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Effects of *Bradyrhizobium japonicum* inoculants on soybean (*Glycine max* (L.) Merr.) growth and nodulation

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ABSTRACT

Responses to inoculation have shown that inoculation is justified in soil where has never been grown soybean, such is the case of El Rincón de Monagas (94° 41' 35" N, 63° 04' 38" W and 39 m.a.s), Monagas State, Venezuela. In this context, two inoculants types of Bradyrhizobium japonicum (RIZO-LIQ, water-based product and RIZOPLUS SUPER, peat-based product, both from RIZOBACTER Argentina, S.A.) were tested in soybean variety Tracaja (EMBRAPA, Brazil) throughout growth (shoot height and dry weight, root length and dry weight, and leaf area) and nodulation (red nodule number and dry weight) parameters measurements. Chemical fertilizer as NPK (10-10-10) was added at planting which supplied 20kg nitrogen/ha as starter dose. Also was applied Legumol (8,0% Mo, 0,7% Co, 5,0% Zn, 0,5% B, 0,5% Cu and 0,1% Mn), at a rate of 200g/g seed as source of micronutrients. Data were subjected to variance analysis, using the statistical package Statistix and to the least significant difference (LSD) test at 5% probability level to compare treatment means. The inoculants combination (RIZOPLUS + RIZO-LIQ) treatment resulted more favorable to shoot height and dry weight and leaf area; similarly behaved red nodule number and dry weight. The inoculants combination (RIZO-LIQ + RIZOPLUS) was more favorable for growth and nodulation of soybean variety Tracaja. In relation to the results, the factors bacterial number and competitiveness are discussed.

Keywords: Venezuela, soybean, inoculants, Tracaja, *Bradyrhizobium japonicum*.

INTRODUCTION

It is generally accepted that soybean (*Glycine max* (L.) Merr.) originated in China. It has been introduced into other countries, the United States, Brazil, Argentina and Japan, which together with China constitute the major producers [15, 30].

The protein content in soybean seed is approximately 40% and the oil content is 20%. This crop has the highest protein content and the highest gross output of vegetable oil among the cultivated crops in the world [10]. Also, soybean cultivation improves soil health because its ability to fix nitrogen fixation and its deep root [19].

Nitrogen fixation in soybean has been widely studied using different methodologies which reveal that soybean shows a strong demand for nitrogen (N) for optimal development and grain productivity [9, 11, 21].

Inoculation of soybean with the appropriate rhizobia bacteria provides high numbers of viable effective rhizobia to the rhizosphere to allow rapid colonization and nodulation [23, 24]. Responses to inoculation in research experiments have shown clearly that inoculation is justified, at least in soil that has never grown this legume before or has not grown this for many years [28].

Soybean is mainly nodulated by *Bradyrhizobium japonicum* and *B. elkanii* [11]. Soils usually lack the species strains unless soybean is grown on them for at least five or more years. Hiltbold [2] reported that numbers of *B. japonicum* in 52 Iowa fields were correlated with whether soybeans had been grown at the site within the previous 13 years. It is therefore important to inoculate seeds with relevant strains of bacteria before sowing, especially if the crop is to be grown for the first time on the land. Inoculation responses are associated primarily with the first planting of a legume in soil having no prior history of the crop [5, 14]. In any case, to get the maximum benefit out of inoculation there is a need to follow correct and careful inoculation procedures, and the inoculant should carry live and effective bacterial cells [27].

Bradyrhizobium japonicum strains are included on two types of carriers- peat and water. Peat (or humus) is used as a carrier in either a granular form, which is applied in-furrow, or in a powder form, which is applied to the seed at planting. Water-based products include liquid inoculants (seed applied or in-furrow) and frozen concentrates. New inoculants, introduced within the last 10 years, have increased potency due to sterile carriers and new packaging techniques [18]. Besides, there were varying reports on the interaction between variety and strain in soybean. Solomon *et al*. [27] and Thao [28] found a significant interaction between variety and strain on different parameters whereas Munyinda *et al*. [17] reported a no significant interaction.

