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Short Communication

Effect of *Bradyrhizobium* sp. strains in soybean mutant's nodulation

Efecto de cepas de *Bradyrhizobium* sp en la nodulación de mutantes de soya

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ABSTRACT: Soybean is a crop of great importance due to its nutritional value for both human and animals, in addition to its use in multiple economic lines. It is essential to expand its production in Cuba, for which work is being done on the selection of productive varieties adapted to the country's soil and climate conditions. As a legume, soybeans associate in a symbiotic interaction with bacteria of the rhizobia family, forming nodules on their roots that allow the nitrogen biological fixation and that way supply it to the plant. Among the most studied and validated inoculants in the international market are those based on *Bradyrhizobium*, and their starting point is the strains selection for their competitiveness and efficiency in the formation of active nodules. The objective of this work was to select *Bradyrhizobium* strains based on their ability to form nodules in Cuban soybean cultivars. Five strains of *Bradyrhizobium*, from the INCA bacteria collection, were used. They were inoculated on seeds of six soybean cultivars obtained by selection or mutations by gamma rays induction. A bifactorial analysis with the cultivar-strain factors was carried out. The results showed interaction between the factors, also that the resident strains nodulate all the cultivars studied, and that the interaction of *B. elkanii* ICA 8001 strain with the cultivars Cuvin 22 and Cuvi 99 was highlighted.

Key words: Symbiosis, *Glycine max*, rhizobia, selection.

RESUMEN: La soya es un cultivo de gran importancia por su valor nutritivo tanto para el hombre como para los animales, además de su uso en múltiples renglones económicos. Resulta imprescindible ampliar su producción en Cuba, para lo cual se trabaja en la selección de variedades productivas y adaptadas a las condiciones edafoclimáticas del país. Como leguminosa, la soya se asocia en una interacción simbiótica con bacterias de la familia de los rizobios, formando nódulos en sus raíces que permiten la fijación biológica del nitrógeno y que la planta pueda asimilarlo de esta manera. Entre los inoculantes más estudiados y validados en el mercado internacional se encuentran aquellos a base de *Bradyrhizobium*, y tienen como punto de partida la selección de cepas por su competitividad y eficiencia en la formación de nódulos activos. Este trabajo tuvo como objetivo: seleccionar cepas de *Bradyrhizobium* en función de su capacidad para formar nódulos en cultivares cubanos de soya. Se utilizaron cinco cepas de *Bradyrhizobium*, procedentes de la colección de bacterias del INCA, las que se inocularon sobre semillas de seis cultivares de soya obtenidos en el INCA mediante selección o inducción de mutaciones por rayos gamma. El análisis bifactorial de los factores cultivar y cepa permitió definir interacción entre ambos, destacándose la nodulación de los cultivares Cuvin 22 y Cuvi 99 cuando se inoculan con la cepa *B. elkanii* ICA 8001. Se comprobó, además, que las cepas residentes en el suelo nodulan todos los cultivares estudiados.

Palabras clave: simbiosis, *Glycine max*, bacteria, selección.

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INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is among the ten most important crops in the world (1), with a cultivable area of more than 53 million hectares (2), known for its wide range of agricultural, food and industrial applications, as well as for its health benefits (3). In Cuba, it has not yet been cultivated in large extensions. However, work continues to select and develop national and foreign genotypes that are adapted to the soil and climatic conditions of the country and that have good productive potential. One way to obtain new cultivars with agriculturally desirable properties, such as higher yield potential and greater adaptability to abiotic stress conditions, is the induction of mutations using gamma rays. The use of this method has made it possible to obtain a set of cultivars at the National Institute of Agricultural Sciences, which stand out for their resistance to viruses and good yields (4).

Soybean plants associate with nodulating bacteria, among which are those of the *Bradyrhizobium* genus, through an exchange of signals that results in a close relationship in which both symbionts benefit (5). Farmers inoculate soybean seeds with commercial products containing these nodulating strains to ensure high yields and replace the use of nitrogen fertilizer (6). For this purpose, strains related to the crop are selected, which stand out for their ability to induce nodule formation and have a high nitrofixing activity under the prevailing soil and climatic conditions (7).

