BIOFERTILIZATION USING RHIZOBACTERIA AND AMF IN THE PRODUCTION OF TOMATO (*Lycopersicon esculentum* Mill.) AND ONION (*Allium cepa* L.) SEEDLINGS. II. ROOT COLONIZATION AND NUTRITIONAL STATUS

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ABSTRACT. As a complement to preceding studies on biofertilization of horticultural plant seedlings, on eutric, compacted Red Ferralitic soils in experimental areas of the University of Ciego de Ávila, the effects of coinoculation and single inoculation by means of seed coating and without mineral fertilizers were evaluated, using plant growth promoting rhizobacteria- PGPR- (*Azospirillum brasilense*, *Azotobacter chroococcum* and *Burkholderia cepacia*) and arbuscular mycorrhizal fungi- AMF- (*Glomus clarum* and *G. fasciculatum*) on some indicators of root colonization by microorganisms, as well as the nutritional status of onion and tomato seedlings. Results proved that, for both crops, the populations of *A. chroococcum*, *B. cepacia* and *A. brasilense* increased considerably in those treatments inoculated with these rhizobacteria; in general, the highest values were recorded in coinoculated treatments. Concerning mycorrhization, the highest percentages of mycorrhizal colonization and endophyte weight in tomato were achieved by means of coinoculation using *A. brasilense* with both AMF species, whereas in onion, the highest colonization was achieved by the single inoculation of *G. fasciculatum*, even though a higher endophyte weight was obtained by *G. clarum* + *A. chroococcum* coinoculation. Regarding tomato plant nutritional status, the treatments using *A. brasilense* together with both AMF species extracted the highest N and were included among those extracting the greatest P and K. In onion, all the treatments inoculated with both types of microorganisms were able to extract greater amounts of N, P, and K. These results can justify why seedlings with adequate quality are obtained through biofertilization, without using mineral fertilizers.

Key words: rhizobacteria, arbuscular mycorrhizae, roots, nutritional status, onion, tomato, root colonization

RESUMEN. Como complemento a estudios precedentes de la biofertilización en posturas de plantas hortícolas sobre suelos Ferralíticos Rojos compactados, eútricos y en áreas experimentales de la Universidad de Ciego de Ávila, se evaluaron los efectos de la inoculación simple y la coinoculación, mediante el recubrimiento de semillas y sin aplicar fertilizantes minerales, con rizobacterias promotoras del crecimiento vegetal –RPCV- (*Azospirillum brasilense*, *Azotobacter chroococcum* y *Burkholderia cepacia*) y hongos micorrízicos arbusculares –HMA- (*Glomus clarum* y *G. fasciculatum*) en algunos indicadores de la colonización radical por los microorganismos y el estado nutricional de plántulas de tomate y cebolla. A partir de los resultados, se evidenció que, para ambos cultivos, las poblaciones de *A. chroococcum*, *B. cepacia* y *A. brasilense* se incrementaron significativamente en aquellos tratamientos inoculados con estas rizobacterias, encontrando, en general, los mayores valores en los tratamientos que fueron coinoculados. Respecto a la micorrización, los mayores porcentajes de colonización micorrízica y masa del endófito en tomate se obtuvieron mediante la coinoculación de *A. brasilense* con ambas especies de HMA y, para la cebolla, la máxima colonización la realizó *G. fasciculatum* aplicada de forma independiente, mientras que la masa del endófito fue mayor en la coinoculación de *G. clarum* + *A. chroococcum.* En relación con el estado nutricional de las plantas, en tomate, los tratamientos con presencia conjunta de *A. brasilense* y ambas especies de HMA fueron los que hicieron mayores extracciones de N y estuvieron entre los que realizaron mayores extracciones de P y K. En cebolla, todos los tratamientos inoculados con ambos tipos de microorganismos fueron capaces de extraer mayores cantidades de N, P y K. Todos estos resultados permiten explicar las causas de la obtención de posturas de adecuada calidad mediante la biofertilización sin el uso de fertilizantes minerales.

Palabras clave: rizobacterias, micorrizas arbusculares, raíces, estado nutricional, tomate, cebolla, colonización radical

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INTRODUCTION

Seeking and evaluating alternative sources to mineral fertilization, which satisfy crop nutritional needs and allow to obtain adequate levels of crop yield and quality, as well as to increase soil biological processes, as a sustainability index of the agricultural process, is a highly important current task (1). Microbial inoculants are one of such alternative sources.