In Venezuela, soybeans demand is around 1 million 200 thousand MT per year. National production supplies only a fraction (27.71%) of that national demand, having a high dependence of imported soybeans; so to ensure food sovereignty, it is necessary to make great efforts to increase domestic production. Through the agreement between Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) and the Instituto Nacional de Investigaciones Agrícolas de Venezuela (INIA) [10], it can be exchanged the experiences of Brazil in the cultivation of soybeans in tropical conditions and it will also gain access to genetic materials developed by Brazil which can be used in Venezuelan similar conditions [4, 13].

In Venezuela, studies on soybean inoculation are scarce but necessary to evaluate different inoculants types in the varieties recommended and various soil conditions. On this basis, the specific objectives of this research include studying the main effects of inoculants types on soybean growth parameters and nodulation.

MATERIALS AND METHODS

In this study, it was established a randomized complete design with four replications of two commercial inoculants of *Bradyrhizobium japonicum* (RIZO-LIQ, water-based product and RIZOPLUS SUPER, peat-based product, both from RIZOBACTER Argentina, S.A.) along with one uninoculated treatment and the variety of soybean, Tracaja, originating from EMBRAPA, Brazil.

The experiment was carried out on a sandy soil without history of soybean cultivation from the rural community of El Rincon de Monagas: 94° 41' 35" N, 63° 04' 38" W and 39 m.a.s (Monagas state, Venezuela), in the greenhouse of Postgraduate Center at Universidad de Oriente, Maturín, Venezuela, using the soybean cultivars specified above. Soil analysis showed that the soil was moderately acidic in reaction (pH = 5.6) and sandy in texture with low cation exchange capacity (2.3), organic matter (0.70%) and phosphorus content (2.5 ppm).

Each experimental unit (plastic bag) consisted of 4 kg of soil and 5 soybean seeds, which were superficially sterilized with commercial bleach (containing 5% of sodium hypochlorite) for 20 min and then rinsed five times with sterile water. Chemical fertilizer as NPK (10-10-10) was added at planting which supplied 20kg nitrogen/ha as starter dose. Also was applied Legumol (8,0% de Mo, 0,7% de Co, 5,0% de Zn, 0,5% de B, 0,5% de Cu and 0,1% de Mn), at a rate of 200g/g seed as source of micronutrients.

For symbiotic inoculation, two procedures were used: a liquid formulation inoculant (RIZO-LIQ, 5 x 10^9 cells/ml) at a rate of 300 ml/50 kg seed plus sugar (15%) to ensure that all the seeds receive a thin coating of the inoculant; and a peat formulation inoculant (RIZOPLUS SUPER, 1.5 10^9 cells/ml) at a rate of 150 g/50 kg seed; using wet method, that is, with seeds previously wetted with sterile water (250ml water/50kg seed). All inoculations were made just before planting under shade to maintain the viability of bacterial cells. Seeds were allowed to air dry for a few minutes and then sown. The plants were thinned to two/bag after 10 days of growth.

At 80 days after sowing, all plants were harvested. Plant parts were separated into leaves plus stems, roots, and nodules. There were dated: plant height (distance between cotyledonal nude and the base of the youngest expanded leave), root length (distance between cotyledonal nude and the tip of the more distal root) and leaf area (disk method) [20]. Nodulation was evaluated by red nodule counting and dry weight. All plant parts were oven dried at 72 ^{o}C for up to 72 h, and then weighed. Collected data were subjected to variance analysis, using the statistical package Statistix and to the least significant difference (LSD) test at 5% probability level, performed to compare treatment means.

RESULTS AND DISCUSSION

Measuring the benefits of rhizobial inoculation should include several traits of legume performance as strains may vary in their symbiotic effectiveness [3]. Accordingly, it is used a selection of variables to effectively discriminate the different *Bradyrhizobium japonicum* inoculants tested.

