

Effect of Inorganic Nitrogen and *Bradyrhizobium japonicum* Inoculation on Growth and Yield of Soybean

Abdel-Fattah I. El-Shaarawi, Atef Z. Sabh, Sawsan M. Abou-Taleb and Ahmed E. Ghoniem

Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Egypt.

Abstract: The current investigation was carried out during the two successive seasons 2006 and 2007 at The Agricultural Experimental and Research Station, Faculty of Agriculture, Cairo University, Giza, Egypt to study the effect of different levels of inorganic nitrogen with and without *Bradyrhizobium japonicum* inoculation on vegetative growth, internal structure and yield of soybean plant. Three levels of inorganic nitrogen 20, 30 and 40 kg/fed. were applied. Two rates of *B. japonicum* were used as seed inoculant. Different combinations between mineral nitrogen and the inoculant were carried out. Vegetative growth, yield and yield components were negatively affected by lowering the applied dose of inorganic nitrogen. Inoculation with *B. japonicum* tended to compensate the adverse effect of applying lower doses of mineral nitrogen. The combinations between the inoculant and the lower doses tended to enhance most growth and yield characters compared to the lower doses applied alone. The beneficial effect of *B. japonicum* on total dry weight of shoots and pods and seed yield was increased by decreasing the applied level of mineral nitrogen, reached the maximum with the lowest rate. The combination between 20kg MN/fed. with the higher rate of *B. japonicum* induced the highest increase in seed yield/fed. (19.2 and 31 %) over that of the highest rate of mineral nitrogen alone in the two successive seasons, respectively. Transections made in main stem and leaflet blade exhibited that the whole stem and pith diameter, thickness of xylem and phloem of stem and leaflet midvein were negatively affected by lowering the applied rate of MN. Combination between *B. japonicum* with 20 kg MN/fed. slightly increased stem xylem and phloem thickness whereas pith diameter was reduced compared to those of 20 kg MN/fed. alone. Thickness of blade tissues slightly increased by such treatment. Thickness of stem xylem and phloem, diameter of stem and thickness of blade tissues were negatively affected by *B. japonicum* combined with 40 kg MN/fed. compared with those of 40 kg MN/fed. alone. Three centimeters below the soil surface, inoculated roots had wider vascular cylinder and more thickness of both xylem and phloem. Higher numbers of wider vessels were obviously noted in inoculated root compared to those of uninoculated one. Two centimeters below the soil surface, the inoculated root showed an increase in diameter of the whole section, thickness of cortex, diameter of vascular cylinder and thickness of both xylem and phloem compared with those of uninoculated one. On the other hand, pith diameter was decreased in inoculated roots. One centimeter below the soil surface, most tissues were enhanced in inoculated plant compared to uninoculated one.

Key words: Inoculation, *Bradyrhizobium japonicum*, inorganic nitrogen, soybean growth and yield.

INTRODUCTION

Glycine is a well known genus belonging to the family Fabaceae. Soybean (*Glycine max* (L.) Merr.) is the most important species of the genus. It is a source of edible oil, 20-25% and high protein contents, 42-45%. It used as a good source of unsaturated fatty acids, minerals (Ca and P) and vitamins A, B, C and D (Rahman, 1982) & Jones and Luchsinger, 1987). Leguminous root nodulation presented a symbiotic association between soybean plant root and *Bradyrhizobium japonicum* where symbiotic nitrogen fixation (SNF) takes place. The main products in the process, namely ureids (allantoin and allantoic acid) are exported from soybean root nodules to the rest of the plant where it is incorporated into amino acids and protein formation (Bordeleau and Prevost, 1994). The efficiency of inoculation by *B. japonicum* on nodulation and nitrogen fixation in soybean was reported by many investigators (Saleh and Shalaby, 1993; Okereke and Onochie, 1996; Groppa *et al.*, 1998; Bai *et al.*, 2002 and Redzepovic *et al.*, 2004). This positive effect of inoculation by *B. japonicum* was accompanied with increase in seed yield of soybean (Jaarsveld *et al.*, 2002; Redzepovic *et al.*, 2004; Mabood *et al.*, 2006b; Son *et al.*, 2007 and Meghvansi *et al.*, 2008).

In bio-organic farming systems, chemical fertilization is often replaced (at least partly) by biofertilization, using different N-fixing and P-solubilizing bacteria (Burris, 1976) and using some plant growth promoting rhizobacteria (Antoun *et al.*, 1998 and Ragab *et al.*, 2006). The efforts to decrease chemical fertilizers by using biofertilizers might reduce the high costs and environmental pollution. Hence biological N₂ fixation has great attention to cover N-requirements and improve the soil fertility (Okon and Lanabdera, 1994). It was found that using biofertilizers alone, without stimulative rates from mineral fertilizers (25%, 33%, 50% or 75% from the

recommended level of mineral fertilizers, according to soil fertility or organic manure) was less effective than the recommended rates of chemical fertilizers (Gomaa, 1989; Abdel-Ati *et al.*, 1996 and Awad, 1998).

Therefore, the present work was carried out to study the effect of inoculation by *B. japonicum* combined with different levels of inorganic nitrogen on morphological, anatomical and yield characters of soybean. Reducing the amounts of mineral fertilization is of great importance in reducing soil pollution approaching to sustainable agricultural development. Partial replacement of biofertilization instead of mineral one could be considered to avoid the expected soil pollution due to the residual effect of mineral fertilization.