The inoculation of rhizobial strains more efficient in nodulation and nitrogen fixation in more productive and adapted soybean cultivars constitutes an attractive biotechnological alternative for soybean production and for reducing crop fertilization and production costs. The present work had as objective to select *Bradyrhizobium* strains according to their capacity to form nodules in six Cuban soybean cultivars.

MATERIALS AND METHODS

The experiment was carried out under semi-controlled conditions, with pots containing 0.55 kg of red Ferrallitic soil. A completely randomized design was used, 10 plants were used per treatment and the experiment was repeated twice.

Six cultivars obtained at INCA were used, four of them: Cuvi-02, Cuvi-22, Cuvi-84, Cuvi-99; by individual selection, from mutants from the Institute of Agricultural Genetics of Vietnam. The other two: Cuvin-22 and Cincuentenario, were obtained by mutation induction with ^{60}Co gamma rays (200Gy).

The soybean cultivars were inoculated with five strains of the genus *Bradyrhizobium*, from different countries and conserved in the bacterial collection of the Department of Plant Physiology and Biochemistry of INCA: *Bradyrhizobium elkani* ICA 8001 and *Bradyrhizobium* sp. S1 (Cuba); *Bradyrhizobium japonicum* E-109 (Argentina), *Bradyrhizobium japonicum* USDA110 (United States) and *Bradyrhizobium elkanii* 6134 (Belgium). All with a concentration higher than 10^8 CFU mL.

A bifactorial arrangement was performed, where the factors were: cultivar with six levels (Cuvi-02, Cuvi-22, Cuvi-84, Cuvi-99, Cuvin-22 and Cincuentenario) and the strain factor with five levels (*B. elkani* ICA 8001, *Bradyrhizobium* sp. S1, *B. japonicum* E-109, *B. japonicum* USDA110 and *B. elkanii* 6134). At 35 days post inoculation (dpi) the following were determined: total nodule number (u), nodule effectiveness (%) and total nodule dry mass (mg) per plant. Data were tested for normality (Bartlett's test) and homogeneity of variance (Kormogorov-Smirnov test). Simple rank analysis of variance was applied. Tukey's mean comparison test ($p<0.05$) was used to discriminate differences between means. The SPSS 21 program was used for statistical processing of the data.

RESULTS AND DISCUSSION

The results showed that there was interaction between the factors *Bradyrhizobium* strain and soybean cultivar in the three variables analyzed ($p\leq0.05$). Although mostly no significant differences were found among the treatments in the variables analyzed, there were some that stood out over others. In the number of total nodules, the cultivar Cuvin 22 stood out when inoculated with strains ICA 8001, S1 and BJE-109; as well as the cultivar Cuvi 22 inoculated with strains S1 and USDA 6134. Something similar was found in the values of nodule effectiveness for biological nitrogen fixation, with the use of strain S1 in the cultivar Cuvi 22 and the non-inoculated treatment of the same cultivar. Only the inoculation of strain *B. elkanii* ICA 8001 on cultivars Cuvin 22 and Cuvi 99 produced a higher effect than the non-inoculated treatment on both cultivars in the dry mass of nodules, which shows a more efficient interaction of this strain with these cultivars. The dry mass of nodules is indicative of the bacteroid content within the nodule, so that a greater amount of these functional cells should result in a higher nitrogen supply to the plant (8). Strain *B. elkanii* ICA 8001 has been characterized for having positive attributes such as the capacity to fix nitrogen, to produce phytohormones such as indole acetic acid and to possess ACC deaminase enzyme activity (9), which confers advantages in the promotion of plant growth (Table 1).

