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Original article

Study of *Bradyrhizobium* strains and mycorrhizal application on soybean (*Glycine max* (L.) Merrill)

Estudio de cepas de *Bradyrhizobium* y la aplicación de micorriza en soya (*Glycine max* (L.) Merrill)

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ABSTRACT: On a Leached Red Ferrallitic soil, located in the National Institute of Agricultural Sciences of Cuba areas, an experiment was carried out under field conditions, with the objective of evaluating the effect of different *Bradyrhizobium* strains and a strain of arbuscular mycorrhiza, on the growth and yield of the soybean cultivar INCAsoy-27, sown in summer season. A randomized block design with four replicates per treatment was used, which consisted of the inoculation of microorganisms in their simple forms and the combination of each *Bradyrhizobium* strain with the mycorrhiza used. In addition, control treatments, absolute and with mineral fertilization, were evaluated. The results showed a positive effect of the different use of *Bradyrhizobium* strains on the growth and development of the cultivar evaluated, with very similar results among them and yield increases in relation to the absolute control between 25.14 and 26.26 %. Increases that were higher when the joint inoculation of both biofertilizers was used, without significant differences between treatments, regardless of the *Bradyrhizobium* strain evaluated (between 40.22 - 44.13 % against the absolute control and between 11.06 - 14.16 % against the fertilized control). It demonstrated the synergistic and beneficial effects of *Bradyrhizobium*-arbuscular mycorrhizae co-inoculation on this crop.

Key words: biostimulants, inoculation, legume, yield, legumes.

RESUMEN: Sobre un suelo Ferralítico Rojo Lixiviado, ubicado en las áreas del Instituto Nacional de Ciencias Agrícolas de Cuba, se realizó un experimento en condiciones de campo, con el objetivo de evaluar el efecto de diferentes cepas de *Bradyrhizobium* y una cepa de micorriza arbuscular, en el crecimiento y rendimiento del cultivar de soya INCAsoy-27, sembrado 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 *Bradyrhizobium* con la micorriza empleada. Además, se evaluaron tratamientos controles, absoluto y con fertilización mineral. Los resultados mostraron un efecto positivo del empleo de las diferentes cepas de *Bradyrhizobium* en el crecimiento y el desarrollo del cultivar evaluado, con resultados muy similares entre ellas e incrementos del rendimiento con relación al control absoluto entre 25,14 y 26,26 %, incrementos que fueron superiores cuando se utilizó la inoculación conjunta de ambos biofertilizantes, sin diferencias significativas entre los tratamientos, independientemente de la cepa de *Bradyrhizobium* evaluada (entre 40,22 y 44,13 % contra el control absoluto y entre 11,06 % y 14,16 % contra el control fertilizado), lo que demostró los efectos sinérgicos y beneficiosos de la coinoculación *Bradyrhizobium*-micorrizas arbusculares en este cultivo.

Palabras clave: bioestimulantes, inoculación, leguminosa, rendimiento.

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INTRODUCTION

The Food and Agriculture Organization of the United Nations (FAO) estimates a world population growth of 13 % in 2030 and 30 % in 2050, which will require a 70 % increase in agricultural production to solve problems of malnutrition and ensure food security (1).

Soybean (*Glycine max* (L.) Merrill) is among the ten most important crops 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 (2). It is one of the strategic crops in food security worldwide, occupying a privileged position as the main oilseed for animal and human food, due to the high nutritional value of its seeds. It contains 20 % oil and 40 % protein (3). 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, grains that are considered very versatile, since they can be consumed as seeds, sprouts, and can be processed to obtain derivatives such as soy milk, tofu, soy sauce and flour (4,5).

In processes of sustainable agricultural production, special interest has been given to the use of beneficial soil microorganisms, which through symbiotic activity induce to improve plant nutrition, help to tolerate adverse production conditions and consequently promote the growth and production of crops. For these reasons, beneficial microorganisms have been considered as biological fertilization agents or biofertilizers. Among these microorganisms are bacteria of the rhizobia group and arbuscular mycorrhizal fungi, plant growth promoters, which exert beneficial effects on soil and plants and have a positive influence on soybean yield (6-8).