MATERIALS AND METHODS

The present investigation was carried out at The Agricultural Experimental and Research Station, Faculty of Agriculture, Cairo University, Giza, Egypt during two successive seasons 2006 and 2007. Soybean (*Glycine max* L. Merr.) cv. Giza3 was used in this study. Seeds were obtained from The Crop Research Section, Agricultural Research Center, Ministry of agriculture, Giza, Egypt. *B. japonicum* carried on vermiculite was obtained from Production Unit for Biofertilizers, Agricultural Research Center, Giza, Egypt. One gram of vermiculite contains 10^7 - 10^8 cells of *B. japonicum*. Soybean seeds were inoculated at sowing with two rates of the inoculant, the first was the recommended dose, 300 g, and the second one 450 g of the inoculant carrier (vermiculite)/fed.

Three levels of inorganic nitrogen (ammonium nitrate, 33.5%) namely 20, 30, 40 kg MN/fed. were used. The amount of each level was divided into two halves. One half was applied before the first irrigation and the other half before the second one. Fertilization with 50 kg/fed. potassium sulphite (48% K₂O) before the second irrigation and 200 kg/fed. triplesuper phosphate (35.5%) before sowing, followed the normal fertilization of cultivated soybean in vicinity. Nine treatments included mineral nitrogen and inoculation with *B. japonicum* were carried out (as shown in the tables)

The layout of the experiment was Randomized Complete Block Design with three replicates for each treatment. Seeds were sown in May 28th in the two seasons in ridges 2.5m long and 60 cm apart. The usual farming procedures for the crop in the vicinity were followed. Growth characters e.g. main stem length (MSL), main stem diameter (MSD), number of branches (No. B), number of leaves (No. L), total leaf area per plant (TLA) and dry weight of leaves, stems and green pods were examined at the age of 11 weeks after sowing. Yield and yield components were estimated at maturity. Morphological and yield characters subjected to conventional methods of analysis of variances as reported by Snedecor and Cochran (1982).

It was intended to carry out a comparative microscopic analysis on plant materials which showed the most prominent response of plant growth to the different investigated treatments. Specimens were taken from the middle portion of main stem (6th internode) and leaf blade at the age of 64 days in the first season. For studying the response of root tissues to inoculation with *B. japonicum*, specimens were taken from the main taproot 1, 2 and 3 cm below the soil surface (at the age of 10 days). Specimens were killed and fixed at least 48 hrs. in FAA (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). The selected materials were washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax of melting point 56°C, sectioned to a thickness of 15-20 microns, double stained with safranin- light green, cleared in xylene and mounted in Canada balsam (Willey, 1971). Sections were microscopically analyzed and photomicrographed.

RESULTS AND DISCUSSION

Morphological Characters:

Data in Table (1) illustrate the influence of applying three rates of mineral nitrogen (MN) with or without *Bradyrhizobium japonicum* inoculation on some morphological characters of soybean plant. Main stem length & diameter, number of branches & leaves and total leaf area per plant were estimated at the age of 11 weeks after sowing in the two successive seasons 2006 and 2007. Data show that lowering the applied rate of MN down to 20 kgMN / fed. negatively affected all morphological characters under study in both growing seasons. When the lowest rate (20 kg MN/fed.) of mineral nitrogen combined with any of the two rates of *B. japonicum* produced stems with no significant differences in length compared with those received 30 or 40 kg N/fed. alone. However the differences significantly increased compared to plants received the lowest rate alone. Inoculation of plants with the lower rate of *B. japonicum* and fertilization with 30 kg/fed. of inorganic nitrogen significantly promoted the growth of stem in length in comparison with those fertilized with the highest rate alone. The highest values of main stem diameter was obtained when plants inoculated with *B. japonicum*, 450 g/fed. And then fertilized with 20 kg MN/fed. in the first season and with 30 kg MN/fed. in the second one. In other words such stems surpassed, in their growth in thickness, those received the corresponding or the recommended doses without inoculation. However the differences were statistically significant only in the second season.

Inoculation of plants received any of the three applied rates of MN with *B. japonicum* enhanced branching. A pronounced effect was obtained at this age in the first season where the difference between each level of

nitrogen combined with *B. japonicum* and the corresponding level used alone was statistically significant. Moreover, most combinations between the inoculants and inorganic nitrogen especially at the higher rate of *B. japonicum* significantly surpassed the highest rate of inorganic nitrogen 40 kg MN/fed., when used alone. Inoculation of plants by any of the two rates of *B. japonicum*, 300 or 450 g/fed. with application of any of the two lower rates of inorganic nitrogen increased the number of leaves per plant comparing with those of the corresponding rates of inorganic nitrogen when used alone. However the differences were statistically significant only in the second season. Furthermore these combinations surpassed the adequate rate of inorganic nitrogen (40 kg/fed.) alone. When the rate of inorganic nitrogen was raised to 40 kg/fed. The promotive effect of *B. japonicum* on number of leaves/plant decreased.

The lowest rate of mineral nitrogen combined with 300g/fed. of the inoculants induced the maximum increase in total leaf area per plant. The increments were 30.50 and 47.75% over the corresponding rate without inoculation in the first and second seasons, respectively. When the rate of 40 kg MN/fed. Was applied in combination with *B. japonicum*, no increase or even decrease in total leaf area/plant was recorded, compared with the same rate used alone. No clear differences were observed in this respect between the two applied rates of *B. japonicum*. No significant differences in this aspect were recorded between the two treatments of 20 kg MN/fed. + *B. japonicum* and 40 kg MN/fed. alone.

