J. Agric. Sci. Mansoura Univ., 28 (7): 5661 - 5668, 2003

EFFECT OF FOLIAR SPRAYS OF MULTI-NUTRIENT MATERIALS AND N-FERTILIZATION ON SYMBIOTIC PERFORMANCE OF *Bradyrhizobium japonicum* AND GROWTH AND YIELD OF SOYBEAN

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ABSTRACT

Two field experiments were conducted at Sakha Agric. Res. Station during the two successive seasons 2000 and 2001 to measure the symbiotic performance of *Bradyrhizobium japonicum* (St 1577, RJG and SBG) when used to inoculate soybean seeds in combination with mineral N_2-fertilization (60 kg N/fed.) and two foliar sprays, each of which contains different quantity and quality of macro and micronutrients.

Results were recorded after 45 and 80 days of planting time. The uninoculated treatments exhibited the lowest number of nodules on soybean roots, because the soil under investigation could be consider free of native rhizobia. It is, therefore of vital importance to continue inoculation of soybean seeds with effective, competitive and persistent strains of soybean rhizobia inoculation of soybean enhanced in general nodule formation, growth of nodular tissue which reflects it is effect on seed yield and their total N_2 content. The improvement of nodulation and biological N_2-fixation was further strengthed by foliar spray especially with comp. 2. In the absence of rhizobial inoculation foliar application achieved less improvement in seed yield and their total N, P and K.

INTRODUCTION

The fertilizers production in Egypt falls short and this gap is going to continue. The high cost of N_2-fertilizers, the widening gap between supply and demand, the law purchasing power of small and marginal farmers beside the deleterious effect of using only chemical fertilizers on environments led to a great attention to the biological N_2-fixation concerning its importance in the crop production of legumes.

Soybean, *Glycine max* (L.) Merr. was introduced in Egypt in the 1960's. Farmers grow it as a non-legume and rely on heavy N_2-fertilizer to obtain high yield. Egyptian soils are void of native *Bradyrhizobium japonicum* (Cassman *et al.*, 1983 and Chobrial *et al.*, 2002), in contrast to the tradition food legume crops as their native rhizobia are ubiquitous in Egyptian soils. For these reasons, the need for a better understanding and application of factors that increase biological N_2-fixation of soybean, seems worthy to save food and nitrogen fertilizers.

It is quite obvious that the growth of both plant and bacteria are greatly affected by environmental factors and any factor that adversely affects plant growth will also profoundly affect nodule formation on the root of soybean plants. To allow nitrogen fixation to occur in the root nodule, there must be extensive metabolic balance between bactericides and the different plant cells (Michiel and Hooker, 1988).

The potential response of grain legumes to the application of the major fertilizers is affected by (a) the level of supply in an available form of nutritional elements required at trace levels both by the plant and by nodule system; (b) soil levels of N, P, K; (c) by the presence or absence of strains of rhizobia capable of forming effective nodules on the legume concerned; (d) by competition between the plant and nodule for nutrient elements present limited supply; (e) by variation in the quantity and quality of trace elements in the seed used in the experiment and (f) by the method by which trace elements or bacterial inoculation are applied (Hallsworth, 1972).

Therefore, inoculation of soybean with effective strains or its own, is not guarantee of the development of effective nodulation. High level of N_2-fertilization or the lack of trace elements as Mo, Cu, Co, Fe and Mn in sufficient quantities, prevent the nodule system for fixing a significant quantities of N_2, even in the present of effective strains of rhizobia.

The Egyptian alluvial soils is thought to be deficient in plant microelements due to low percentage of soil organic matter, addition of NPK fertilization without considering microelements need, intensive cropping, reducing added amount of mud to the soil after constricting the High Dam and the alkaline conditions of soil which decreased the availability of some trace elements such as Mn, Fe, B and Zn.