At 80 days after sowing, variance analysis for soybean shoots height showed statically significant differences (F= 3.38) between treatments (Figure 1). Thus, the inoculants combination (RIZOPLUS + RIZO-LIQ) treatment resulted more favorable to shoot height (53 cm plant^{-1}), while the least beneficial treatment was RIZOPLUS (25 cm plant^{-1}), being this value 50% lower than the value found at the combined treatment.

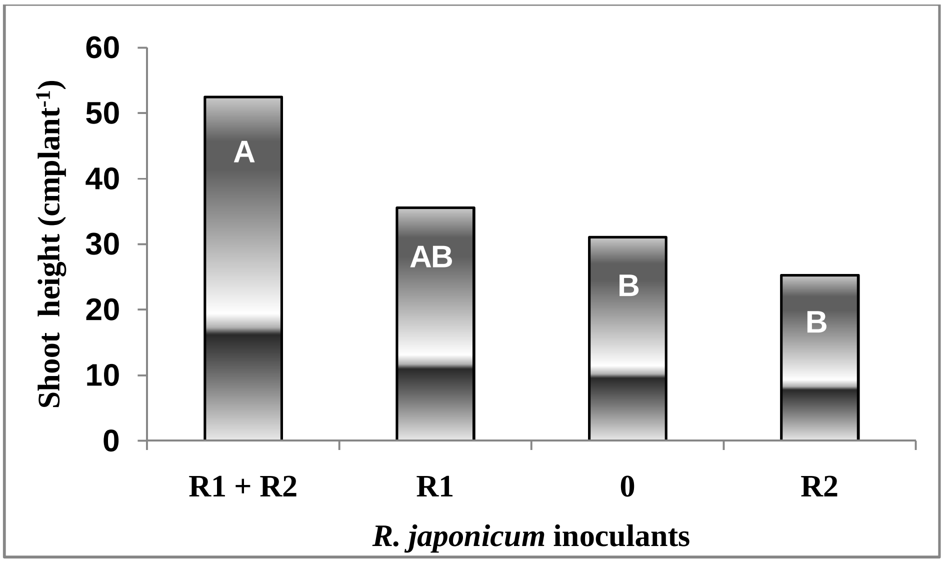

Figure 1. Shoot height variation in relation to inoculant treatments
***R1**: RIZO-LIQ, **R2**: RIZOPLUS, **R1 + R2**: RIZOPLUS + RIZO-LIQ,**0**: Uninoculated.*

Variance analysis of shoot dry weight showed significant differences between treatments (F= 7.08, $P<0.05$). The highest value corresponded to the inoculants combination treatment (15.1 g plant^{-1}), which was statistically different to the others treatments applied. Shoot dry weight of plants inoculated with RIZOPLUS or RIZO-LIQ or uninoculated were similar, it varied between 7.6 and 9.9 g plant^{-1} (Figure 2).