It could be conclude therefore that, morphological characters were clearly enhanced by combination between the lower rates of mineral nitrogen and *B. japonicum*. In other words, inoculation with *B. japonicum* could be compensate, to some extent, the adverse effect of using lower amounts of inorganic nitrogen on soybean growth. Increasing the used amount of *B. japonicum*, or inoculation of plants received the highest rate of inorganic nitrogen did not induce beneficial effect on all of morphological traits under study. These results are more or less in accordance with the findings of Pal and Gana (1991) and El-Naggar (1998) on mungbean; Elsheikh and Elzindany (1997a) and Mohamed (2000) on broad bean; Abd El-Fattah and Arisha (2000) on common bean; Dashti *et al.* (1997), Mabood *et al.* (2006) and Meghvansi *et al.* (2008) on soybean. In this connection Hassan (1981) stated that the height of soybean plants was increased by increasing mineral nitrogen alone. He noticed also that the number of branches per plant was significantly increased with increasing nitrogen for uninoculated soybean plants. Whereas nitrogen application with inoculation by *B. japonicum* had no significant effect on this trait.

Table 1: Main stem length (MSL), main stem diameter (MSD), number of branches (No. B), number of leaves (No. L) and total leaf area (TLA)/plant at the age of 11 weeks as affected by different levels of inorganic nitrogen with and without *Bradyrhizobium japonicum* inoculation.

Treatments	First season (2006)					Second season (2007)				
	MSL (cm)	MSD (mm)	No. B	No. L	TLA (cm ²)	MSL (cm)	MSD (mm)	No. B	No. L	TLA (cm ²)
40 kgN/fed.	102.9	8.7	2.6	30.9	4226.9	102.7	8.9	3.0	29.4	4193.9
30 kgN/fed.	103.3	8.5	2.3	30.7	3366.2	98.1	8.7	2.7	28.3	3285.6
20 kgN/fed.	90.5	8.2	2.1	27.5	2886.6	94.2	8.4	2.4	26.4	2980.7
40 kgN/fed. + B ₁	95.0	8.5	3.3	32.1	4268.5	103.8	9.6	4.4	34.7	4356.9
30 kgN/fed. + B ₁	109.5	8.6	3.1	34.3	3525.6	106.6	9.2	4.3	34.1	4523.7
20 kgN/fed. + B ₁	99.2	8.5	2.7	30.9	3767.2	101.1	9.0	4.6	32.2	4404.0
40 kgN/fed. + B ₂	100.4	8.6	3.5	30.9	3863.0	104.0	9.6	4.0	31.7	3970.7
30 kgN/fed. + B ₂	104.1	8.5	4.3	32.8	3754.2	105.0	9.7	4.6	33.6	4112.9
20 kgN/fed. + B ₂	102.5	8.7	3.9	32.5	4170.4	101.0	9.4	4.2	33.7	4413.8
Average	100.8	8.5	3.1	31.4	3758.7	101.8	9.2	3.8	31.6	4026.9
L.S.D. 0.05	5.1	ns	0.5	ns	570.8	3.0	0.3	ns	2.4	596.2

B₁ = 300 g/fed., B₂ = 450 g/fed. of *B. japonicum* carrier

Dry Weight of Shoot and Green Pods:

It is clear from Table (2) that without inoculation, the rate of 40 kg MN/fed. produced the highest values of dry weight of leaves and stems. Lowering the level of MN reduced both traits. In comparison with the corresponding rates of MN used alone, combination between 20 kg MN/fed. and the lower rate of *B. japonicum*, 300gm per fed. induced the maximum increase in dry weight of leaves (40 and 41%), stems (36 and 48%) and green pods (74.2 and 48.5%) per plant in the first and second seasons, respectively.

The table clearly shows that combinations of 20 or 30 kg MN/fed. and *B. japonicum* decreased the dry weight of leaves in the first season and showed an increase in the second one compared with the adequate rate (40 kg MN/fed.) alone. Combination between 20 kg MN/fed. and 300 g/fed. of the inoculant produced stems higher in their dry weight than those of 40 kg MN/fed. alone by 4 and 12% in the first and second seasons respectively.

Table 2: Dry weight of leaves, stems and pods per plant (gm) of soybean plant at the age of 11 weeks as affected by different levels of inorganic nitrogen with and without *B. japonicum* inoculation.

Treatments	First season (2006)				Second season (2007)			
	Leaves	Stems	Pods	Total	Leaves	Stems	Pods	Total
40 kgN/fed.	9.0	23.8	4.7	37.6	13.8	19.9	17.4	51.2
30 kgN/fed.	7.6	21.8	4.9	34.4	13.5	19.3	15.8	48.5
20 kgN/fed.	6.5	16.7	3.1	26.4	11.3	17.6	13.2	42.1
40 kgN/fed. + B ₁	10.8	24.0	4.9	39.8	16.3	23.4	18.5	58.5
30 kgN/fed. + B ₁	8.9	25.4	5.6	39.9	16.6	25.1	20.2	61.8
20 kgN/fed. + B ₁	8.9	24.8	5.4	39.1	16.0	24.0	19.6	59.7
40 kgN/fed. + B ₂	7.4	18.3	4.2	29.9	16.9	23.9	19.5	60.4
30 kgN/fed. + B ₂	7.2	20.8	4.2	32.2	15.6	25.0	21.3	61.9
20 kgN/fed. + B ₂	6.7	22.8	7.2	36.7	17.2	22.9	21.6	61.8
Average	8.1	22.0	4.9	35.1	15.2	22.4	18.6	56.2
L.S.D. 0.05	n.s.	4.05	1.58	5.35	n.s.	n.s.	1.41	3.79

B₁= 300 g/fed., B₂ = 450 g/fed. of *B. japonicum* carrier

Without inoculation, a significant decrease in dry weight of green pods per plant was recorded when the rate of 20 kg M/fed. was applied alone, compared with the two higher rates. Inoculated plants received 20 or 30 kg MN/fed. proved an increase in this trait, in comparison to the recommended rate (40 kg MN/fed.). The increments were statistically insignificant in the first season and significant in the second one.