In the light of the presumptive evidences, the present investigation was conducted to assess the optimal combinations between rhizobial inoculation and two commercial foliar sprays, each, of which have different concentrations of N, P, K and trace elements, aiming to achieve maximum nodulation and yield of soybean. The effect of either inoculation, fertilization and their interaction on the characteristics of the growing plants and the seed quantity and quality under the prevailing field conditions were also, studied.

MATERIALS AND METHODS

Two field trials on soybean, *Glycin max* (L.) Merr. Clark variety were conducted during the two successive seasons of 2000-2001 at Sakha Agricultural Research Station. The soil was clayey in texture, with organic matter 1.63%, total nitrogen content of 0.097%. The pH of soil-water suspension (1: 2.5) was 8.10.

Forms of N, P, Fe, Mn and Zn nutrients of soil under investigation were found as follows:

Available-N (Amm sulfate extract N)	65 ppm
Available-P (Olsen extractable-P)	11.51 ppm
Available-Fe (DTPA-Fe)	1350 ppm
Available-Mn (DTPA-Mn)	11.61 ppm
Available-Zn (DTPA-Zn)	9.17 ppm.

All the soil characteristics were determined according to the standard methods mentioned by Black (1965), Chapman and Pratt (1961) and Jackson (1958).

A complete randomized blocks (CRB) design with 4 replicates as used. The treatments were as follows:

1. Control (without inoculation and without fertilizers).
2. 60 kg N/fed.
3. 20 kg N/fed. + foliar compound No. 1.
4. 20 kg N/fed. + foliar compound No. 2.
5. 20 kg N/fed. + rhizobium inoculation.
6. 20 kg N/fed. + foliar rhizobium + compound No. 1.
7. 20 kg N/fed. + foliar rhizobium + compound No. 2.

Soybean inoculation:

Mixture of three strains of *Bradyrhizobium japonicum* 1577 (W. Germany), Sbb (USA) and RJG (local strain) were obtained form Biological Nitrogen Fixation Unit Agric. Res. Centre, Sakha Cultures wee maintained on yeast extract mannitol (YEM) Vineent 1970) agar slant Each rhizobial-strain was grown in YEM broth on rotary Shaker at 25°C. Prior to inoculation plate dilution count of viable all were made (initial number of cells used for inoculation was about 3 x 10^a cell/ml). Six-days old culture from the three strains was added to the fine nitrogen peat at rate of 1: 1 (v/w) before planting.

Seeds of soybean were sown in rows 60 cm apart and hills spaced 20 cm in the last week of May in the two seasons of experimentation. The form of N-fertilizer was urea 46% N and P-fertilizer as single super phosphate 15% P_2O_5 were added at sowing. Two commercial foliar compounds (1) and (2) were sprayed twice, the first 30 days after planting and the second 15 days later.

Foliar compound No. 1:

N %	P %	K %	Mg %	Fe %	Zn %	Mn %	Cu %
7	3	4	0.01	0.1	0.005	0.01	0.001

Foliar compound No. 2:

N %	P %	K %	S %	Mg %	Mn %	Fe %	Cu %	Zn %	Mo %	Bo %	Co %
22	21	17	0.167	0.079	0.0395	0.037	0.076	0.0068	0.005	0.0033	0.002

Data of nodules number and weight and dry matter of plants at 45 and 80 days, from planting, N in plants at 45 and 80 days from planting N, P and K of seeds and seed yield were recorded. The statistical analysis (for all data) was carried out according to Snedecor and Cochran (1971). The mean values for the studied treatments were compared using L.S.D. 0.01 test.

RESULTS AND DISCUSSION

1. Effect of inoculation, N_2-fertilization and foliar application on nodulation status of plants:

The effect of inoculation of soybean plants with *Bradyrhizobium japonicum* in association with N_2-fertilizer and two foliar compounds (comp-1 and 2) on number and dry weight of nodules was determined at the two investigation periods 45 and 80 days after planting time (Table 1).