Numerous authors have shown that the combined inoculation of rhizobia-arbuscular mycorrhizal fungi (AMF) increases biological nitrogen fixation (BNF) and plant development largely than the inoculation of each of these microorganisms separately. The 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 pathogen incidence (9,10).

The results of these investigations in Cuba and worldwide, with the use of combined inoculations of rhizobia and mycorrhizal fungi in soybean cultivation have provided increases in the growth and yield of plants, highlighting the importance of this joint practice in the production of this important food item.

Taking into account this background, this study was conducted to evaluate the effect of different strains of *Bradyrhizobium* and a strain of AMF on the growth and yield of the soybean cultivar INCAsoy 27, planted in summer.

MATERIALS AND METHODS

The study was developed in 2017 summer, 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 and on a Leached Red Ferrallitic soil (11) (Table 1).

The soybean cultivar INCAsoy-27, planted in October 2017, was used in an experimental area of about 0.11 ha, with 52 plots of 14 m^2 (four furrows x 5 m long) and 7 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:

1. *Bradyrhizobium*: Strain 1 (ICA 8001), Strain 2 (6134), Strain 3 (BJE-109), Strain 4 (S-5079) and Strain 5 (S-5080). (Coming from different countries and characterized in the Microbiology Laboratory of the Plant Physiology and Biochemistry Department from INCA, certified inocula in liquid support with a cell concentration of 5 x10^8 CFU mL^{-1}.
2. 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 from INCA, with a guaranteed minimum composition of 20 spores per gram of inoculant and 50 % of root colonization.

The biofertilizers were applied through Seed Coating Technology (12), 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 test was used for the comparison of means.

The following evaluations were carried out:

- Flowering stage (at 48 days after sowing (das), taking 10 plants per treatment):

Height (cm), aerial and root dry mass (g), % N, P and K in trifolioles, nodulation (number, dry mass (g) and effectiveness (%) of total nodules, according to the

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

Type of soil	pH	P_2O_5	OM	$cmol^{(+)}$ Kg^{-1}			
	(H_2O)	(mg $100g^{-1}$)	(%)	Na	K	Ca	Mg
Leached Red Ferrallitic	7.80	42.65	2.96	0.03	0.65	12.88	2.50

pH (H_2O): potentiometric method. Soil-solution ratio 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)

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

No.	Treatments	Flowering	Harvest
1	Absolute control	33.35 c	44.45 c
2	Fertilized control	36.02 bc	49.03 d
3	Strain 1	36.81 abc	50.95 bcd
4	Strain 2	37.88 abc	52.28 abcd
5	Strain 3	38.71 ab	53.30 abc
6	Strain 4	38.76 ab	51.13 abcd
7	Strain 5	38.76 ab	50.73 cd
8	AM	37.62 abc	50.58 cd
9	Strain 1 + AM	39.16 ab	54.73 ab
10	Strain 2 + AM	38.50 ab	54.48 ab
11	Strain 3 + AM	40.44 ab	54.67 ab
12	Strain 4 + AM	41.07 a	54.73 ab
13	Strain 5 + AM	40.64 a	54.83 a
	Total mean	38.28*	52.06*
	Standard error	1.90	1.64

Means with common letters in oneself column doesn't differ significantly p <0,05

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

No.	Treatments	Root dry mass	Aerial dry mass
1	Absolute control	0.41 f	5.28 e
2	Fertilized control	0.53 abc	7.71 abc
3	Strain 1	0.45 ef	6.78 d
4	Strain 2	0.46 ef	6.99 cd
5	Strain 3	0.47 de	7.18 bcd
6	Strain 4	0.47 de	7.72 abc
7	Strain 5	0.47 de	7.87 abc
8	AM	0.49 bcde	7.66 abc
9	Strain 1 + AM	0.52 abcd	7.95 ab
10	Strain 2 + AM	0.53 ab	8.31 a
11	Strain 3 + AM	0.54 a	8.49 a
12	Strain 4 + AM	0.55 a	8.21 a
13	Strain 5 + AM	0.55 a	8.23 a
	Total mean	0.49*	7.57*
	Standard error	0.02	0.40

Means with common letters in oneself column doesn't differ significantly p <0,05

coloration inside the nodules through the cross section). Fungal variables were evaluated using the root staining technique (13): mycorrhizal frequency (%) and colonization intensity (%), according to the described methodologies (14,15).