Effect of inoculation with *B. japonicum* at its lower rate, 300g/fed. on total dry weight of shoot and pods per plant varied according to the used level of mineral nitrogen. It induced maximum promotive effect when used with the rate of 20 kg MN per fed. The increments were about 48 and 42% over those of plants received 20 kg MN/fed. alone in the two successive seasons, respectively. This effect decreased by raising the rate of mineral nitrogen and declined to its minimum degree or even disappeared when the highest rate (40 kg MN/fed.) was used. Combination between any rate of inorganic nitrogen and inoculation with the lower rate of *B. japonicum* showed an increase in dry weight of shoot and pods per plant compared to the recommended rate (40 kg MN/fed) used alone with significant differences only in the second season. It is worthy to notice that, no beneficial effect was obtained by raising the rate of the inoculant from 300 to 450 g/fed. concerning dry weight of different plant parts. The recorded enhancement of total dry weight of shoot and pods of soybean plant due to inoculation with *B. japonicum* could be attributed to its promotive effect on stem growth in length and width, number of branches and leaves, and leaf area/plant. The promotive effects recorded in the present study due to inoculation with *B. japonicum* confirm the findings of Arayangkoon *et al.*, (1990) on guar, Bell *et al.*, (1990), Okereke and Onochie (1996), Bai *et al.*, (2002), Egamberdiyeva *et al.*, (2004b) and Meghvansi *et al.*, (2008) on soybean. Whereas Groppa *et al.*, (1998) and Shamsuddin and Ang (1999) reported insignificant differences in total dry matter production when soybean plants were inoculated by *B. japonicum*.

Anatomical Study:

Transverse sections of main stem, 64-day-old, revealed that application of low level of mineral nitrogen (20 kg MN/fed) decreased the amount of different main stem tissues (diameter of the whole section, pith diameter, thickness of both xylem and phloem) compared with those of 40 kg MN/fed. Cortex thickness was not affected by lowering the applied rate of MN (Figs. 1A and 2A). Inoculation of plants by *B. Japonicum* combined with 20 kg MN/fed, increased thickness of both xylem and phloem and decreased pith diameter of main stem. Diameter of the whole section and cortex thickness were not affected compared with those of 20 kg MN/fed. alone (Figs. 1A and B). Combination between *B. Japonicum* and 40 kg MN/fed. decreased the thickness of both xylem and phloem and diameter of the whole section compared with those of 40 kg MN per fed. alone. Thickness of cortex and pith diameter showed no differences (Figs. 2A and B).

Transverse sections of leaflet blade 64-day-old, showed that the blade tissues specially xylem and phloem of midvein were negatively affected by decreasing the level of mineral nitrogen from 40 to 20 kg MN/fed (Figs. 3A&4A). Inoculation with *B. Japonicum* showed a slight increase in thickness of blade tissues of plants received low level (20 kg MN/fed.) of mineral nitrogen (Figs 3A and B), whereas a slight decrease was observed in those of inoculated plants fertilized with the highest level of mineral nitrogen, compared with those of the corresponding rates of mineral nitrogen alone (Figs. 4A and B).

Transverse sections were made in the main root, one two and three cm below the soil surface at the age of 10 days after sowing to examine the response of root tissues to *B. Japonicum* inoculation. At a level of three cm. below the soil surface, there were no clear differences in thickness of cortex between inoculated and uninoculated roots. The inoculated roots had wider vascular cylinder and more thickness of both xylem and phloem. Higher numbers of wider vessels were obviously noted in inoculated root compared to that of uninoculated one (Figs. 5A and B).

At the higher level, two cm. below the soil surface, the inoculated root showed an increase in diameter of the whole section, thickness of cortex, diameter of vascular cylinder and thickness of both xylem and phloem compared with those of uninoculated one. On the other hand pith diameter was decreased in inoculated roots.

Nodules are well developed in inoculated roots (Figs. 6A&B). One cm. below the soil surface, the different root tissues were enhanced in inoculated plant compared to uninoculated one. An exception was noticed concerning vascular cylinder diameter (Figs. 7A and B).

In conclusion, the development of stem and blade tissues especially the vascular ones was enhanced as the inoculated plants received the lower dose of mineral nitrogen. No beneficial effect of inoculation was recorded on stem and leaf structure when the highest dose (40 kg MN/fed.) of inorganic nitrogen was used. Decreasing the amount of stem tissues due to such treatment might be attribute to the negative effect of the higher dose of mineral nitrogen fertilization on nodulation and nitrogen fixation and consequently on plant tissue development. Inoculation of soybean seeds with *B. japonicum* enhanced seedling root growth in thickness by increasing the amount of the developed vascular tissues, specially the primary and secondary xylem. These results are more or less in agreement with the findings of Mohammed (2000) on broad bean and Swaefy *et al.*, (2007) on peppermint.