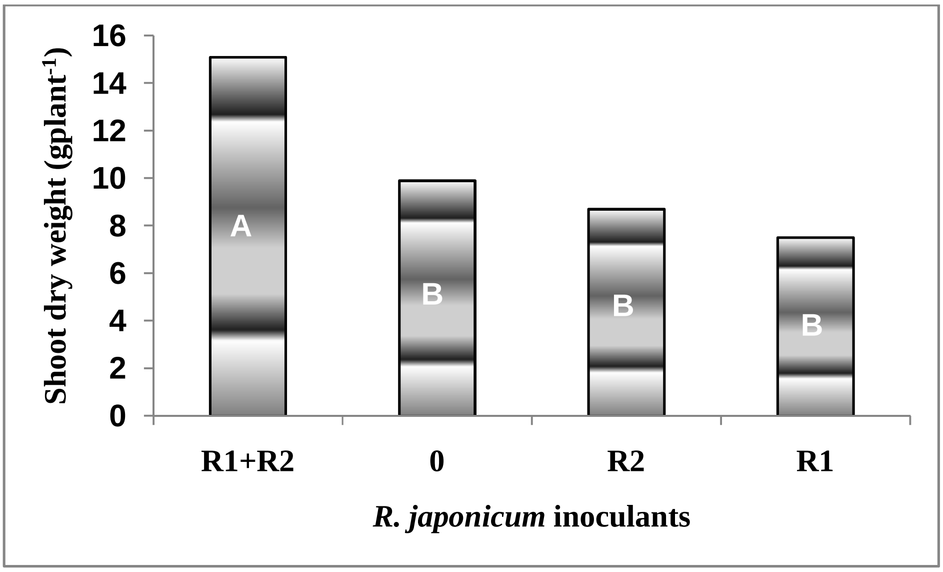

Figure 2. Shoot dry weight of soybean plants under different inoculant treatments
R1: *RIZO-LIQ,* ***R2***: *RIZOPLUS,* ***R1 + R2***: *RIZOPLUS + RIZO-LIQ,* ***0***: *Uninoculated.*

Similar to shoot dry weight, the analysis of variance of leaf area showed significant differences between treatments (F= 4.61, P<0.05). Mean comparisons revealed the inoculants combination treatment as the more favorable; these plants showed the highest value (57.5 cm^2), which was statistically different to the others treatments applied. Leaf area of plants inoculated with RIZOPLUS or RIZO-LIQ, or uninoculated were similar, these values ranged from 28.8 to 37.6 cm^2 (Figure 3).

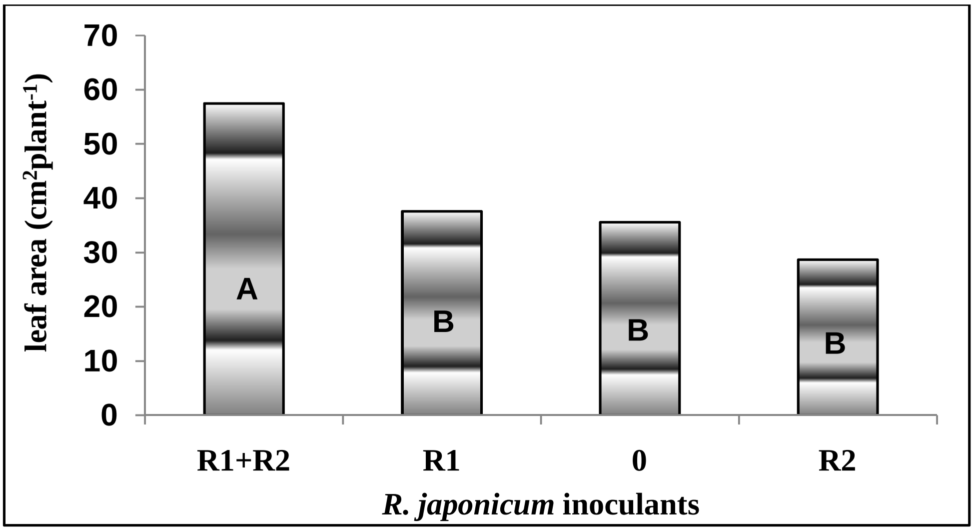

Figure 3. Influence of *R. japonicum* inoculants on leaf area of soybean plants.
R1: RIZO-LIQ, R2: RIZOPLUS, R1 + R2: RIZOPLUS + RIZO-LIQ, 0: Uninoculated.

Accordingly with the resulting data of shoot height and dry weight, and leaf area, the combined treatment (RIZOPLUS + RIZO-LIQ) was the more favorable treatment to shoot growth.

Variance analysis of root dry weight showed no significant differences between treatments (F= 2.21, P>0.05), thus, they were similar in dry weight; the average was 1.03 g $plant^{-1}$. Similarly, it occurred with root length, the values were similar between treatments (F= 2.34, P>0.05); the average was 1.02 cm $plant^{-1}$ (Table 1).