Plant rhizosphere zone is inhabited by a group of microorganisms, which may have a profitable influence on plants. From the ecological point of view, it is important to know the bacterial community components, in order to favor the application, as inoculants, of those causing no harm to the ecological balance of soil-plant systems (2). Within those microorganisms are plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF), from which many have been used as biofertilizers, due to their proved positive influence on plant growth and yield following different mechanisms (3), as well as on soil fertility (4).

A previous study was developed as a result of evaluating the effects of inoculation using both types of microorganisms on tomato and onion seedling production (5) and it was found that both, single and combined inoculations using rhizobacteria and mycorrhizal fungi, were able to promote growth, even without the presence of mineral fertilizers, obtaining seedlings with adequate quality.

The present work was aimed at studying, under the same experimental conditions, the behavior of root colonization using PGPR and AMF, as well as nutrient extraction, in tomato and onion seedlings inoculated with those microorganisms.

MATERIALS AND METHODS

Trials were carried out under similar conditions to the ones described in the first part of this paper (5). Starting from the selection of the most effective AMF and PGPR species as inoculants, two field experiments were conducted at seedbed stage, focused on evaluating the effects of single and combined inoculations, using such microorganisms, on root colonization effectiveness, as well as nutrient extraction in seedlings. Once again, tomato (*Lycopersicon esculentum*, Mill) cv. Roma VF/ P73 and onion (*Allium cepa*, L) cv. Red creole crops were used, preparing seedbeds in the period between 20 and 25 of October, using a randomized block design with three replications. Table II shows the treatments studied, including an absolute check (without mineral fertilization) and a check with the recommended mineral fertilization (production check), which were not inoculated.

Except *Azotobacter chroococcum* (INIFAT 12 strain), by which seeds were imbibed in liquid medium with titer 1.10×10^{8} ufc.mL^{-1}, inoculation with the remaining PGPR was performed by means of seed coating, applying 1.5 g of inoculant to 1.5 and 5.0 g of tomato and onion seeds, respectively. Strains and titers of each inoculum were *Azospirillum brasilense* (Sp 7)= 3,55x 10^{9} ufc.g^{-1}and *Burkholderia cepacia* (0057)= 3.24×10^{9} ufc.g^{-1}, both of them in solid medium (peat). Arbuscular micorrhizal fungi were also inoculated through seed coating at a dose of 10 % seed weight using high quality inoculum (>20 spore.g^{-1}).

Table I. Treatments used for evaluating the effects on root colonization and nutritional status in tomato and onion

Treatment	Variant
1	Absolute check
2	Production check
3	*G. clarum*
4	*G. fasciculatum*
5	*A. chroococcum*
6	*B. cepacia*[1]
7	*A. brasilense*
8	*G. clarum* + *A. chroococcum*
9	*G. clarum* + *B. cepacia*[1]
10	*G. clarum* + *A. brasilense*
11	*G. fasciculatum* + *A. chroococcum*
12	*G. fasciculatum* + *B. cepacia*[1]
13	*G. fasciculatum* + *A. brasilense*

[1] Treatment with *B.cepacia* only in tomato crop

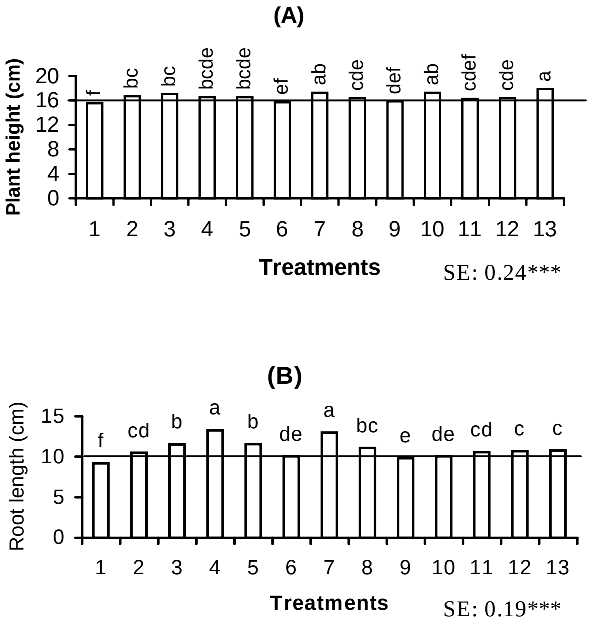
Each treatment took up a total area of 1m² (evaluated area of 0.25 m²) per replication and seeding patterns were 1.5 and 5.0 g.m^{-2} seeds for tomato and onion, respectively. Mineral fertilization was applied to none of the treatments, except the production check, to which the recommended dose for each treatment (150-94-125 and 140-90-125 kg.ha^{-1} N- P_2O_5-K_2O, for tomato and onion, respectively) was applied. Crop cultivation followed what is recommended in technical patterns (7, 8).