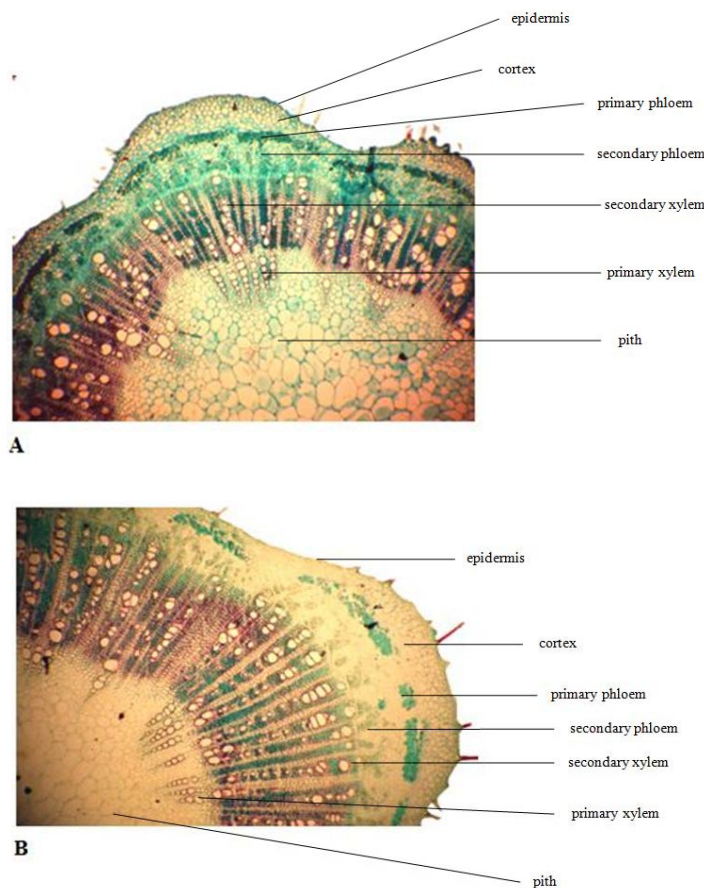


Fig. 1: Transverse sections of main stem, 64-day-old as affected by inoculation with *B. japonicum* under MN level of 20kg/fed. A: 20 kg N/fed.; B: 20 kg N/fed. + 450 g /fed. *B. japonicum*.

Yield and Yield Components:

Table (3) illustrates the effect of inorganic nitrogen fertilization with or without *B. japonicum* inoculation on yield and yield components of soybean plants in the two successive seasons. Without inoculation, lowering the applied rate of mineral nitrogen down to the rate 20 kg MN/fed. negatively affected yield and most of yield components.

Combinations of 20 or 30 kg MN/fed. with any of the two rates of *B. japonicum* significantly increased the number of pods per plants, compared with the corresponding two rates or with the highest rate (40 kg MN/fed.) alone. Plants inoculated by the lower rate of *B. japonicum* then fertilized with 40 kg MN/fed. induced significant increase in this aspect and vice versa with the higher one. The data indicate that the highest number of seeds per pod was recorded in the first season at 30 kg MN/fed. with the higher rate of *B. japonicum* and at 20 kg MN per fed with the same rate of the inoculant in the second one. Combination between the inoculant with 40 kg MN/fed. showed no effect in this respect. The data indicate also that plants inoculated by the higher rate of *B. japonicum* then fertilized by the lowest rate of mineral nitrogen produced the highest weight of 100 seed.

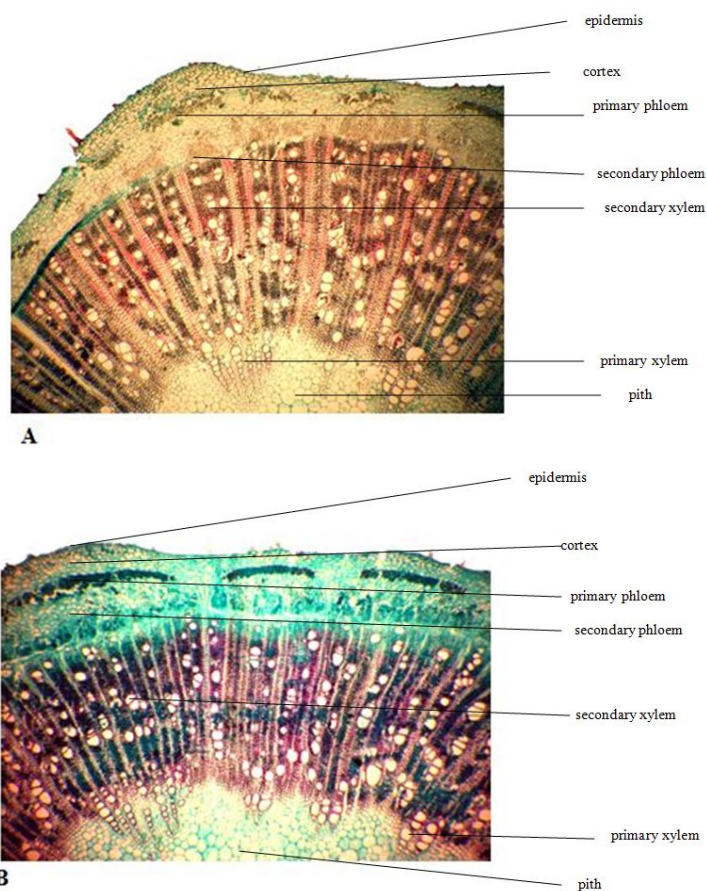


Fig. 2: Transverse sections of main stem, 64-day-old as affected by inoculation with *B. japonicum* under MN level of 40 kg/fed. A: 40 kg N/fed.; B: 40 kg N/fed. + 450 g /fed. *B. japonicum*.