- Harvest stage (at 97 days after sowing (das), 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

Table 2 shows the behavior of plant height in the flowering and harvest stages, where significant responses were observed among treatments, with positive responses for the biofertilized treatments and differences in relation to the absolute control. All *Bradyrhizobium* strains evaluated were effective. Those treatments where the biofertilizers were inoculated together are highlighted.

For the harvest stage, treatments with co-inoculation application showed significant differences, even with the NPK mineral fertilization treatment, where all Bradyrhizobium strains in combination with AM were effective. Similar results, in relation to the joint application of both biofertilizers, have been reported by other authors (8,16).

For the case of the root and aerial dry mass of plants (Table 3), similar results were observed as for the previously mentioned indicator; although the response of the joint application of both biofertilizers was much higher; but without significant differences with the NPK-fertilized treatment. The *Bradyrhizobium* strains used were superior to the absolute control, mainly in aerial dry mass.

On the other hand, taking into consideration the effect of treatments on nodulation, evaluated from the variables

Table 4. Effect of the treatments on nodulation

No.	Treatments	No. Nodules for plant	Nodular dry mass (g)	Nodular effectiveness (%)
1	Absolute control	5.15 bc	0.033 ef	31,42 c
2	Fertilized control	2.73 c	0.015 f	26,22 d
3	Strain 1	14.55 a	0.070 cd	93,17 ab
4	Strain 2	15.63 a	0.070 cd	93,71 ab
5	Strain 3	16.12 a	0.073 cd	97,56 a
6	Strain 4	15.29 a	0.060 de	94,11 ab
7	Strain 5	15.84 a	0.078 bcd	97,35 a
8	AMF	7.03 b	0.038 ef	91,21 b
9	AM	17.22 a	0.090 abc	94,68 ab
10	Strain 1 + AM	17.56 a	0.095 abc	94,33 ab
11	Strain 2 + AM	18.09 a	0.110 a	97,75 a
12	Strain 3 + AM	17.70 a	0.103 bc	94,32 ab
13	Strain 4 + AM	17.70 a	0.098 abc	97,29 a
	Total mean	13,89	0.070	84.85
	Standard Error	1,50 *	013 *	2.16 *

Means with different letters do not differ significantly (Duncan's Multiple Range Test, p≤0.05)

Table 5. Effect of treatments in the fungical variables

No.	Treatments	Mycorrhizal colonization (%)	Visual density (%)
1	Absolute control	12.00 f	0.12 f
2	Fertilized control	15.00 ef	0.15 f
3	Strain 1	23.00 cd	0.25 de
4	Strain 2	21.00 de	0.22 e
5	Strain 3	28.00 c	0.30 c
6	Strain 4	26.00 cd	0.26 de
7	Strain 5	22.00 cd	0.24 de
8	AM	36.00 b	0.36 bc
9	Strain 1 + AM	39. 97 ab	0.41 ab
10	Strain 2 + AM	40.03 ab	0.42 ab
11	Strain 3 + AM	42.33 ab	0.42 ab
12	Strain 4 + AM	45.13 a	0.45 a
13	Strain 5 + AM	42.00 ab	0.42 ab
	Total mean	30.19	0.31
	Standard error	3.01*	0.03*

Means with equal letters do not differ from each other (Duncan's Multiple Range Test, p≤0.05)

nodule number, nodule dry mass and nodule effectiveness (Table 4), significant responses were observed especially with *Bradyrhizobium* application for the mentioned variables, highlighting those where *Bradyrhizobium*-AM co-inoculation was applied, fundamentally in its effects on nodule mass and its effectiveness to fix nitrogen.