Evaluations consisted of measuring in 20 plants per replication from each treatment, at seedling transplantation (25 and 55 days after tomato and onion seed germination, respectively) the following parameters: a) two growth indicators: height (cm) from root neck to the youngest leaf axil, and main root length (cm) of tomato, as well as of root group of onion; b) some indicators of root colonization by inoculated microorganisms: mycorrhizal colonization (%) and arbuscular endophyte weight (mg.g^{-1} rootlets) (9), as well as rhizobacteria population (ufc.g^{-1} rhizosphere soil) in the rhizosphere, by means of sampling, diluting, and seeding, in differential media, according to conventional methods (10); c) extraction of N, P, and K (kg.ha^{-1}) by calculating, dry weight and each nutrient content.

Original data, corresponding to the population variables of each rhizobacteria (ufc.g^{-1} soil) and mycorrhizal colonization (%) were transformed according to log x y arcsen √% functions, respectively; results were subjected to a variance analysis, according to the experimental design used. In the cases where significant differences among means were recorded, Duncan's multiple range test was used as a discriminant criterion. Critical levels for root colonization indicators followed a described mathematical procedure (11).

RESULTS AND DISCUSSION

Starting from rhizobacteria and AMF species, selected in former investigations (5), the effects of single and combined inoculations on seedling growth indicators were again evaluated. Figures 1 and 2 show the results for tomato and onion crops, respectively.



1: Absolute check
2: Production check
3: *G. clarum*
4: *G. fasciculatum*
5: *A. chroococcum*
6: *B. cepacia*
7: *A. brasilense*
8: *G. clarum + A. chroococcum*
9: *G. clarum + B. cepacia*
10: *G. clarum + A. brasilense*
11: *G. fasciculatum + A. chroococcum*
12: *G. fasciculatum + B. cepacia*
13: *G. fasciculatum + A. brasilense*

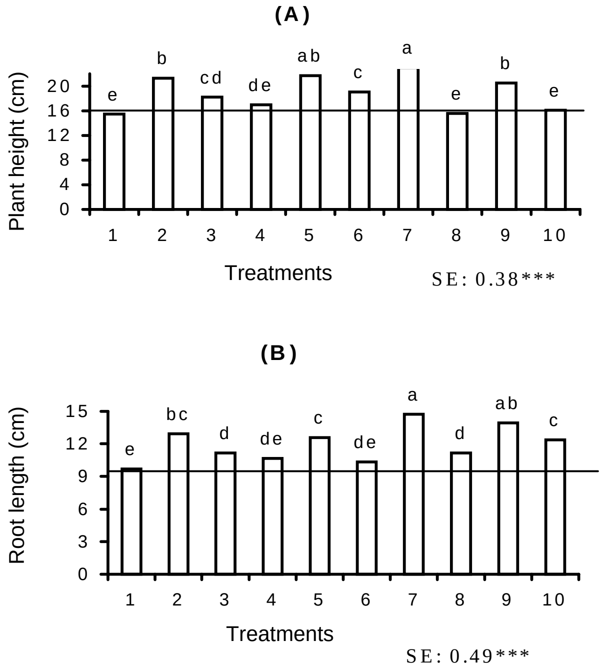
Common letters do not differ at p<0.05, according to Duncan's multiple range test

Figure 1. Tomato seedling growth under the effect of inoculations and coinoculations using PGPR and AMF. 1999-2000 season

In tomato, all variants allowed to obtain seedlings with appropriate height (Figure 1A), except for treatments inoculated with *B. cepacia*, *G. clarum + B. cepacia*, *G. fasciculatum + A. chroococcum*, and the absolute check. *A. brasilense* and *A. chroococcum* stood out again among PGPR's, with a similar behavior to that showed by the two AMF selected. There was not strengthening of the effect of each microorganism through their joined application, that suggests the existence of certain additional factors influencing PGPR and AMF behavior, which could not be determined.