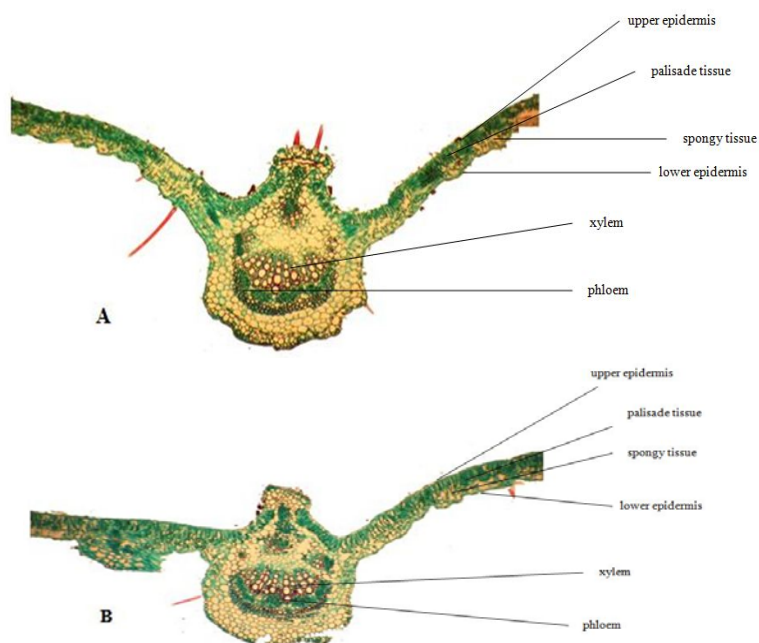


Fig. 3: Transverse sections of leaflet blade, 64-day-old as affected by inoculation with *B. japonicum* under MN level of 20 kg/fed. A: 20 kg N/fed.; B: 20 kg N/fed. + 450 g /fed. *B. japonicum*.

Fertilization of plants with any of the three levels of inorganic nitrogen in addition to inoculation by any of the two rates of *B. japonicum* induced an increase in number of seeds/plant compared to the corresponding rates of inorganic nitrogen without inoculation in the two seasons. The differences were statistically significant only in the second season. The higher rate of the inoculant was more effective in this respect, especially when used with the lower two levels of inorganic nitrogen. Plants inoculated with *B. japonicum* and fertilized with 20 or 30 Kg MN/fed. surpassed those fertilized with 40 kg MN/fed. alone, in this respect.

Comparing with the corresponding rates of MN used alone, inoculation by *B. japonicum* induced promotive effect in weight of seeds per plant depending on the level of mineral nitrogen, rate of *B. japonicum* and to some extent the season. The increase in weight of seeds per plant due to inoculation with *B. japonicum* was significantly enhanced by lowering the level of MN. The highest degree of increment was recorded with the lowest rate (20 kg MN/fed.) when used with any of the two rates of *B. japonicum* in the two seasons, except with the lower rate of *B. japonicum* in the first season where the medium rate showed the highest percentage of increase. The rate of 40 kg MN/fed. showed the lowest effect in this respect. The differences between plants fertilized with the highest rate of MN with inoculation by *B. japonicum* and those received 40 kg MN/fed. alone were statistically insignificant in both seasons.

In comparison with the recommended rate, 40 kg MN/fed., plants inoculated by *B. Japonicum* and fertilized with the lower rates (20 or 30 kg MN/fed.) of mineral nitrogen not only produced seed equivalent in their weight to those of plants fertilized with the rate of 40 kg MN/fed. alone, but also showed exceeding in this respect. The differences were statistically significant when the lowest rate of MN (20 kg/fed.) was used with the higher rate of *B. japonicum* in both seasons and when the rate of 30 kg MN/fed. was used with the higher rate of *B. japonicum* in the first season and with the lower one in the second season.

Inoculation of plants, fertilized with mineral nitrogen, by *B. japonicum* improved their productivity. The highest stimulative effect of the biofertilizer was estimated when used at the higher rate, 450 g/fed. with the lowest level (20 kg/fed.) of MN. This combination increased the seed yield/fed. by 36.3 and 112.6% over that of the corresponding rate of MN alone in the first and second seasons, respectively. However the differences were statistically significant only in the second season. In comparison to the recommended rate (40 kg MN/fed.), the beneficial effect of *B. Japonicum* on seed yield increased by decreasing the level of applied MN, and reached its maximum with the lowest rate (20 kg MN/fed.). This combination induced an increase in seed yield per fed. by 19.2 and 31.0% in the first and second seasons respectively.

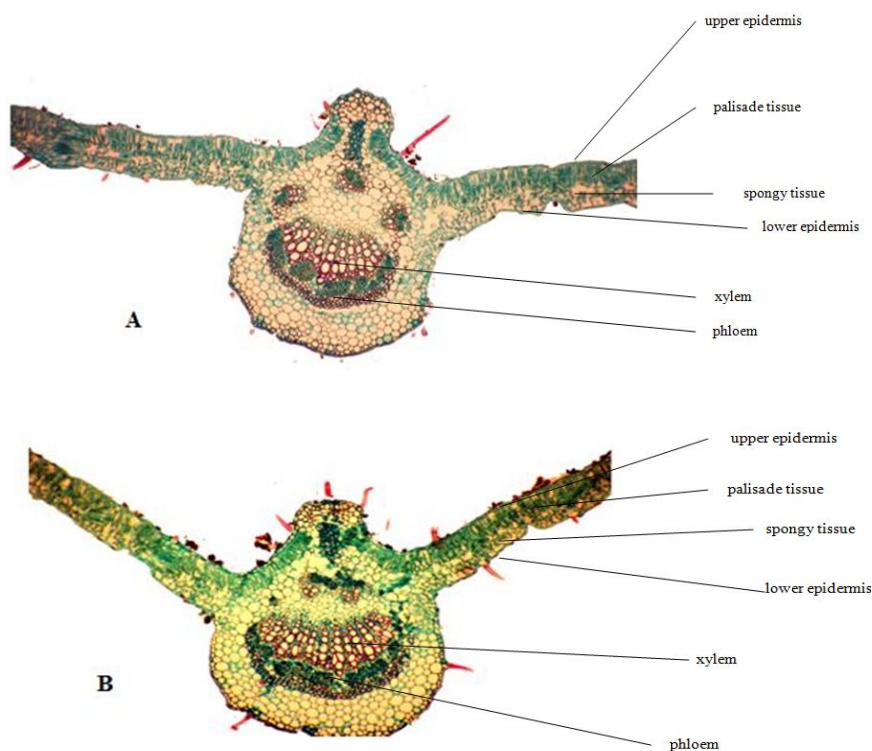


Fig. 4: Transverse sections of leaflet blade, 64-day-old as affected by inoculation with *B. japonicum* under MN level of 40 kg/fed. A: 40 kg N/fed.; B: 40 kg N/fed. + 450 g /fed. *B. japonicum*.