Table(1): Effect of nitrogen fertilization, foliar application and *Bradyrizobium japonicum* on No. of nodules and dry weight of nodules (gm/plant) of soybean plants at 45 and 80 days in 2000 and 2001 season.

Treatments	No. of nodules/plant				Dry weight of nodules			
	2000		2001		2000		2001	
	45 days	80 days	45 days	80 days	45 days	80 days	45 days	80 days
Control	2.331	3.332	2.671	3.672	0.008	0.012	0.010	0.020
60 kg N/fed.	1.330	1.670	1.672	2.332	0.005	0.006	0.006	0.018
20 kg N/fed. + comp. (1)	3.672	4.000	4.333	5.000	0.013	0.015	0.014	0.025
20 kg N/fed. + comp. (2)	2.670	3.671	3.334	4.673	0.010	0.013	0.013	0.023
20 kg N/fed. + rhizobia	46.672	67.673	50.332	67.674	0.183	0.350	0.195	0.384
20 kg N/fed. + rh. + comp. (1)	53.000	73.000	58.000	75.000	0.198	0.459	0.201	0.451
20 kg N/fed. + rh. + comp. (2)	56.331 **	79.000 **	61.000 **	83.000 **	0.204 **	0.486 **	0.206 **	0.480 **
L.S.D at 0.01	1.677	2.334	1.765	3.723	0.010	0.102	0.011	0.008

The data show that inoculation increased significantly the number of nodules especially for soybean plants that sprayed with macro and micronutrients of the two foliar applied. The uninoculated exhibited a very lowest number of nodules, being on the average 2.7 and 3.5 nods/plant after 45 and 80 days after planting. Such results might indicate that the soil under investigation could be consider free from native soybean rhizobia. These results are in accordance with obtained by many Egyptian investigators (Hamdi *et al.*, 1974, El-Haddad *et al.*, 1984 and Ghobrial *et al.*, 1995 and 2002). It is therefore, of vital importance to continue inoculation of soybean seeds successively with effective, competitive and persistent strains of soybean rhizobia. The comparable inoculated treatments recorded higher number, being after 80 days of planting 74.0 nod./plant. The improvement of nodulation was further strengthed by the application of both foliar sprays, but the superiority was evident with comp-2 than that of comp-1.

These might be due to the high content of macro and micronutrients elements in comp-2 compared with those present in comp-1 as evident from their structure (Sec. of materials and methods) or the balance of its components which may be more suitable for the growth of plant and bacteria. In addition the comp-2 is supplemented with S (0.167%), Fe (0.37%), Mo (0.005%), Bo (0.0033%) and Co (0.002%). A more specific role has been demonstrated for there elements in biological N_2-fixation (Hamdi, 1982). The nitrogen fixing enzyme nitrogenase is composed of molybedenum and iron, without adequate quantities of these elements nitrogen fixation can not occur (Howrd and Leonard, 1972). Also iron is constituent of the leghaemoglobin that protects nitrogenase from oxygen inactivation (Apleby, 1988 and Fornasieri *et al.*, 1988). Boron deficiency affects the vascular system leading to the nodule and hence causes a shortage of carbohydrate at fixation sites (Hamdi, 1988 and Abd El-Kodoos *et al.*, 2002).

With regard to the dry weight of nodules data presented in Table 1 clarified that the total mass of nodules showed similar as shown in nodule number it remains however, that the highest improvement in nodule tissue was combined with foliar spry with comp-2 with age progress to 80 days the interaction between rhizobial inoculation and foliar application showed a continuous increase in dry weight of nodules. The results reported coincide with those obtained by Hashimoto and Yamaskai (1976), El-Essawi and Abadi (1983); Hegazy y *et al.* (1990) and Murek and Kobust (1990).