There was evidence of an effect of the resident strains in the soil used (non-inoculated controls), which also showed the ability to associate with the cultivars under study and achieve similar nodulation results to those that were inoculated. The symbiotic efficiency of naturalized strains with a better adaptation to the environment constitutes a promising genetic material for the selection of new isolates (10), which could exert a superior effect in symbiosis with these cultivars. It is important to consider that there are no indigenous or native strains in Cuba because it is not the center of origin of soybean. However, successive sowings of this legume inoculated with introduced rhizobia have generated the appearance of naturalized strains that have managed to adapt to the new edaphoclimatic conditions and that constitute a source of biological diversity for processes of selection of more effective strains in N fixation.

Table 1. Combined effect of soybean cultivar and *Bradyrhizobium* strain factors on nodulation variables of soybean plants under semi-controlled conditions

Treatments	Total nodules (u)	Effectiveness (%)	Dry mass total nodules (mg)
Cuvi 99*ICA8001	7.0 bc	7.0 abcdef	28.93 abcd
Cuvi 99*S1	4.6 bc	4.6 bcdef	19.70 abcdefg
Cuvi 99*USDA110	11.3 abc	8.3 abcdef	22.23 abcdefg
Cuvi 99*BJE-109	11.3 abc	10.3 abcdef	25.86 abcdef
Cuvi 99*USDA6134	8.0 abc	7.3 abcdef	11.30 bcdefg
Cuvi 99* non-inoculated	0.6 c	0.3 f	2.86 fg
Cuvin 22*ICA 8001	12.6 ab	11.3 abcde	34.30 ab
Cuvin 22*S1	12.6 ab	12. 0 abcd	30.63 abc
Cuvin 22*USDA110	6.0 bc	5.3 bcdef	18.33 abcdefg
Cuvin 22*BJE-109	13.0 ab	12.3 abc	27.16 abcd
Cuvin 22*USDA6134	6.3 bc	5.6 abcdef	17.16 abcdefg
Cuvin 22* non-inoculated	5.3 bc	4.0 bcdef	6.02 cdefg
Cuvi 22*ICA 8001	11.0 abc	10.0 abcdef	37.900 a
Cuvi 22*S1	18.3 a	16.3 a	27.600 abcd
Cuvi 22*USDA110	6.0 bc	5.6 abcdef	26.967 abcd
Cuvi 22*BJE-109	5.0 bc	5.0 bcdef	14.367 bcdefg
Cuvi 22*USDA6134	2.3 ab	2.0 cdef	3.267 fg
Cuvi 22* non-inoculated	13.0 bc	13.0 ab	34.133 ab
Cincuentenario*ICA 8001	6.0 bc	2.6 bcdef	9.333 cdefg
Cincuentenario*S1	4.3 bc	3.3 bcdef	22.933 abcdefg
Cincuentenario*USDA110	8.6 abc	1.6 cdef	27.967 abcd
Cincuentenario*BJE-109	7.3 abc	7.3 abcdef	11.100 bcdefg
Cincuentenario*USDA6134	7.3 abc	4.6 bcdef	16.433 abcdefg
Cincuentenario* non-inoculated	2.0 bc	0.3 f	1.233 g
Cuvi 84*ICA 8001	6.6 bc	6.6 abcdef	23.833 abcdefg
Cuvi 84*S1	4.3 bc	2.6 bcdef	6.300 cdefg
Cuvi 84*USDA110	7.0 bc	6.0 abcdef	24.367 abcdefg
Cuvi 84*BJE-109	2.6 bc	1.3 def	7.200 cdefg
Cuvi 84*USDA6134	8.0 abc	6.6 abcdef	14.767 abcdefg
Cuvi 84* non-inoculated	6.0 bc	1.0 ef	4.067 efg
SE x ANOVA	**2.81**	**2.720**	**5.938**

Anova means and standard error (SE) are shown. Different letters in the same column show significant differences (Tukey HSD $p<0.05$, n=3)

CONCLUSIONS

Different strains showed similar behavior in the number of nodules and their effectiveness on the six soybean cultivars evaluated. Differences were only obtained with the inoculation of *B. elkanii* ICA 8001 on the cultivars Cuvin 22 and Cuvi 99, with respect to the non-inoculation of these cultivars. All cultivars showed a positive predisposition to associate with inoculated or resident strains, which should be evaluated in further studies under field conditions.

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