The lowest values were found for the controls (absolute and fertilized) and the treatment where only AM was inoculated, which shows the low presence of native *Bradyrhizobium* and also that they were not very effective. The higher effectiveness of the nodules was either related to the treatments where *Bradyrhizobium* was applied, the independent strains or combined with AM. Similar results have been reported by other authors, regarding better responses with the joint application of both biofertilizers (16,17).

Table 5 shows the results for the mycorrhizal variables where a significant effect of inoculation was found in the different indicators of fungal performance evaluated, with a greater effect of the treatments where joint inoculation of the bioproducts was used, independently of the *Bradyrhizobium* strain evaluated.

All inoculated treatments, either in their simple or combined form, showed higher indexes than the absolute and mineral fertilization controls. A low activity of the native mycorrhiza was observed under the conditions in which the study was carried out, given by the values found in the variables evaluated for the treatments in which the AM-based product was not applied, mainly in control treatments.

The foliar contents of nitrogen, phosphorus and potassium (N, P and K), as a reflection of the nutritional status of the plants (Table 6), generally showed significant differences only with the absolute control.

The biofertilized treatments, in none of their evaluation forms, showed differences between them or with the

Table 6. Treatment effect in percentages of N, P and K foliar

No.	Treatments	(%)		
		N	P	K
1	Absolute control	2.44 b	0.37 b	1.37 e
2	Fertilized control	3.34 a	0.52 a	1.42 de
3	Strain 1	2.97 a	0.50 a	1.44 de
4	Strain 2	3.00 a	0.52 a	1.46 cde
5	Strain 3	3.26 a	0.53 a	1.49 bcd
6	Strain 4	3.13 a	0.52 a	1.46 cde
7	Strain 5	3.14 a	0.52 a	1.46 cde
8	AM	3.01 a	0.52 a	1.52 abcd
9	Strain 1 + AM	3.13 a	0.53 a	1.49 bcd
10	Strain 2 + AM	3.35 a	0.53 a	1.60 a
11	Strain 3 + AM	3.40 a	0.53 a	1.63 a
12	Strain 4 + AM	3.19 a	0.53 a	1.58 ab
13	Strain 5 + AM	3.20 a	0.53 a	1.56 abc
	Total mean	3.12	0.51	1.50
	Standard error	0.19 *	0.03 *	0.05 *

Means with equal letters do not differ from each other (Duncan's Multiple Range Test, p≤0.05)

Table 7. Treatment effect in grain yield and their components

No.	Treatments	No. Pods per plant	Mass of 1000 grains (g)	Yield (t ha^{-1})	% % increase in relation to controls:	
					Absolute	Fertilized
1	Absolute control	43.90 d	146.00 b	1.79 c	-	-
2	Fertilized control	52.00 abc	148.00 ab	2.26 b	26.26	-
3	Strain 1	49.93 bc	150.00 ab	2.25 b	25.70	-
4	Strain 2	49.63 bc	154.25 ab	2.26 b	26.26	-
5	Strain 3	49.93 bc	149.25 ab	2.25 b	25.70	-
6	Strain 4	49.73 bc	152.00 ab	2.24 b	25.14	-
7	Strain 5	49.75 bc	150.50 ab	2.25 b	25.70	-
8	AM	48.70 c	149.75 ab	2.18 b	21.79	-
9	Strain 1 + AM	54.48 ab	156.00 a	2.57 a	43.57	13.72
10	Strain 2 + AM	54.88 a	155.50 a	2.55 a	42.46	12.83
11	Strain 3 + AM	55.57 a	156.33 a	2.58 a	44.13	14.16
12	Strain 4 + AM	55.45 a	155.50 a	2.53 a	41.34	11.95
13	Strain 5 + AM	54.07 ab	156.00 a	2.51 a	40.22	11.06
	Total mean	51,38	152.38	2.33	-	-
	Standard error	2,16 *	3.70 *	0.05 *	-	-

Means with equal letters do not differ from each other (Duncan's Multiple Range Test, p≤0.05).

fertilized control for N and P. However, for foliar K, those treatments where the combination of biofertilizers was applied resulted with the highest values and significant differences.

