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Original article

Rhizobia evaluation and the use of AMF in soybeans (*Glycine max* (L.) Merrill)

Evaluación del efecto de rizobios y de un HMA en soya (*Glycine max* (L.) Merrill)

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ABSTRACT: The experiment was developed under field conditions on a Lixiviated Red Ferralitic soil in the central experimental area of the National Institute of Agricultural Sciences, in order to evaluate strains of rhizobia and a strain of arbuscular mycorrhizal fungi, on the growth and development of the soybean cultivar INCAsoy-27, sown in summer. For this, a randomized block design was used with four repetitions per treatment, which consisted of the inoculation of microorganisms, in their simple forms and the combination of each rhizobium strain with the arbuscular mycorrhiza used, as well as two control treatments, absolute and with mineral fertilization. Results showed a positive effect of the use of the different strains of rhizobia on the growth and yield of the soybean cultivar evaluated, with similar results between them and increases in yield in relation to the absolute control between 11.01 and 14.68 %, those that were made higher when both biofertilizers were co-inoculated (between 31.19 and 38.53 % in relation to the absolute control and between 13.49 and 19.84 % in relation to the fertilized control), with little significant differences between them, regardless of the rhizobium strain evaluated. These results demonstrate the synergistic and beneficial effects of rhizobia- arbuscular mycorrhizal co-inoculation in this culture.

Key words: inoculation, legume, yield.

RESUMEN: El experimento fue desarrollado en condiciones de campo, sobre un suelo Ferralítico Rojo Lixiviado, en el área experimental central del Instituto Nacional de Ciencias Agrícolas de Cuba, con la finalidad de evaluar cepas de rizobios y una de hongo micorrízico arbuscular, sobre el crecimiento y desarrollo del cultivar de soya INCAsoy-27, sembrada en época de verano. Para ello se empleó un diseño de bloques al azar con cuatro repeticiones por tratamiento, los cuales consistieron en la inoculación de los microorganismos, en sus formas simples y la combinación de cada cepa de rizobio con la micorriza arbuscular empleada, así como dos tratamientos controles, absoluto y con fertilización mineral. Los resultados mostraron un efecto positivo del empleo de las diferentes cepas de rizobios en el crecimiento y el rendimiento del cultivar de soya evaluado, con resultados similares entre ellas e incrementos del rendimiento con relación al control absoluto entre 11,01 y 14,68 %, los que se hicieron superiores cuando se coinocularon ambos biofertilizantes (entre 31,19 y 38,53 %, en relación con el control absoluto y entre 13,49 y 19,84 %, en relación con el control fertilizado), con diferencias poco significativas entre ellos, independientemente de la cepa de rizobio evaluada. Estos resultados demuestran los efectos sinérgicos y beneficiosos de la coinoculación rizobio-hongo micorrízico arbuscular en este cultivo.

Palabras clave: inoculación, leguminosa, rendimiento.

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INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is the most important oilseed worldwide because from its grains, which constitute the main source of vegetable protein, several essential products are obtained in human and animal food (1,2), it has a high nutritional value, with values of 38-42 % of proteins and 18-20 % of oil. Its consumption is increasing every day, due to the need to use the grain as raw material in the elaboration of concentrated food for animals and for human consumption (1). This crop is among the ten most important in the world; it is planted on more than 90.2 million hectares, whose world production exceeds 345.96 million tons, which represents an increase of 10.52 % in world production in recent years (3). The soybean crop ended with 348 million tons in the 2016-2017 season, constituting a historical record worldwide (4).

Among the agroecological alternatives proposed today in Cuba and the world, is the application of biostimulants, which include various formulations of compounds, substances, microorganisms and other products that when applied to plants or soils, regulate and improve the physiological processes of the crop (absorption and assimilation of nutrients, tolerance to biotic or abiotic stress), making them more efficient and improving some agronomic characteristics (5). Among them are the beneficial microorganisms that play a fundamental role in naturally sustainable agroecosystems. Some of them can be used as inoculants to benefit plants, since they develop activities that involve promoting their growth and protection. Rhizobium-arbuscular mycorrhizal (AM) associations act synergistically on infection levels, mineral nutrition and plant growth (6).

Several studies have demonstrated the positive influence of rhizobial bacteria and arbuscular mycorrhizal fungi on soybean yield. The combined inoculation of rhizobia and AMF can increase biological nitrogen fixation (BNF) and plant development to a greater extent than the inoculation of each of these microorganisms separately. Beneficial effects of their combination can supply the enormous phosphorus demand required by nitrogenase in the BNF process, alleviate deficiencies associated with certain environmental stress conditions and reduce the incidence of pathogens. It is known that the main effect of AMs to enhance Rhizobium activity is through a generalized stimulation of host nutrition, although some more localized effects may occur at the root or nodule level (6,7).