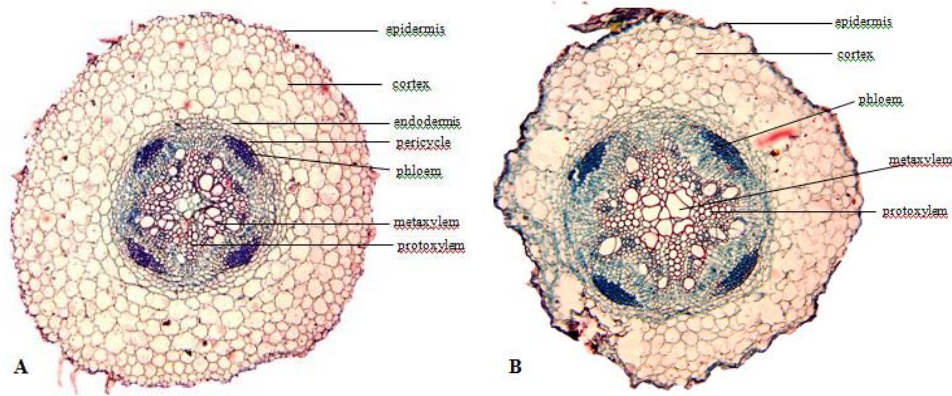


Fig. 5: Transverse sections of main root, 3 cm below the soil surface at the age of 10 days as affected with *B. japonicum*. A: uninoculated.; B: inoculated with 300 g/fed. of *B. japonicum*.

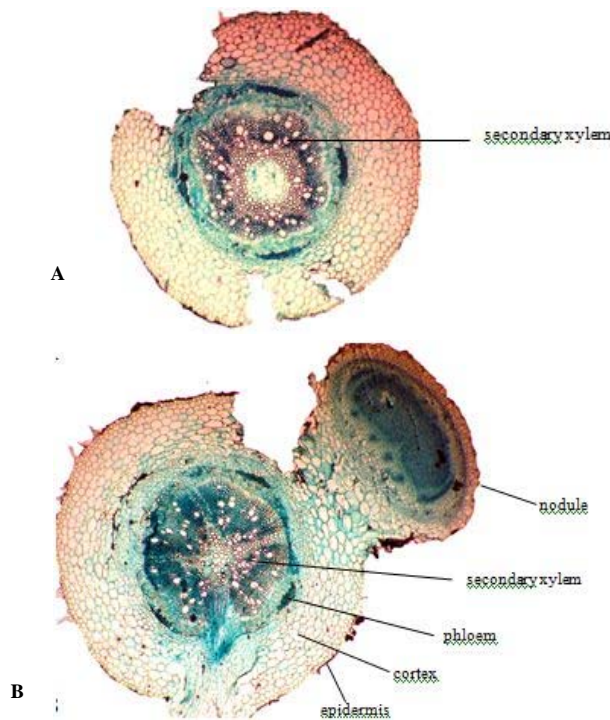


Fig. 6: Transverse sections of main root, 2 cm below the soil surface at the age of 10 days as affected with *B. japonicum*. A: uninoculated.; B: inoculated with 300 g/fed. of *B. japonicum*.

Inoculation with *B. japonicum* with different levels of inorganic nitrogen enhanced the number of pods/plant. This positive effect of inoculation with *B. japonicum* might be due to its promotive effect on fruit setting and/or decreasing flower and young pods abscission. Inoculation by *B. japonicum* with the lower rates of mineral nitrogen induced an increase in the number of seeds per pod. Inoculation of plants, fertilized with mineral nitrogen by *B. japonicum* improved their productivity. Moreover, the beneficial effect of inoculation with *B. japonicum* on seed yield increased by decreasing the level of applied MN. It could be stated also that the promotive effect of *B. japonicum* on seed yield increased by raising the rate of the biofertilizer, especially when used with the lower two levels of mineral nitrogen. These results confirm the finding of Hume and Blair, (1992). They found an increment of 19% in seed yield of soybean as the inoculation rate raised from 10^{-5} to 10^{-6} *B. japonicum* cells per seed.

The positive effects of inoculation by *B. japonicum* on yield and yield components in the present work are in agreement with the findings of Dashti *et al.*, (1997), Jaarsveld *et al.*, (2002), Salih and Nawar, (2003), Mabood *et al.*, (2006), Meghvansi *et al.*, (2008) and Elsheikh *et al.*, (2009) on soybean; Pal and Jana, (1991), El-

Naggar, (1998) on mungbean; Elsheikh and Elzidany, (1997a) and Mohamed, (2000) on faba bean; El-Habbasha (2005) on groundnut; El-Hadi and Elsheikh, (1999) on Chickpea; El-Sheikh and Ibrahim, (1999) on guar and Abd El-Fattah and Arisha, (2000) and Kucuk and Kivanc, (2008) on common bean. In disaccordance, Barsoum and Abd El Gawad, (1990) on soybean noticed that number and weight of seeds/pod were not significantly affected with inoculation by *B. japonicum*. Elsheikh and Ibrahim, (1999) found no significant increase in the weight of 1000 seeds with inoculation of guar by *Rhizobium*.