As it is evident from the data presented in Figure 4, the nodule number was affected significantly by the main effect of inoculants (F= 10.7, P<0.05). The combined treatment (RIZO-LIQ + RIZOPLUS) produced significantly higher number of nodules (17.5) than RIZO-LIQ (7.0) or RIZOPLUS (9.5 nodules plant^{-1}) independently. The uninoculated control did not produce any nodule at all, this is an indication that native strains of *Bradyrhizobium japonicum* bacteria that could form symbiotic relation with soybean are absent in the soils of El Rincón de Monagas, and that a cross-contamination did not occur. Moreover emphasize that when growing a new legume species on a soil, it is necessary that the appropriate rhizobial culture be applied. The favorable effect of inoculation on soybean nodulation and consequently on growth has been shown in many studies [3, 27, 29].

Table 1. Variations in root dry weight and length of soybean plants treated with two inoculant types

***B. japonnicum* inoculants**	**Root dry weight (g plant^{-1})**		**Root length (cm)**	
RIZO-LIQ	1.21	A	11.8	A
Uninoculated	1.18	A	11.9	A
RIZO-LIQ+ RIZOPLUS	0.93	A	11.5	A
RIZOPLUS	0.81	A	11.6	A

Also, nodule dry weight follows the similar trend that nodule number, thus the nodule dry weight was affected significantly by the type of inoculant used (F= 14.9, P<0.05). The combined treatment (RIZO-LIQ + RIZOPLUS) produced significantly higher nodule dry weight than RIZO-LIQ or RIZOPLUS independently (Figure 5).

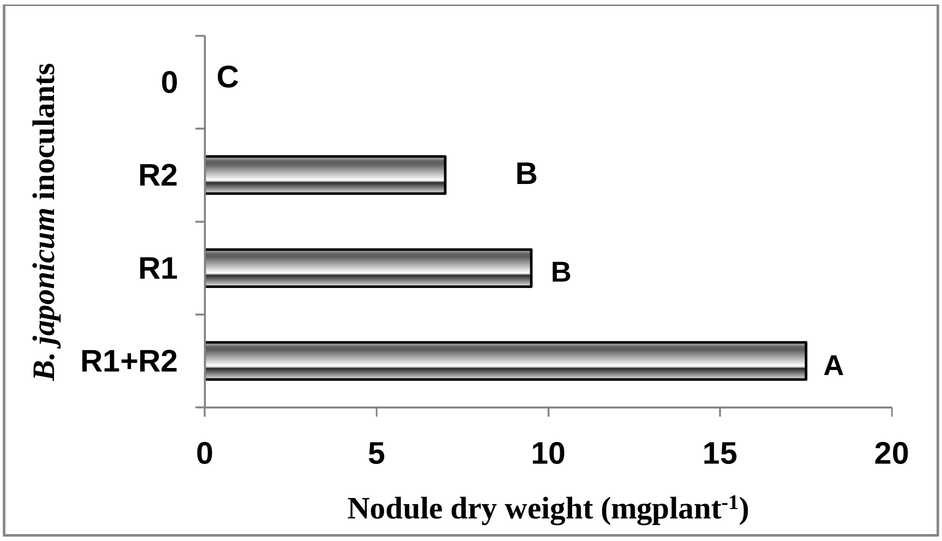

Figure 4. Main effects of *B. japonicum* inoculants on nodule number of soybean plants
***R1**: RIZO-LIQ, **R2**: RIZOPLUS, **R1 + R2**: RIZOPLUS + RIZO-LIQ,**0**: Uninoculated.*

It is clear from the data that inoculants combination (RIZO-LIQ + RIZOPLUS) was more favorable for growth and nodulation of soybean variety Tracaja. Some approaches can be considered, bacterial number and competitiveness.