Values for the elements were found to correspond to those reported in the international literature as sufficient for the crop, except for nitrogen where the percentages shown were below the established sufficiency range. Nevertheless, the values for all elements correspond to those obtained under similar conditions in Cuba for this crop (16,17).

Table 7 shows the results of grain yield and its components, where the number of pods per plant showed significant differences among treatments, with values higher than the absolute control of the biofertilized treatments. The treatments where the seeds were co-inoculated with both biofertilizers showed the highest values, similar to the fertilized control.

For the mass of 1000 grains, significant differences were only observed with the absolute control of the co-inoculated treatments. Treatments with application of bioproducts, in their simple forms, did not differ among themselves, nor with the controls used, which could indicate that this variable in the crop is little influenced by the application of these products.

Grain yield as a result of crop growth and development also showed significant differences between treatments, highlighting those where *Bradyrhizobium* strains and *Glomus cubense* arbuscular mycorrhiza were inoculated together, which showed increases in yield for the cultivar studied, between 40.22 - 44.13 % in relation to the absolute control and between 11.06 - 14.16 % in relation to the control with mineral fertilization.

Different *Bradyrhizobium* strains studied, in their simple forms, showed positive results, similar to the fertilized control and superior to the absolute control, with yield increases between 25.14 - 26.26 %, according to the strain used, which shows the effectiveness of each of strains. Several authors have reported positive results with the use of rhizobia strains on legume growth and yield (18-21). The treatment with only AMF strain application was also superior to the absolute control, with a yield increase of 21.79 %.

The application of biofertilizers showed positive responses for the study conditions and the cultivar evaluated, expressed in grain yield increases as a fundamental variable, highlighting the joint management of these products for the development of soybean cultivation in soil and climatic conditions similar to those of the study. Similar results in soybean cultivation have been reported by several authors (9,16,17,22-25), as well as in other crops such as alfalfa (26), which corroborate the benefits of the tripartite rhizobia-mycorrhizae-legumes symbiosis.

CONCLUSIONS

- The application of the biofertilizer products positively influenced the growth and yield of the soybean crop, cultivar INCAsoy-27, both in their simple and combined forms.
- The combined application of different *Bradyrhizobium* strains and the AM *Glomus cubense* strain produced the best results, achieving yield increases of 40.22 - 44.13% and 11.06 - 14.16%, in relation to the absolute and fertilized controls, respectively.
- The use, in their simple forms, of the different bioproducts showed superior results to the absolute control, with yield increases between 25.14 - 26.26 % for *Bradyrhizobium* and 21.79 % for mycorrhiza.