Both worldwide and in Cuba, research results with the use of combined inoculations of rhizobia and mycorrhizal fungi in the cultivation of soybean have provided increases in plant growth and yield, as well as the efficient use of soil nutrients and fertilizers, highlighting the usefulness of this joint practice in the production of such an important food item.

Taking into account this background, this study was carried out with the objective of evaluating different rhizobia strains and one AMF strain, as well as the combined use of both biofertilizers, on the growth and yield of the soybean cultivar INCAsoy 27, during the summer season.

MATERIALS AND METHODS

The study was developed in the summer of 2019, under field conditions, in the central area of the National Institute of Agricultural Sciences, located in San José de las Lajas municipality, Mayabeque province, Cuba, on a Ferrallitic Red Leached soil (8) (Table 1).

The soybean cultivar INCAsoy-27, planted in July 2019, was used in an experimental area of about 0.10 ha, with 52 plots of 12.6 m^2 (4 furrows x 4.5 m long) and 6.3 m^2 of computational area (two central furrows), using 20 plants per linear meter and a distance between furrows of 0.70 m.

The following bioproducts were evaluated:

- Rhizobia: Strain 1 (ICA 8001), Strain 2 (6134), Strain 3 (BJE-109), Strain 4 (S-5079) and Strain 5 (S-5080) (from different countries and characterized in the Microbiology Laboratory of the Department of Plant Physiology and Biochemistry of INCA, certified inocula in liquid support with a cell concentration of 5 x 10^8 CFU mL^{-1}).
- Arbuscular mycorrhiza (AM): *Glomus cubense* species, INCAM-4 strain (from the commercial mycorrhizal inoculum production plant, in solid support, of the Department of Biofertilizers and Plant Nutrition of INCA, with a guaranteed minimum composition of 20 spores per gram of inoculant and 50 % root colonization).

The biofertilizers were applied through Seed Coating Technology (9), at a rate of 4 mL kg^{-1} of seed (200 mL ha^{-1}) for the bacteria and 10 % of the seed weight for the mycorrhiza (5 kg ha^{-1}).

A Randomized Block design was used, with four replicates per treatment, evaluating the results through an analysis of variance (statistical package IBM-SPSS Statistics 19 for Windows), where Duncan's multiple range

Table 1. Some components of the initial chemical fertility of the soil (0-20 cm)

Soil type	pH (H_2O)	P_2O_5 (mg $100g^{-1}$)	OM (%)	Na	K	Ca	Mg (cmol$^{(+)}$ kg^{-1})	No. spores (50 g $soil^{-1}$)
F. R. Leached	7.45	123.03	3.42	0.16	0.66	14.75	2.38	90.5

pH (H2O): potentiometric method. Ratio soil - solution 1:2.5 OM (%): Walkley-Black. Assimilable P (mg 100 g^{-1}): Oniani (extraction with H_2SO_4, 0.1N). Assimilable K (cmol $^{(+)}$ kg^{-1}): Oniani (extraction with H_2SO_4, 0.1N). Exchangeable cations (cmol$^{(+)}$ kg^{-1}): Maslova (Ammonium acetate 1N, pH 7), determination by complexometry (Ca and Mg) and by flame photometry (Na and K).

test was used to discriminate the difference between means.

The following evaluations were performed:

- Flowering stage (at 37 das, taking 10 plants per treatment)

Height (cm), aerial and root dry mass (g), % of N, P and K in trifolioles, nodulation (number, dry mass (g) and effectiveness (%) of total nodules (according to the coloration inside the nodules through the cross section). Fungal variables were evaluated using the root staining technique (10): mycorrhizal frequency (%) and intensity of colonization (%), according to the methodology described (11,12).

- Harvest stage (at 100 days, taking 10 plants per treatment)

Plant height (cm), number of pods per plant, mass of 1000 grains (g) and grain yield (t ha^{-1}) based on the plot calculation area.

RESULTS AND DISCUSSION

Plant height at the flowering and harvest stages (Table 2), showed significant and not very marked responses among the biofertilized treatments, with differences between them in relation to the absolute control. All the rhizobial strains evaluated were effective, especially in the flowering stage, the treatments where co-inoculation was used. For the harvest stage, the treatments with inoculant application did not show significant differences with the NPK mineral fertilization control treatment. Similar results have been reported by other authors (13,14).

Table 3 shows the effects of treatments on the dry mass of the root and aerial part of plants, with results similar to plant height, where the inoculated treatments were superior to the absolute control, with a superior response in aerial dry mass when both biofertilizers were applied together, without significant differences with the NPK fertilized control.