Inoculation of plants with *B. japonicum* with application of different levels of mineral nitrogen affected the vegetative and reproductive growth characters as well as seed yield per plant and per fed. This effect varied according to the applied level of mineral nitrogen. When the lower levels of MN specially the lowest one (20 kg MN/fed.) were used, inoculation with *B. japonicum* improved different growth and yield characters with mostly significant differences comparing with those of plants fertilized with the corresponding doses of mineral nitrogen without inoculation by *B. japonicum*. The decrease in beneficial effect of inoculation with *B. japonicum* by raising the level of mineral nitrogen might be due to the negative effect of the high level of nitrogen on nodulation and consequently, nitrogen fixation. In this respect, Hassan, (1981) mentioned that increasing nitrogen fertilization level depressed nodule development in both inoculated and uninoculated faba bean plants. Bell *et al.*, (1990) reported that inoculation of soybean with *B. japonicum* at low inorganic supply increased shoot dry weight per plant. No significant effects were recorded concerning *B. japonicum* inoculation with adequate inorganic nitrogen. Saleh and Shalaby, (1993) noticed that inoculated soybean plants received 10 kg N/fed. recorded the highest number and mass of nodules. Increasing the amount of applied nitrogen led to significant decrease in nodulation. Shahaby *et al.*, (2000) noticed that fertilization with 45 kg N/fed. decreased nodule production of peanut plants.

The influence of high doses of mineral nitrogen (MN) on legume-rhizobium symbiosis has been widely discussed (Streeter, 1988; Carroll and Mathews, 1990 and Kirichenko, 2001). One possible way to inhibit symbiosis is activation of defense systems of the host plant under a sufficient supply of soil MN that prevents symbiosis formation (Kirichenko, 2001). In other words, the host plant recognizes nodule bacteria as pathogens under these conditions. This is sensible because the energetic and plastic costs for a plant to form symbiotic structures and to fix N_2 exceed the costs of soil MN assimilation by a plant (Kretovich, 1994).

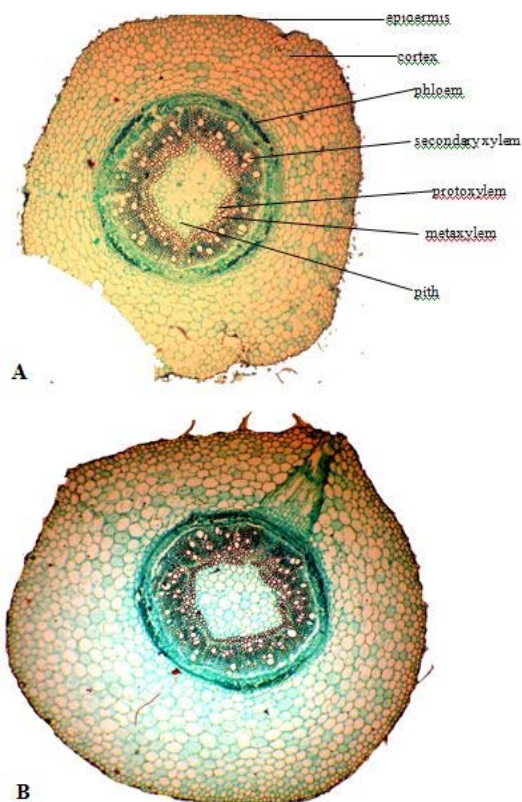


Fig. 7: Transverse sections of main root, 1 cm below the soil surface at the age of 10 days as affected with *B. japonicum*. A: uninoculated.; B: inoculated with 300 g/fed. of *B. japonicum*.

Table 3: Seed yield and yield components of soybean plant as affected by different levels of inorganic nitrogen and inoculation with *Bradyrhizobium japonicum*.

Treatments	First season (2006)						Second season (2007).					
	No. pods/ plant	No. seeds /Pod	No. seeds/ Plant	Seed index (g)	Seed yield/ plant (g)	Seed yield/ feddan (kg)	No. pods/ plant	No. seeds /Pod	No. seeds/ plant	Seed index (g)	seed yield/ plant (g)	seed yield/ fed. (kg)
40 kgN/fed.	71.87	2.83	203.4	15.43	22.52	851.67	103.89	2.75	285.70	19.39	42.90	1202.00
30 kgN/fed.	67.00	2.53	169.5	15.85	20.00	822.73	75.67	2.65	206.58	19.30	35.00	891.80
20 kgN/fed.	58.73	2.58	151.5	14.86	19.96	745.27	72.11	2.73	191.09	18.97	30.53	740.80
40 kgN/fed. +B ₁	94.40	2.77	261.5	16.57	22.88	982.33	117.67	2.77	325.95	21.08	52.95	1458.00
30 kgN/fed. +B ₁	79.80	2.79	222.6	15.97	24.94	913.50	128.56	2.77	356.11	20.16	54.57	1476.00
20 kgN/fed. +B ₁	69.27	2.80	193.9	16.16	22.19	841.00	112.33	2.87	322.39	20.41	50.70	1320.00
40 kgN/fed. +B ₂	83.93	2.81	235.8	16.11	24.94	924.00	109.11	2.78	303.33	18.86	47.91	1294.00
30 kgN/fed. +B ₂	105.2	2.86	300.9	16.55	28.29	977.67	132.22	2.74	362.28	19.73	52.03	1348.00
20 kgN/fed. +B ₂	109.8	2.73	299.8	17.30	30.19	1015.47	133.89	2.88	385.60	21.08	56.23	1575.00
Average	82.22	2.74	226.5	16.24	24.49	897.06	109.49	2.79	304.34	20.00	49.04	1256.09
L.S.D. 0.05	20.84	n.s.	60.24	n.s.	4.94	n.s.	11.15	0.16	32.57	n.s.	11.14	244.20

B₁= 300 g/fed., B₂ = 450 g/fed. of *B. japonicum*. Carrier.

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