Root length also increased under the effect of the treatments (Figure 1B). Only by means of the absolute check, seedlings that complied with the minimal quality indicator were not established. As to the remaining treatments, it should be highlighted the fact that inoculation with *B. cepacia*, as well as the combined applications of G*. clarum* + *B. cepacia* and *G. clarum + A. brasilense* showed the lowest root growth among those inoculated with the different microorganisms. Synergism was not found with coinoculation either.

Treatments that allowed to obtain seedlings with the demanded minimal height for onion were: the production check, inoculation with both PGPR (A*. chroococcum* and *A. brasilense*) and both AMF (G*. clarum* and *G. fasciculatum*), as well as coinoculations using *A. chroococcum* with both AMF (Figure 2A). On the other hand, all treatments produced seedlings, of which root length achieved the required minimal values (Figure 2B).



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7:*G. clarum + A. chroococcum*
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9: *G. fasciculatum + A. chroococcum*
10: *G. fasciculatum + A. brasilense*

Common letters do not differ at p<0.05, according to Duncan's multiple range test.

Figure 2. Onion seedling growth under the effect of inoculations and coinoculations using PGPR and AMF. 1999-2000 season

Results of both species through the application of rhizobacteria showed differences between them. However, they confirm the results of other authors (12), who explained the increase of plant development by means of the additive hypothesis, according to which, more than one mechanism is likely to be involved in the association. No preponderance is showed among them, working simultaneously or in succession, either by means of an increase of water and nutrient uptake, phytohormone production, phytopathogen biological control, or changes in vegetable cell metabolism. Starting from such hypothesis, quiescence of one or more mechanisms brings about a total or partial failure, which is usually seen on crop inoculation using these bacteria; issues also noticed by other investigators (13). The appropriate vigor manifested by onion seedlings inoculated with *A. chroococcum* has been attributed to the contribution of growth biostimulant substances, such as cytokinins, auxins, gibberellins, amino acids, and vitamins, which allow accelerating seedling development (14, 15, 16).

By the same manner, the influence of inoculation on root development and functions is, probably, one of the issues giving higher crop benefits. In this effect, a leading role is assigned to the production of growth-regulating substances either by inoculated species or by roots, as a response to bacterial colonization (17). Therefore, the species used for obtaining microbial inoculants produce IAA auxins, which could be involved in the results. Regarding this matter, it has been determined that *B. cepacia* 0057 and *A. brasilense* Sp 7 produce 20,1 and 11.92 $mg.mL^{-1}$ of IAA, respectively (18, 19).