BIBLIOGRAPHY

1. FAO. El estado de la seguridad alimentaria y la nutrición en el mundo - 2019 | FAO | Organización de las Naciones Unidas para la Alimentación y la Agricultura [Internet]. 2019 [cited 23/01/2022]. Available from: https://www.fao.org/publications/sofi/2019/es/
2. FAO. Estadísticas mundiales de soya [Internet]. Blog Agricultura. [cited 23/01/2022]. Available from: https://blogagricultura.com/estadisticas-soya-produccion/
3. Díaz Franco A, Magallanes Estala A, Aguado Santacruz A, Hernández Mendoza JL. Respuesta de la soya a inoculantes microbianos en el norte de Tamaulipas, México. Revista mexicana de ciencias agrícolas [Internet]. 2015;6(2):227-38. Available from: http://www.scielo.org.mx/scielo.php?pid=S2007-09342015000200001&script=sci_abstract&tlng=pt
4. FAOSTAT. No Title [Internet]. 2018 [cited 23/01/2022]. Available from: https://www.fao.org/faostat/es/#data/QC/visualize
5. Moreano ICF, Falquez NPT, Gutiérrez MCM, Bermúdez FLL. Evaluación agronómica de materiales de soya (*Glycine max* (L) Merril) de hilium claro. RECIAMUC [Internet]. 2017;1(4):850-60. Available from: https://www.reciamuc.com/index.php/RECIAMUC/article/view/210
6. Barroso Frómeta L, Abad Michel M, Rodríguez Hernández P, Jerez Mompié E. Aplicación de FitoMas-E y EcoMic® para la reducción del consumo de fertilizante mineral en la producción de posturas de cafeto. Cultivos Tropicales [Internet]. 2015;36(4):158-67. Available from: http://scielo.sld.cu/scielo.php?pid=S0258-59362015000400021&script=sci_arttext&tlng=pt
7. Juge C, Prévost D, Bertrand A, Bipfubusa M, Chalifour F-P. Growth and biochemical responses of soybean to double and triple microbial associations with Bradyrhizobium, Azospirillum and arbuscular mycorrhizae. Applied Soil Ecology [Internet]. 2012;61:147-57. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0929139312001345
8. Hernández AF. La coinoculación *Glomus hoi like-Bradyrhizobium japonicum* en la producción de soya (*Glycine max*) variedad Verónica para semilla. Cultivos tropicales [Internet]. 2008;29(4):41-5. Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0258-59362008000400006
9. Abd-Alla MH, El-Enany A-WE, Nafady NA, Khalaf DM, Morsy FM. Synergistic interaction of *Rhizobium leguminosarum* bv. viciae and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. Microbiological research [Internet]. 2014;169(1):49-58. Available from: https://www.sciencedirect.com/science/article/pii/S0944501313001171
10. Ibiang YB, Mitsumoto H, Sakamoto K. Bradyrhizobia and arbuscular mycorrhizal fungi modulate manganese, iron, phosphorus, and polyphenols in soybean (*Glycine max* (L.) Merr.) under excess zinc. Environmental and Experimental Botany [Internet]. 2017;137:1-13. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0098847217300242
11. Hernández Jiménez A, Bosch Infante D, Pérez Jiménez JM, Castro Speck N. Clasificación de los suelos de Cuba 2015. Libro [Internet]. 1st ed. Instituto Nacional de Ciencias Agrícolas, Cuba: Ediciones INCA; 2015 [cited 23/01/2022]. Available from: https://isbn.cloud/9789597023777/clasificacion-de-los-suelos-de-cuba-2015/
12. Fernández F, Gómez R, Vanegas LF, Noval BM de la, Martínez MA. CU22641 Producto inoculante micorrizógeno. Patente No. 22641 [Internet]. 2000 [cited 23/01/2022]. Available from: https://patentscope.wipo.int/search/en/detail.jsf;jsessionid=ED1D72BCCE74B2DBD801D4D94327D0B1.wapp2nA?docId=CU4401498&recNum=98&office=&queryString=&prevFilter=%26fq%3DOF%3ACU%26fq%3DICF_M%3A%22A01N%22&sortOption=Pub+Date+Desc&maxRec=135

