THE EFFECT OF INOCULATING WITH ARBUSCULAR MYCORRHIZA AND BRADYRHIZOBIUM STRAINS ON SOYBEAN (*Glycine max* (L) Merrill) CROP DEVELOPMENT

Marlen Hernández✉ and F. Cuevas

ABSTRACT. The present work was accomplished in "Los Palacios" Rice Research Station, belonging to the National Institute of Agricultural Sciences (INCA) in spring, 2001. The response of soybean G7-R-315 cv to single and combined inoculations with *Bradyrhizobium japonicum* and *Glomus fasciculatum* strains was evaluated. Therefore, an experiment was conducted on a randomized block design with six treatments and four replications. Results showed the positive influence of microorganisms on soybean plant height and yield. The best results were recorded in the treatments of seed inoculation combined with both strains, as well as the one of nitrogen fertilization replaced by *Bradyrhizobium japonicum*.

RESUMEN. El presente trabajo se realizó durante la primavera del 2001, en áreas de la Estación Experimental del Arroz "Los Palacios", perteneciente al Instituto Nacional de Ciencias Agrícolas. Se evaluó la respuesta de la variedad de soya G7-R-315, ante las inoculaciones simple y combinada de las cepas de *Bradyrhizobium japonicum* y *Glomus fasciculatum*. Para ello se realizó un experimento empleando un diseño de bloques al azar con seis tratamientos y cuatro réplicas. Los resultados mostraron influencia positiva de los microorganismos utilizados sobre la altura y el rendimiento de las plantas de soya, obteniéndose los mejores resultados en los tratamientos donde se combinó la inoculación de la semilla con ambas cepas, así como en el tratamiento donde se sustituyó la fertilización nitrogenada por *Bradyrhizobium japonicum*.

Key words: bradyrhizobium, soybean, arbuscular mycorrhizae, inoculation

Palabras clave: bradyrhizobium, soya, micorrizas arbusculares, inoculación

INTRODUCTION

Soybean is among mankind´s ancient crops and constitutes a very important source of plant protein and oil at present. Its grain contains from 18 to 21 % fat and 38 to 40 % protein (1, 2).

According to FAO reports, in 1994 soybean was grown in about 62 million hectares all over the world and its production surpassed 136 million tons of grains, reaching an average yield of 2.8 t.ha^{-1} (3).

Cuban government is interested in widening this commercial crop once its technical and economic feasibility is proved, not only due to its great internal demand but also its high world market prices.

Agriculture, as a multidisciplinary and integral system, requires the knowledge and application of several sciences, with the viewpoints of study influencing definitely its production sustainability and environmental conservation.

In this sense, nitrogen profitability as a result of rhizobium bacteria has been deeply studied and become a common practice in those countries where leguminous crop is established; on the other hand, there are many research papers on the use of mycorrhizae (mutual symbiotic associations), which has converted hardly mobile nutrient absorption into a more efficient one (such as phosphorus, that influences N nodulation and fixation), also provides some protection against certain fungal pests, and eventually increases crop yield (4).

Thus, this paper was aimed at studying the effect of single and combined inoculations with *Bradyrhizobium japonicum* and *Glomus fasciculatum* strains on soybean yield and its components.

MATERIALS AND METHODS

This experiment was conducted in "Los Palacios" Rice Research Station of spring, 2001, on a Ferruginous Nodular Gley Hydromorphic soil (5), as it is shown in Table I.

Table I. Some soil fertility components at 0-20 cm

pH (H_2O)	P (ppm)	Ca (cmol.kg^{-1})	K (cmol.kg^{-1})	CCB (cmol.kg^{-1})	OM (%)
6.50	8.0	12.0	0.40	26.0	2.80

Soybean seeds from G7-R-315 cv were selected to evaluate single and combined inoculations with *Bradyrhizobium japonicum* ICA 8001, the latter at a

Marlen Hernández, Especialista y Ms.C. F. Cuevas, Investigador Agregado de la Estación Experimental del Arroz "Los Palacios", Instituto Nacional de Ciencias Agrícolas, Gaveta Postal 1, San José de las Lajas, La Habana, Cuba. CP 32 700.