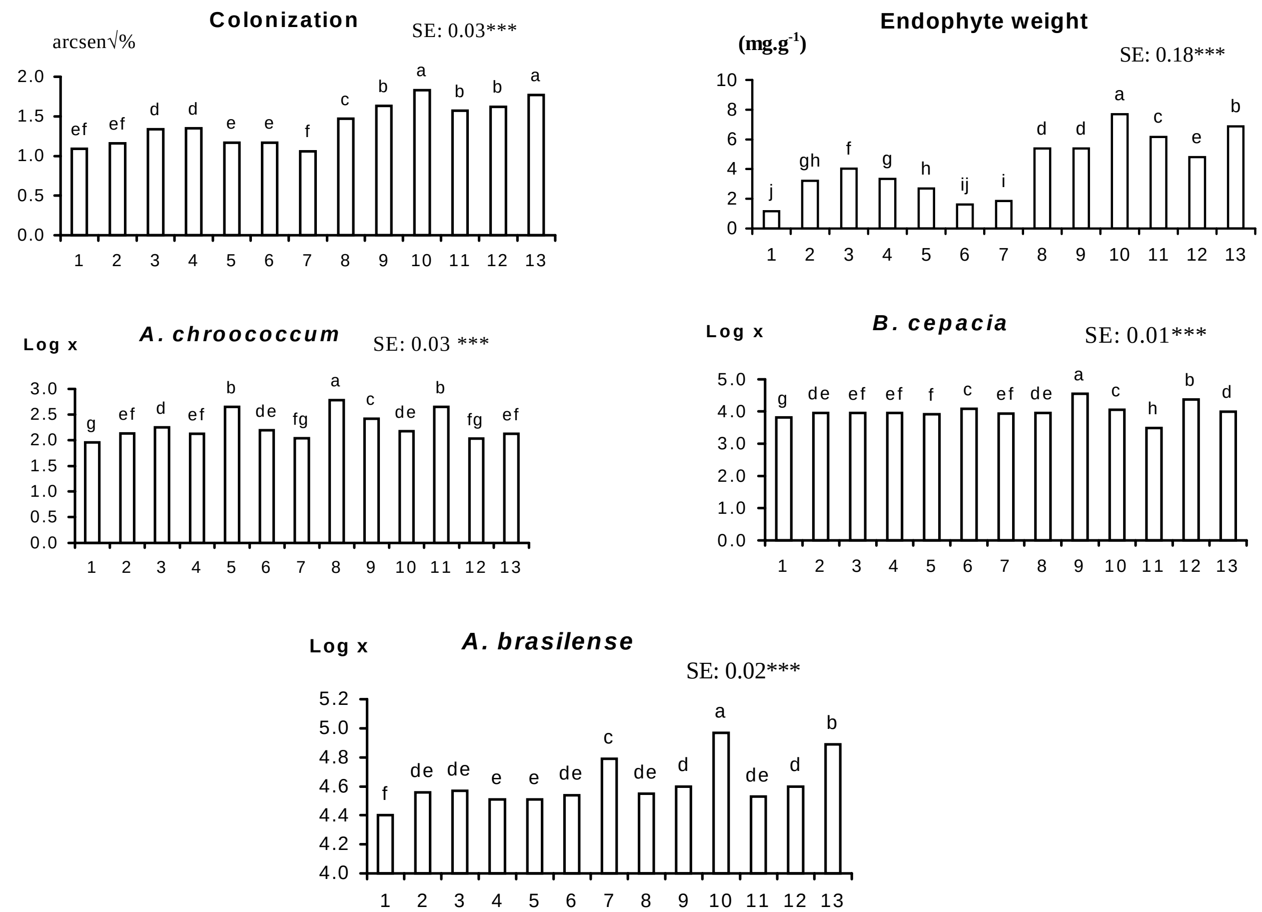
Some authors (20, 21) have pointed out a relationship between tomato root exudates and colonization by *A. brasilense* and *A. chroococcum*. In the same way, a strong interaction between *A. chroococcum* and onion was found (22). All this confirms the importance of studies on plant-PGPR specificity, in order to obtain beneficial effects by inoculating with such microorganisms.

The comparative analysis of the effect of coinoculations, with regard to that of the treatment provided with the required nutrients (production check) showed that, in general, the joined application of both groups of microorganisms did not have a better effect either on plant height or root length since both values were the same or lower than the check, except *G. fasciculatum* + *A. brasilense* for tomato (only in the case of height) and *G. clarum* + *A. chroococcum* for onion (in both indicators evaluated), since those treatments did showed superiority over the production check.

Effects on rhizosphere colonization

Tomato. The highest percentages of colonization and endophyte weight were obtained by means of coinoculation using *A. brasilense* with both AMF species (Figure 3).

It is highlighted that, for both fungal efficiency indicators (colonization and endophyte weight), rhizobacteria inoculated independently did not bring about, in general, any increase on their values, compared to treatments inoculated with AMF and, mainly, to those where inoculations were carried out with both groups of microorganisms. Thus, comparing values achieved by PGPR, root colonization did not increase with respect to the absolute check. However, endophyte weight increased slightly, except for *B. cepacia*, which showed similar values.



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5: *A. chroococcum*
6: *B. cepacia*
7: *A. brasilense;*
8: *G. clarum* + *A. chroococcum*
9: *G. clarum* + *B. cepacia*
10: *G. clarum* + *A. brasilense*
11: *G. fasciculatum* + *A. chroococcum;*
12: *G.fasciculatum* + *B. cepacia*
13: *G. fasciculatum* + *A. brasilense*

Common letters do not differ statistically at p<0.05, according to Duncan's multiple range test

Figure 3. Transformed microbiological variables in tomato seedlings after seedbed stage

A. chroococcum, *B. cepacia*, and *A. brasilense* populations increased considerably in treatments inoculated with these PGPR; and higher values of ufc.g^{-1} soil were found in coinoculated treatments, except for coinoculation with *A. chroococcum*, where one of its combinations was similar to the individual application. In all cases, populations of the respective rhizobacteria outnumbered values reached by the absolute check.