13. Rodríguez Yon Jy, Arias Pérez L, Medina Carmona A, Mujica Pérez Y, Medina García LR, Fernández Suárez K, et al. Alternativa de la técnica de tinción para determinar la colonización micorrízica. Cultivos Tropicales [Internet]. 2015;36(2):18-21. Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0258-59362015000200003
14. Trouvelot A, Kough JL, Gianinazzi-Pearson V. Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthode d'estimation ayant une signification fonctionnelle. In: Physiological and genetical aspects of mycorrhizae: proceedings of the 1st european symposium on mycorrhizae, Dijon, 1-5 July 1985 [Internet]. 1986. p. 217-21. Available from: https://www.semanticscholar.org/paper/Mesure-du-taux-de-mycorhization-VA-d%27un-systeme-de-Trouvelot/5482eab5f682dd814ac986ccce94b30e7e7ffb0e
15. Herrera-Peraza RA, Furrazola E, Ferrer RL, Valle RF, Arias YT. Functional strategies of root hairs and arbuscular mycorrhizae in an evergreen tropical forest, Sierra del Rosario, Cuba. Revista CENIC. Ciencias Biológicas [Internet]. 2004;35(2):113-23. Available from: https://www.redalyc.org/pdf/1812/181226079010.pdf
16. Corbera Gorotiza J, Nápoles García MC. Efecto de la inoculación conjunta *Bradyrhizobium elkanii*-hongos MA y la aplicación de un bioestimulador del crecimiento vegetal en soya (*Glycine max* (L.) Merrill), cultivar INCASOY-27. Cultivos Tropicales [Internet]. 2013;34(2):05-11. Available from: http://scielo.sld.cu/scielo.php?pid=S0258-59362013000200001&script=sci_arttext&tlng=en
17. Corbera J, Nápoles MC. Evaluación de la inoculación conjunta *Bradyrhizobium japonicum*-hongos MA y la aplicación de un bioestimulador del crecimiento vegetal en soya, cultivada en época de primavera. Cultivos Tropicales [Internet]. 2011;32(4):13-9. Available from: http://scielo.sld.cu/scielo.php?pid=S0258-59362011000400002&script=sci_arttext&tlng=pt
18. Granda-Mora KI, Alvarado-Capó Y, Torres-Gutiérrez R. Efecto en campo de la cepa nativa COL6 de *Rhizobium leguminosarum* bv. viciae sobre frijol común cv. Percal en Ecuador. Centro Agrícola [Internet]. 2017;44(2):5-13. Available from: http://scielo.sld.cu/scielo.php?pid=S0253-57852017000200001&script=sci_arttext&tlng=en
19. Díaz Pérez B. Influencia de diferentes variantes de biofertilización sobre variables morfofisiológicas y el rendimiento del frijol común (*Rhizobium leguminosarum* L.) [Internet]. Universidad Central" Marta Abreu" de Las Villas. Facultad de Ciencias …; 2017. 38 p. Available from: https://dspace.uclv.edu.cu/handle/123456789/7933
20. Chipana V, Clavijo C, Medina P, Castillo D. Inoculación de vainita (*Phaseolus vulgaris* L.) con diferentes concentraciones de *Rhizobium etli* y su influencia sobre el rendimiento del cultivo. Ecología aplicada [Internet]. 2017;16(2):91-8. Available from: http://www.scielo.org.pe/scielo.php?script=sci_arttext&pid=S1726-22162017000200003
21. Segura HC, Joaquín AH, Davila DEZ. Efectividad simbiótica de dos cepas de Rhizobium sp. en cuatro variedades de frijol (*Phaseolus vulgaris* L.) en Perú. Idesia [Internet]. 2019;37(4):73-81. Available from: https://dialnet.unirioja.es/servlet/articulo?codigo=7438339
22. González JES, Cuéllar AE, Espinosa RR. Manejo, integración y beneficios del biofertilizante micorrízico EcoMic® en la producción agrícola. Agricultura Tropical [Internet]. 2021;6(3). Available from: https://www.researchgate.net/publication/340223155_Manejo_integracion_y_beneficios_del_biofertilizante_micorrizico_EcoMicR_en_la_produccion_agricola
23. Hernández A, Hernández AN. Efecto de la *interacción Rhizobium*-MA en el cultivo de la soya (*Glycine max* (L.) Merrill). Cultivos Tropicales. 1996;17(1):5-7.
24. Delgado Moreno HO. Análisis de la combinación de microorganismos bioestimulantes (Micorrizas y Rhizobium) en el cultivo de soya (*Glycine max*) [Internet]. Babahoyo: UTB, 2019; 2019. 27 p. Available from: http://dspace.utb.edu.ec/handle/49000/6129
25. Sauvu-Jonasse C, Nápoles-García MC, Falcón-Rodríguez AB, Lamz-Piedra A, Ruiz-Sánchez M. Bioestimulantes en el crecimiento y rendimiento de soya (*Glycine max* (L.) Merrill). Cultivos Tropicales [Internet]. 2020;41(3). Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0258-59362020000300002
26. Tovar-Franco J. Incremento en invernadero de la calidad y cantidad del follaje de la alfalfa (*Medicago sativa* L.) variedad Florida 77 causado por la combinación de fertilización biológica y química en un suelo de la serie bermeo de la sabana de Bogotá. Universitas Scientiarum [Internet]. 2006;11(Esp):61-71. Available from: https://www.redalyc.org/pdf/499/49909905.pdf