Treatment effect on nodulation is reflected in Table 4, where, for the variables number of nodules, nodular dry mass and nodular effectiveness, and significant responses were observed for the treatments with application of rhizobia for these variables, superior to the absolute and fertilized controls, with similar results for the treatment with application of arbuscular mycorrhiza. The treatments where rhizobia-AMF co-inoculation was applied showed superior results. The inoculated treatments showed high values in their effectiveness for nitrogen fixation, except for the one in which only arbuscular mycorrhiza was applied. Similar results have been reported by other authors, regarding better responses with the joint application of both biofertilizers (14,15).

The controls (absolute and fertilized) and the treatment inoculated only with mycorrhizae showed the lowest values in the nodulation variables evaluated, which demonstrates a low presence of native rhizobia, which were also not very effective. The greatest effectiveness of nodules was related to treatments where the rhizobia-based inoculation was applied, either with the independent strains or combined with AMF.

Table 5 shows the results for the mycorrhizal variables, where the treatments with inoculation showed a significant effect on the different indicators of fungal performance evaluated, with higher rates than the absolute controls and those with mineral fertilization. The greatest effect of the treatments was observed when joint inoculation of the bioproducts was used, independently of the rhizobia strain evaluated.

Lower values were observed in the variables evaluated for the treatments in which the AMF-based product was not applied, fundamentally in the control treatments, which presupposes low or little effectiveness of the native mycorrhiza in the conditions where the study was carried out. The values found coincide with those reported by other authors in the combined use of biofertilizers (15-17).

Table 2. Effect of treatments on plant height (cm)

No.	Treatments	Flowering	Harvest
1	Absolute control	36.78 c	93.45 b
2	Fertilized control	52.90 a	108.50 a
3	Strain 1	47.23 b	101.03 ab
4	Strain 2	47.10 b	101.73 ab
5	Strain 3	47.30 b	101.05 ab
6	Strain 4	48.68 b	102.58 ab
7	Strain 5	48.65 b	102.33 ab
8	AMF	47.73 b	101.98 ab
9	Strain 1 + AMF	53.18 a	107.43 ab
10	Strain 2 + AMF	54.43 a	106.05 ab
11	Strain 3 + AMF	52.23 a	107.58 a
12	Strain 4 + AMF	52.30 a	112.28 a
13	Strain 5 + AMF	52.00 a	107.03 ab
	X	49.27	104.08
	SE x	1.25 *	5.92 *

Means with common letters in the same column do not differ significantly at $p<0.05$

Table 3. Effect of treatments on dry mass (g plant^{-1})

No.	Treatments	Root dry mass	Aerial dry mass
1	Absolute control	0.34 b	2.12 d
2	Fertilized control	0.44 a	3.16 a
3	Strain 1	0.41 a	2.42 cd
4	Strain 2	0.41 a	2.47 c
5	Strain 3	0.42 a	2.57 bc
6	Strain 4	0.40 a	2.58 bc
7	Strain 5	0.41 a	2.48 c
8	AMF	0.41 a	2.45 c
9	Strain 1 + AMF	0.43 a	2.88 ab
10	Strain 2 + AMF	0.45 a	2.87 ab
11	Strain 3 + AMF	0.45 a	2.89 ab
12	Strain 4 + AMF	0.45 a	2.94 a
13	Strain 5 + AMF	0.45 a	2.91 ab
	X	0.42	2.67
	SE x	0.02 *	0.15 *

Means with common letters in the same column do not differ significantly at p<0.05

Table 4. Effect of treatments on nodulation

No.	Treatments	No. Nodules per plant	Nodular dry mass (g)	Nodular effectiveness (%)
1	Absolute control	2.00 d	0.023 f	60.00 e
2	Fertilized control	1.25 e	0.010 g	50.00 f
3	Strain 1	13.55 b	0.103 d	93.33 c
4	Strain 2	13.70 b	0.103 d	94.17 bc
5	Strain 3	13.90 b	0.108 cd	96.22 ab
6	Strain 4	13.98 b	0.110 bcd	96.04 ab
7	Strain 5	13.53 b	0.103 d	95.48 abc
8	AMF	3.78 c	0.040 e	62.36 d
9	Strain 1 + AMF	15.15 ab	0.118 abc	96.97 a
10	Strain 2 + AMF	15.25 ab	0.120 ab	96.67 a
11	Strain 3 + AMF	15.48 ab	0.125 a	97.22 a
12	Strain 4 + AMF	15.55 a	0.128 a	97.22 a
13	Strain 5 + AMF	15.50 ab	0.123 a	97.18 a
	X	11.74	0.093	87.14
	SE x	0.20 *	0.005 *	1.10 *

Means with common letters in the same column do not differ significantly at p<0.05

Table 6 shows the results of grain yield and its components, where for the number of pods per plant, no significant differences were observed between the treatments with rhizobia application, superior to the evaluated controls. Treatments where seeds were co-inoculated with both biofertilizers showed the highest values, higher than the absolute and fertilized controls, with no differences between them. The treatment with simple application of mycorrhiza only showed higher values than the absolute control.