Onion. Maximal fungal colonization was achieved by *G. fasciculatum*, applied independently; whereas endophyte weight was higher when using *G. clarum* + *A. chroococcum* coinoculation than in the rest of the treatments (Figure 4).

It can also be noticed that, when inoculation was performed using each rhizobacteria, populations of the respective PGPR increased. Inoculation using AMF always increased rhizobacteria populations. The highest number of *A. chroococcum* was achieved in coinoculations where this PGPR was present. Something similar occurred with coinoculation using *A. brasilense*, where populations of this rhizosphere species increased.

As to root colonization, two issues must be highlighted. In first place, mainly in the case of the most outstanding treatments, it was found only a certain degree of correspondence between growth index response to inoculation and the corresponding values to mycorrhizal colonization indicators when, generally, a close relationship between estimators of mycorrhizal functioning and effectiveness of inoculation on growth or yield is found. Secondly, even though populations of the inoculated rhizobacteria increased considerably, compared to their original values, these increments were relatively low. Different from this, those increases are told to reach, generally, from two to three degrees of magnitude. These apparent disagreements could be explained, taking into account that the obtained values were found at seedling transplantation, when PGPR and AMF colonization is not at their maximal effectiveness stage and, therefore, its effects are still limited.

On the other hand, results from both vegetable species using the absolute check, evidenced the low and ineffective colonization potential of native species, which implies that there was an agroecosystem with low occurrence of effective propagules under experimental conditions.