2. Dry matter yield and N-content:

Dry matter production of soybean plants was strongly influenced by rhizobial inoculation as shown by striking differences between inoculated and control treatments (Table 2). The two control treatments, namely the uninoculated and uninoculated but supplied with mineral nitrogen fertilizer (60 kg N/fed.) produced the biggest contrast in the dry weight of shoot and their total nitrogen content (up to 2 times). An increase of about 116% in nitrogen yield was recorded for N_2-fertilization as compared to control treatment after 80 d ays a fter p lanting t ime. S uch r esult i ndicate t hat t he a vailable n itrogen was the main factor limiting plant growth.

Table (2): Effect of nitrogen fertilization, foliar application and *Bradyrizobium japonicum* on dry weight of plant (gm) and total nitrogen of soybean plants (%) at 45 and 80 days in 2000 and 2001 season.

Treatments	Dry weight of plants				N% in plants			
	2000		2001		2000		2001	
	45 days	80 days	45 days	80 days	45 days	80 days	45 days	80 days
Control	13.45	18.17	13.59	18.04	1.94	2.38	1.99	2.41
60 kg N/fed.	17.30	28.83	17.07	28.79	2.42	3.21	2.45	3.31
20 kg N/fed. + comp. (1)	14.73	21.43	13.53	21.14	2.06	2.46	2.11	2.49
20 kg N/fed. + comp. (2)	15.00	22.80	19.33	22.51	2.03	2.48	2.22	2.52
20 kg N/fed. + rhizobia	16.63	28.23	16.20	27.09	2.44	3.21	2.48	3.23
20 kg N/fed. + rh. + comp. (1)	16.90	28.40	16.60	28.42	2.47	3.28	2.52	3.30
20 kg N/fed. + rh. + comp. (2)	17.13	28.53	16.77	28.68	2.50	3.32	2.55	3.34
	**	**	**		**	**	**	**
L.S.D at 0.01	0.489	1.697	0.904	1.515	0.3062	0.051	0.047	0.089

It is also, evident that in the absence of rhizobial inoculation foliar application with both comp-1 and 2 did help the plant to accumulate more nitrogen, but the with less magnitude. The percentage of increase in dry matter over control were 20.4 and 25.13%, respectively after 80 days of planting time (average of two years). The parallel values for N_2 % were 3.34 and 4.38%. The c omparable p attern o btained a s a r esult o f a r hizobial i noculation only was slightly lower than N_2-fertilization, but recorded highly significant increase over control treatment. Such a result may be attributed to the fixation of adequate amount of atmospheric nitrogen symbiotically and consequently covers (46%) the requirement of the plants from this particular element. The vital importance of rhizobial inoculation. It is also, noticed that foliar application can enhanced the simulative effect of inoculation. However, it is not clear that the differences in nitrogen yield between inoculation in the presence of foliar sprays are completely attributed to increased N_2fixation activity. As the improved growth due to increased availability of N could be responsible for higher uptake of soil nitrogen, especially application of micronutrients enable the plant to make better use of soil nitrogen. These results are in agreement with those reported by Vasiales *et al.*, 1980; Abd El-Hadi *et al.*, 1984 and Hegazy *et al.*, 1990.

3. Effect of seed yield and seed components (N, P and K):

The yield of soybean was estimated after 130 days of planting time. To evaluate the interactions among different treatments, the percentage of changes in comparison with control treatment in grain yield and their total N, P and K were evaluated and recorded in Table 3 from which the following could he concluded:

1. Further average increase over control were obtained in seed yield and their total N-content as a result of rhizobial inoculation in combination with foliar spray with comp-2 then comp-1. The corresponding values were 27.4 and 24.6% for seed yield and 28.6 and 27.2% from total nitrogen. Inoculation and N_2-fertilization increased proportionally the seed yield and their total N-content.
2. The lowest values were obtained form the treatments which were sprayed with foliar compounds but neither inoculation nor fertilizer nitrogen application took place.
3. The concentration of phosphorus and potassium in seeds showed similar trend as shown in seed yield and their total nitrogen content.