For 1000 grains mass, significant differences were observed between the inoculated treatments in their simple forms and the absolute control. Treatments with joint application of the bioproducts did not differ from each other, being superior to the simple applications and the absolute control, only rhizobia strains 3 and 4 combined with AMF showed differences with the mineral fertilization control.

Grain yield as a result of its components and in general of crop growth and development, also showed significant differences between treatments, highlighting those where rhizobia strains and arbuscular mycorrhiza were inoculated together, with increases in yields for the cultivar studied, between 31.19 and 38.53 % in relation to the absolute control and between 13.49 and 19.84 % in relation to the control with mineral fertilization.

The rhizobia strains studied, in their simple forms, showed positive results, superior to the absolute control and yield increases between 11.01 and 14.68 % according to the strain used, which shows their effectiveness. Positive results with the use of rhizobia strains in the growth and yield of legumes have been reported by several authors (18-20). The treatment with only the application of the AMF strain, although in smaller quantities, was also superior to the absolute control, with 6.42 % yield increase.

Table 5. Effect of treatments on fungal variables

No.	Treatments	Mycorrhizal colonization (%)	Visual density (%)
1	Absolute control	9.00 d	0.09 d
2	Fertilized control	8.75 d	0.09 d
3	Strain 1	13.25 c	0.13 c
4	Strain 2	14.50 c	0.15 c
5	Strain 3	12.25 c	0.12 c
6	Strain 4	13.25 c	0.13 c
7	Strain 5	13.25 c	0.13 c
8	AMF	20.50 b	0.21 b
9	Strain 1 + AMF	33.75 a	0.34 a
10	Strain 2 + AMF	31.00 a	0.31 a
11	Strain 3 + AMF	30.50 a	0.31 a
12	Strain 4 + AMF	32.50 a	0.33 a
13	Strain 5 + AMF	31.00 a	0.31 a
	X	20.27	0.20
	SE x	1.06 **	0.011 *

Means with common letters in the same column do not differ significantly at p<0.05

Table 6. Effect of treatments on grain yield and its components

No.	Treatments	No. pods per plant	Mass 1000 grains (g)	Yield (t ha^{-1})	% increase compared to controls:	
					Absolute	Fertilized
1	Absolute control	43.03 d	101.57 d	1.09 e	-	-
2	Fertilized control	53.78 b	111.49 bc	1.26 c	15.60	-
3	Strain 1	53.58 b	108.99 c	1.21 c	11.01	-
4	Strain 2	53.88 b	108.07 c	1.22 c	11.93	-
5	Strain 3	53.90 b	109.75 c	1.25 c	14.68	-
6	Strain 4	54.35 b	110.17 c	1.25 c	14.68	-
7	Strain 5	53.33 b	109.47 c	1.25 c	14.68	-
8	AMF	49.80 c	109.08 c	1.16 d	6.42	-
9	Strain 1 + AMF	61.10 a	115.34 ab	1.45 ab	33.03	15.08
10	Strain 2 + AMF	61.03 a	115.04 ab	1.43 b	31.19	13.49
11	Strain 3 + AMF	62.00 a	115.22 ab	1.47 ab	34.86	16.67
12	Strain 4 + AMF	62.55 a	117.37 a	1.51 a	38.53	19.84
13	Strain 5 + AMF	62.10 a	116.15 a	1.49 a	36.70	18.25
	X	55.72	111.36	1.31	-	-
	SE x	1.45 *	2.00 *	0.03 *	-	-

Results, for the study conditions and the cultivar evaluated, make possible a biotechnological management of these products for the development of the soybean crop, taking into account the positive responses of the biofertilizers evaluated, expressed by increases in grain yield as a fundamental variable. Similar results in soybean cultivation have been reported by several authors (6,13-16,21), as well as in other crops such as alfalfa (17), which corroborate the benefits of the tripartite symbiosis rhizobia-arbuscular mycorrhizal fungi-legumes.

CONCLUSIONS

- The growth and yield of the soybean crop, cultivar INCAsoy-27, was influenced by the application of biofertilizer products, both in their simple forms and in combination.
- Different bioproducts used in their simple forms showed results that differed from the absolute control, with yield increases between 11.01 and 14.68 % for rhizobia and 6.42 % for arbuscular mycorrhiza.
- The joint application of the different rhizobia strains and the AMF strain *Glomus cubense* produced superior results, achieving yield increases of 31.19 and 38.53 % and 13.49 and 19.84 %, in relation to the absolute and fertilized controls, respectively.

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