✉ palacios@inca.edu.cu

combination of 10^8 UFC.mL^{-1} and a rate of 10 g.kg^{-1} seeds whereas *Glomus fasciculatum* strain with a minimal composition of 100 spores per grain of soil and an application rate of 10 % seed weight. A randomized block design with six treatments and four replications was used.

The following treatments were evaluated:

T1: absolute check (neither fertilized nor inoculated)
T2: (100-80-60) NPK
T3: (PK: 80-60) + *Bradyrhizobium japonicum*
T4: *Bradyrhizobium japonicum*
T5: *Glomus fasciculatum*
T6: *Bradyrhizobium japonicum* + *Glomus fasciculatum*

Urea, triple superphosphate and potassium chloride were respectively employed as NPK carriers and applied as basal dressing by hand at seeding time. A seed-coating technology (6) was used for seed inoculation and coinoculation with biofertilizers.

Seeds were drilled in 8-m^2 plots with four rows each spaced at 0.70 m.

The following evaluations were performed:

★ final plant height
★ nodule number at flowering
★ pod number per plant
★ grain number per pod
★ 100-grain weight (g)
★ crop yield (t.ha^{-1}).

A Double Variance Analysis was applied to the statistical processing of data, also using Duncan´s Multiple Range Test ($p<0.05$) as a discriminant analysis for mean differences.

RESULTS AND DISCUSSION

Soybean response to different fertilizer rates under these conditions is presented in Table II, which also shows that plant height was slightly encouraged by both mineral fertilization and biofertilizers compared to the absolute check (neither fertilized nor inoculated); there were significant differences among them. The treatments T6 (combined inoculation with *Bradyrhizobium japonicum* and *Glomus fasciculatum*) and T3 (N fertilizer was replaced by *Bradyrhizobium japonicum*) were notable. These results are in agreement with others previously obtained (7), which state that, under field conditions, soybean plants usually have two kinds of symbiotic microorganisms on their roots: *Bradyrhizobium* spp and arbuscular mycorrhizal fungi, which are capable to enhance plant growth by supplying nitrogen and mobilizing hardly mobile nutrients, such as P.

Concerning nodule number, the best response was recorded in treatments T3 and T6, without significant differences between them. Despite that T4 (only *Bradyrhizobium japonicum*) differs statistically from the preceding treatments, there is an adequate nodulation effect. Similar results have been reported (8), that proved the high effectiveness of this strain for a great number of soybean varieties, including the one presented in this paper.

With regard to yield components, pod number and 100-grain weight, there were significant differences among treatments; T2 and T3 as well as the combined inoculation of strains in both variables were notable for 100-grain weight. Other results have stated the significant effect of combined strains on these variables with the same variety (8). However, treatments had no influence on grain number per pod, since it is a highly heritable trait, which is in agreement with other experimental results (9).

On the other hand, the treatments of either N fertilizer replaced by Bradyrhizobium strain or the use of coinoculation reached remarkable crop yields, perhaps as a result of the effect of microorganisms on roots, since root system grows thereby nutrient absorption enhances, which implies a greater plant development. Similar results refer to the positive effect of combining inoculation of both biofertilizers on crop growth and yield (9), recording increments of 32.84 and 37.31 %, which proved the synergical and profitable effects of *Bradyrhizobium japonicum*-AM fungus coinoculation to this crop.

Other research works, dealing with native strains of Bradyrhizobium and mycorhizal fungi with P levels in beans, determined the significant infection effect of both endophytes (rhizobium and mycorrhiza) on plant yield (10).

Some studies performed in Guarico, Venezuela, have emphasized the significance of inoculation with Bradyrhizobium to satisfy crop demand and reach good yields (11).

As a result of other experiments, yield increases of 700 to 1 000 kg.ha^{-1} have been reported, compared to the noninoculated check, when inoculating with efficient strains of *Bradyrhizobium japonicum* (12).

