13 | 6.78 | | 14.2 | 9.67 | |

Means in each column followed by the same letter are not significantly different at $P \leq 0.05$
[a] Expressed as % of $^{15}N$ applied
*AS* ammonium sulphate, *Rh Bradyrhizobium japonicum*, *Sp Azospirillum brasilense*

**Table 7** Nitrogen derived from air (Ndfa) by nodulating soybean inoculated with *B. japonicum* either solely or in combination with *A. brasilense* in non-sterile or sterilized soil

| Inoculation | Non-sterilized soil | | | | Sterilized soil | | | |
|---|---|---|---|---|---|---|---|---|
| | Shoots | | Roots | | Shoots | | Roots | |
| | % | mg N $plant^{-1}$ | % | mg N $plant^{-1}$ | % | mg N $plant^{-1}$ | % | mg N $plant^{-1}$ |
| AS (uninoculated) | 11.6 d | 9.13 d | 3.8 d | 0.31 d | 5.5 d | 4.23 d | 1.8 d | 0.15 d |
| AS+Rh | 51.2 b | 45.08 b | 20.7 a | 2.61 a | 77.1 a | 71.27 b | 4.8 b | 0.55 c |
| AS+Rh+Sp | 57.5 a | 67.86 a | 19.3 a | 2.71 a | 78 a | 101.11 a | 7.9 a | 1.11 a |

Means in each column followed by the same letter are not significantly different at $P \leq 0.05$
*AS* ammonium sulphate, *Rh Bradyrhizobium japonicum*, *Sp Azospirillum brasilense*

ity and the enhancement of nodulation, as a result of the growth-promoting substances excreted by *A. brasilense* (Bashan et al. 1990). Values of $N_2$ fixed subjected to regression analysis showed a highly significant correlation between Ndfa and dry mass ($r = 0.89$) and nitrogen uptake ($r = 0.94$) by nodulating soybean.

The significance of the present study lies not only in the confirmation of the importance of inoculation, but also in the demonstration of the ability of associative $N_2$ – fixing bacteria to enhance nodulation and $N_2$ fixation. Also, soil sterilization can be accepted for use in further such studies, in view of the mode of action or competition which may occur between the introduced inoculants and the indigenous bacteria or soil fauna.

**Acknowledgements** The author wishes to express his gratitude to the editor – in – chief and the reviewers for their valuable comments. Thanks are also extended to Prof. Dr. A.F. El-Kholi for his fruitful assistance during the preparation of the revised manuscript.

## References

Bashan Y, Harrison SK, Whitemoyer RE (1990) Enhanced growth of wheat and soybean plants inoculated with *Azospirillum brasilense* is not necessarily due to general enhancement of mineral uptake. Appl Environ Microbiol 56:769–775

Bergersen FJ, Brockwell J, Gualt RR, Morthorpe L, Peoples MB, Turner GL (1989) Effects of available soil N and rates of inoculation on $N_2$ fixation by irrigated soybeans and evaluation of $^{15}N$ methods for measurement. Aust J Agric Res 40:763–780

Buresh RJ, Austin ER, Craswell ET (1982) Analytical methods in $^{15}N$ research. Fert Res 3:37–62

El-Mokadem MT, Helemish FA, Abou-Baker ZYM (1986) Growth and yield parameter responses of two soybean cultivars to inoculation with *Azospirillum* and *Rhizobium.* 2nd Conf. African Association of Biological Nitrogen Fixation 15–19 December, Cairo, Egypt

FAO (1980) Soils Bulletin. Soils and plants as basis of fertilizer recommendations. FAO Soils Bull 38/2

Galal YGM (1993) Evaluation of some biofertilizers for increasing nitrogen efficiency under Egyptian conditions. PhD Thesis, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

Hardarson G, Zapata F, Danso SKA (1984) Effect of plant genotype and nitrogen fertilizer on symbiotic nitrogen fixation by soybean cultivars. Plant and Soil 82:397–405

Hoque MS (1993) *Bradyrhizobium* technology: a promising substitute for chemical nitrogen fertilizer in Bangladesh agriculture. Plant and Soil 155/156:337–340

Kucey RMN, Snitwongse P, Chaiwanakupt P, Wadisirisuk P, Siripaibool C, Arayangkool T, Boonkerd N, Rennie RJ (1988) Nitrogen fixation (N-15 dilution) with soybeans under Thai field conditions. I. Developing protocols or screening *Bradyrhizobium japonicum* strains. Plant and Soil 108:33–41

Kucey RMN, Chaiwanakupt P, Boonkerd N, Snitwongse P, Siripaibool C, Wadisirisuk P, Arayangkool T (1989) Nitrogen fixation (N-15 dilution) with soybeans under Thai field conditions. IV. Effect of N addition and *Bradyrhizobium japonicum* inoculation in soils with indigenous *B. japonicum* populations. J Appl Bact 67:137–144

Mengel K (1994) Symbiotic dinitrogen fixation: Its dependence on plant nutrition and its ecophysiological impact. Z Pflanzenernähr Bodenkd 157:233–241

Ravuri V, Hume DJ (1993) Soybean stover nitrogen affected by dinitrogen fixation and cultivar. Agron J 85:328–333

Rennie RJ (1984) Comparison of N balance and $^{15}N$ isotope dilution to quantify $N_2$ fixation in field-grown legumes. Agron J 76:785–790

Rennie RJ, Dubetz S (1984) Multistrain vs. single strain *Rhizobium japonicum* inoculants for early maturing (00 and 000) soybean cultivars: $N_2$ quantified by $^{15}N$ isotope dilution. Agron J 76:498–502

Safwat MS, Moharram TM, Abdel Moneim A, Ali MZ, Zayed G (1990) The use of N-15 enriched fertilizers for estimating N-fixation in soybean. Minia J Agric Res Dev 12:259–272

SAS Institute Inc (1985) SAS User's guide. Statistics version 5, SAS Inst Inc, Cary, NC

Soliman S, Galal YGM, El-Ghandour IA (1995) Soybean biofertilization in sandy soils of Egypt using $^{15}N$ tracer technique. Folia Microbiol 40:321–326

Singleton PW, Tavares JW (1986) Inoculation response of legumes in relation to the number and effectiveness of indigenous *Rhizobium* populations. Appl Environ Microbiol 51:1013–1018

Vincent JM (1970) Manual for the practical study of root nodule bacteria. Blackwell Scientific Publications, Oxford

Wada E, Imaizumi R, Kabaya Y, Yasuda T, Kanamori T, Saito G, Nishimune A (1986) Estimation of symbiotically fixed nitrogen in field grown soybeans: An application of natural $^{15}N/^{14}N$ abundance and a low level $^{15}N$ tracer technique. Plant and Soil 93:269–286

Williams-Linera G, Ewel JJ (1984) Effect of autoclave sterilization of a tropical andept on seed germination and seedling growth. Plant and Soil 82:263–268

Wolf DC, Dao TH, Scott HD, Lavy TL (1989) Influence of sterilization methods on selected soil microbiological, physical and chemical properties. J Environ Qual 18:39–44