It is clear from the present investigation that:

a. Inoculation soybean with *B. japonicum* enhanced in general, nodule formation, growth of nodular tissue as well as N_2-fixation which reflects its effects on seed yield and the total N, P and K of seeds.

b. The improvement of nodulation and biological N_2-fixation was further strengthened by foliar application (Comp-2).

c. In the absence of rhizobial inoculation foliar application achieved less improvement in seed yield and their total N, P and K.

Table (3): Seed yield (ton/fed.) and their total N, P and K concentration (%) as affected by nitrogen fertilization, foliar applicaitoin and *Bradyrizobium japonicum* during seasons 2000 and 2001.

Treatments	Seed yield ton/fed.		N % of seeds		P % of seeds		K %) of seeds	
	2000	2001	2000	2001	2000	2001	2000	2001
Control	1.05	1.10	5.20	5.28	0.34	0.36	1.23	1.25
60 kg N/fed.	1.21	1.24	6.48	6.59	0.42	0.45	1.33	1.35
20 kg N/fed. + comp. (1)	1.13	1.16	5.60	5.64	0.37	0.39	1.29	1.30
20 kg N/fed. + comp. (2)	1.15	1.18	5.66	5.69	0.38	0.39	1.32	1.33
20 kg N/fed. + rhizobia	1.24	1.27	5.52	6.60	0.43	0.46	1.35	1.36
20 kg N/fed. + rh. + comp. (1)	1.32	1.36	6.65	6.68	0.45	0.47	1.36	1.38
20 kg N/fed. + rh. + comp. (2)	1.35	1.39	6.09	6.72	0.47	0.49	1.37	1.39
	**	**	**	**	**	**	**	**
L.S.D at 0.01	0.021	0.025	0.038	0.206	0.040	0.007	0.0198	0.0018

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تأثير الرش بمركبات ورقية مغذية متعددة العناصر والتسميد النتروجينى على تحسين كفاءة البكتريا العقدية ونمو محصول فول الصويا

فاطمة احمد شريف
معهد بحوث الاراضى والمياه والبيئة ـ محطة البحوث الزراعية ـ بسخا

اقيمت تجربتان حقليتان بمحطة البحوث الزراعية بسخا خلال موسمى ٢٠٠٠/٢٠٠١م وذلك بهدف تقييم التثبيت النتروجينى بواسطة بكتريا العقد الجذرية لنباتات فول الصويا "صنف كلارك" الملقحة بالسلالات "1577, RJG and Sbb" إلى جانب التسميد النتروجينى المعدنى (٦٠ كيلوجرام نيتروجين/فدان) والرش بالمركبين الورقيين الذى يحتوى كل منهما على كميات ونوعيات مختلفة من العناصر الكبرى والصغرى.

وتدل النتائج التى تم تسجيلها بعد ٤٥ ، ٨٠ يوم من الزراعة ان المعاملات الغير ملقحة اعطت اقل القيم فى تكوين العقد على جذور نباتات فول الصويا وهذا يوضح ان ارض التجربة المدروسة تعتبر خالية من الريزوبيا الطبيعية.

ولذلك فأن من الاهمية القصوى استمرار تلقيح بذور فول الصويا بالسلالات الفعالة والتى لها القدرة التنافسية.

واثبتت النتائج أيضا ان تلقيح فول الصويا قد ازداد بشكل عام من تكوين العقد الجذرية وكذلك نمو انسجة العقد والتى بالتالى ينعكس اثرها على زيادة محصول البذور والمحتوى الكلى من النتروجين.

كما أدى استخدام الرش بالمركبات الورقية الى تقوية تكوين العقد الجذرية وعملية التثبيت الحيوى للنتروجين وخاصر باستخدام مركبات الرش الورقى (٢) وأدى غياب التلقيح بالريزوبيا والرش بالمركبات الورقية المغذية الى نقص فى محصول البذور ومحتواها الكلى من النتروجين والفوسفور والبوتاسيوم.