Table II. Effect of treatments on plant height and some yield components

Treatments	Plant height (cm)	Nodule number	Pod number per plant	Grain number per pod	100 grain-weight (g)	Agricultural yield (t.ha^{-1})
T1: (neither fertilized nor inoculated)	72.10 c	75 c	27.80 f	2.21	14.21 b	2.77 d
T2: (100-80-60)NPK	77.30 ab	103 b	49.70 c	2.39	15.80 a	3.85 b
T3: (PK: 80-60)+ Bj	78.10 a	118 a	52.30 b	2.70	15.90 a	4.06 ab
T4 : Bj	75.23 b	92 b	45.20 d	2.59	14.90 b	3.29 c
T5: AM	73.77 c	80 c	33.10 c	2.63	14.38 b	3.02 c
T6: Bj + AM	78.70 a	120 a	59.10 a	2.71	16.04 a	4.22 a
VC	10.25	13.18	5.89	0.02	1.25	2.84
SE x	0.52***	5.21***	2.68***	0.08 ns	0.24***	0.09***

Bj: *Bradyrhizobium japonicum* AM: Arbuscular mycorrhiza
Means followed by the same letter within a column are not significantly different at $p<0.05$

In general, the positive effect of applying biofertilizers was proved; soybean seed coinoculation with *Bradyrhizobium japonicum* and *Glomus fasciculatum* was considered remarkable, as a way to satisfy biofertilizer demands, mainly nitrogen and phosphorus, and achieve higher yields than those normally obtained under these experimental conditions.

REFERENCES

1. INTA. El cultivo de la soya en Argentina. 1997.
2. Batista S. /*et al.*/. La soya en Guantánamo, una realidad para la sostenibilidad de agricultura del territorio. En: Taller Internacional sobre Recursos Fitogenéticos (4:2001), 2001. 122 p.
3. Cuba. Minagri. Cultivo y utilización de la soya en Cuba. La Habana, 1998.
4. Hernández, A. y Hernández, A. N. Efecto de la interacción rhizobium-MA en el cultivo de la soya *(Glycine max* (L) *Merrill)*. *Cultivos Tropicales*, 1996, vol. 17, no. 1, p. 5-7.
5. Cuba. Minagri. Instituto de Suelos. Versión de clasificación genética de los suelos de Cuba. La Habana:Agrinfor, 1999. 64 p.
6. Fernández, F. y Rodríguez, E. L. Estudios básicos de la Tecnología de Recubrimiento de Semilla con inoculantes micorrizógenos V. A. En: Seminario Científico del INCA (10:1996:La Habana), 1996.
7. Zhang, F. /*et al.*/. Root- zone temperature and soybean (*Glycine max* (L) *Merr*.) vesicular arbuscular mycorrhizae: Development and interaction with the nitrogen-fixing symbiosis. *Environmental and Experimental Botany*, 1995, vol. 35, no. 3, p. 287-298.
8. Corbera, J. Coinoculación *Bradyrhizobium japonicum*-micorriza vesículo–arbuscular, como fuente alternativa de fertilización para el cultivo de la soya. *Cultivos Tropicales*, 1998, vol. 19, no. 1, p. 17-20.
9. Corbera J. /*et al*./. Efectos sobre el crecimiento y desarrollo del cultivo de la soya en primavera por la inoculación conjunta *Bradyrhizobium japonicum*-hongos MA y la aplicación de un análogo de Brasinoesteroides. En: Congreso Científico INCA. (13:2002:La Habana), 2002, p. 65.
10. Machado, A. M. Participación de biofertilizantes en la asociación frijol-maíz en la provincia Granma. En: Jornada Científica I.I. Agropecuarias “Jorge Dimitrov” (4:2002:La Habana), 2002, p. 44.
11. Casanova, E. Evaluación de la nutrición y fertilización de la soya en Guárico-Venezuela En: Informaciones Agronómicas, 2001, no. 45.
12. González, N. /*et al*./. Nutrición nitrogenada en *L. giorda* y *H. baigorri*. El cultivo de la soya en Argentina. San Juan : INTA, 1998, 198 p.

Recibido: 6 de noviembre del 2002
Aceptado: 5 de febrero del 2003