Although RIZO-LIQ and RIZOPLUS are high quality commercial inoculants, well recommended for soybeans; in this study, their efficiency have been low to good growth and nodulation in soybean Tracaja when they are use as single inoculant, This situation can be related with a low number of *Bradyrhizobium* in them due to storage conditions, transport, or other factor. In this respect, various researchers have demonstrated the direct relations between *Bradyrhizobium* population in the inoculant or soil and nodulation. Thus, Singleton and Tavares [22] observed that low populations of rhizobia in soil could not sufficiently promote legume nodulation and increase symbiotic N_2 fixation; Hume and Blair [6] found that nodule number and mass, as well as seed yield, increased curvilinearly upward with increasing $\log_{10}$ most probable numbers (MPNs) of *B. japonicum*; Papakoska [7] showed linear relationship between the rate of applied rhizobia and the number of the nodules per plant or the dry weight per nodule; Maurice *et al*. [25] with commercial liquid inoculants, demonstrated that *B. japonicum* cells from old inoculants needed more time to divide and produce visible colonies, indicating that they would probably be unable to grow in soil; more recently, Larson *et al*. [16] indicated that the companies recommend rates for inoculants which often have proven adequate but this is not always consistent, and several cases of inadequate nodulation have been reported in recent years; also, point out that often a 2X rate or combination of different inoculant products is used as added insurance for achieving a good bacterial population near the seedling roots for root colonization.

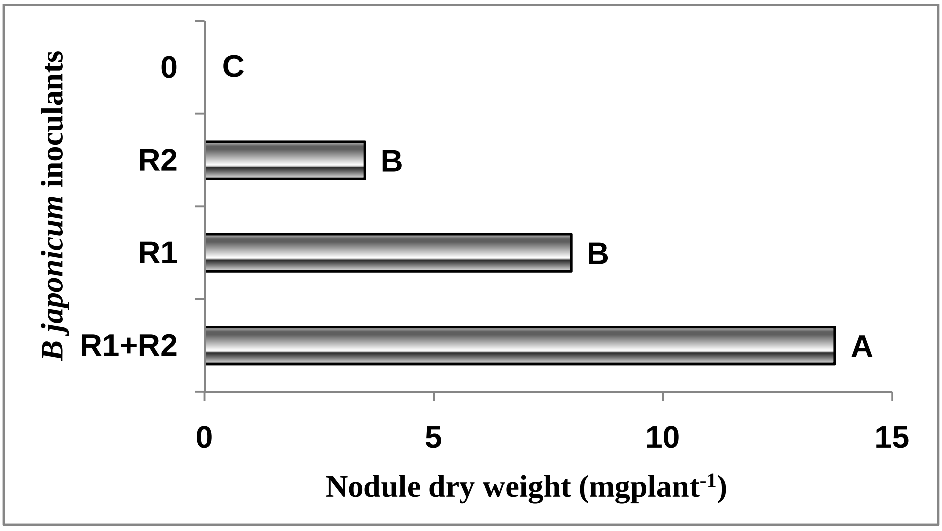

Figure 5. Major effects of *B. japonicum* inoculants on nodule dry weight of soybean plants
***R1**: RIZO-LIQ, **R2**: RIZOPLUS, **R1 + R2**: RIZOPLUS + RIZO-LIQ, **0**: Uninoculated.*

Also, strain competiveness might be considered. Competition between the rhizobia in the inoculant and the microbes already in the soil, the native microflora, affects the success of many inoculants; soil contains billions of different bacteria and other microorganisms, and the process of seed inoculation adds billions more; this causes immediate competition between the microbes, as they are all fighting for the same resources, if the rhizobia in the inoculant can out compete the native population, inoculation will be successful, nodules will form, and they will begin nitrogen fixation [26]. One factor in competitiveness is the bacterial numerical superiority, condition which can account in RIZO-LIQ + RIZOPLUS combination that contrast with the probably lower bacterial number in the inoculants (RIZO-LIQ and RIZOPLUS) when each one is used as a single inoculant. In this context, Hartmann *et al*. [1] and Bromfield *et al*. [8] found that poorly competitive strains may nodulate even if another highly competitive strain is present but in lesser numbers. It is estimated that the field response to rhizobial inoculants diminishes when the number of competing rhizobia in the soil exceed above 10 cells g^{-1} [12].

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