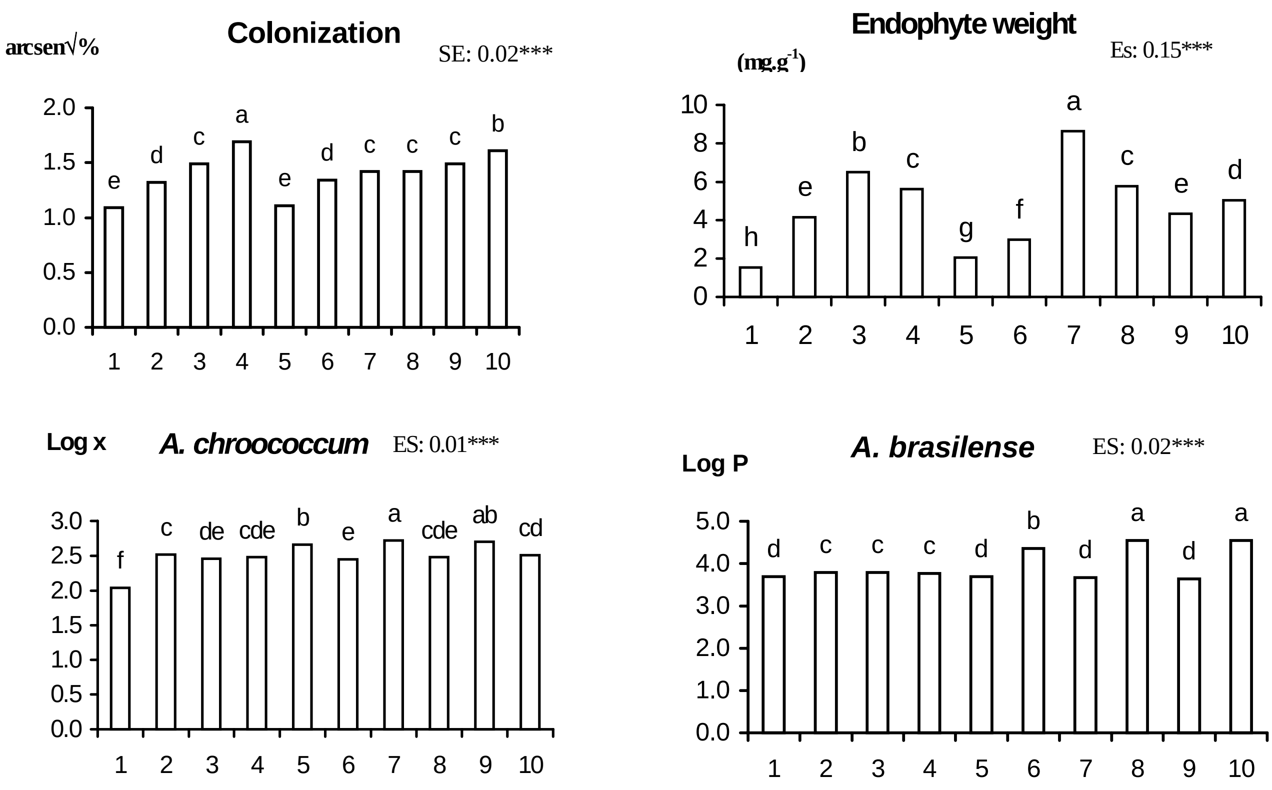

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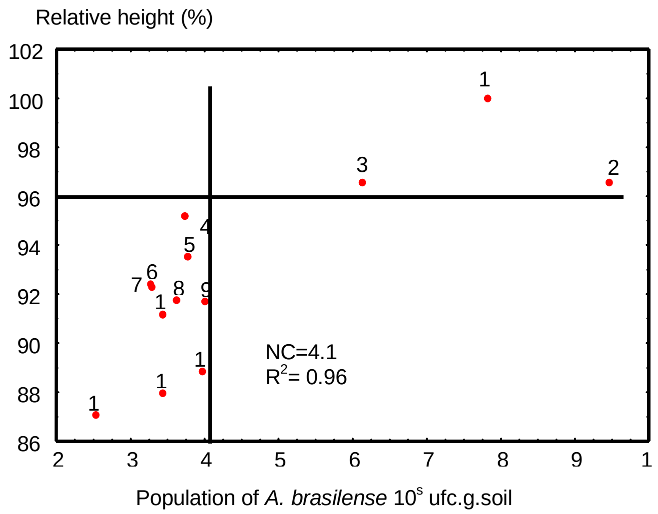
Figure 4. Transformed microbiological variables in onion seedlings after seedbed stage

After determining the influence of the evaluated microorganisms on root growth and colonization of seedlings, an interesting task was considered, that of determining, where possible, response thresholds or kinds of critical levels (by analogy, using a similar concept to that employed on soil fertility and plant nutrition) of some rhizosphere colonization components; in other words, to determine values under which increments in such colonization, by means of inoculation, promoted growth.

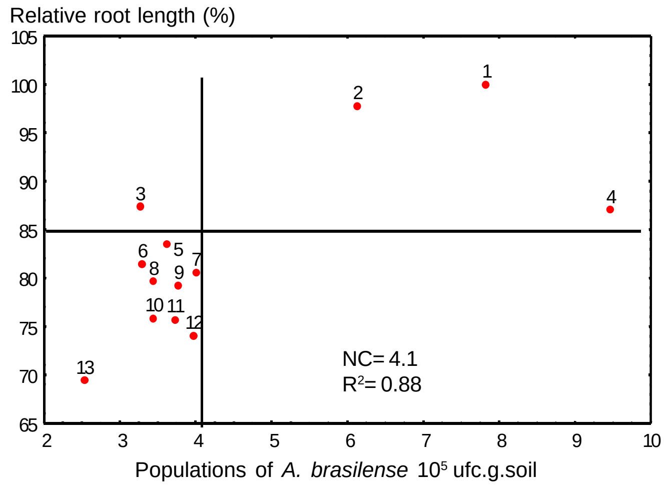
Tomato. Critical level for *A. brasilense* population was 4.1 x 10^5 ufc.g^{-1} sr (Figure 5). It was observed that populations with higher levels were achieved by rhizobacteria inoculation, either independently or coinoculated with *G. clarum* and *G. fasciculatum* AMF, these treatments being, at the same time, those producing the highest seedlings, without differences between treatments. When root length was used as a phenological index, treatments with superior values to that of the critical level, as well as with higher growth, coincided with those determined when height was used as indicator (Figure 6). *G. clarum + G. brasilense* coinoculation is highlighted, which achieved the highest bacterial population, produced seedlings with shorter roots, with respect to the remaining treatments.



1- *G. fasciculatum* **+** *A. brasilense*; 2- *G. clarum* **+** *A. brasilense*; 3- *A. brasilense*; 4- *G. clarum*; 5- Test. producción; 6- *A. chroococcum*; 7- *G. fasciculatum*; 8- *G. clarum* **+** *A. chroococcum*; 9- *G. fasciculatum* **+** *B. cepacia*; 10- *G. fasciculatum* **+** *A. chroococcum*; 11- *G. clarum* **+** *B. cepacia*; 12- *B. cepacia*; 13- T. absoluto

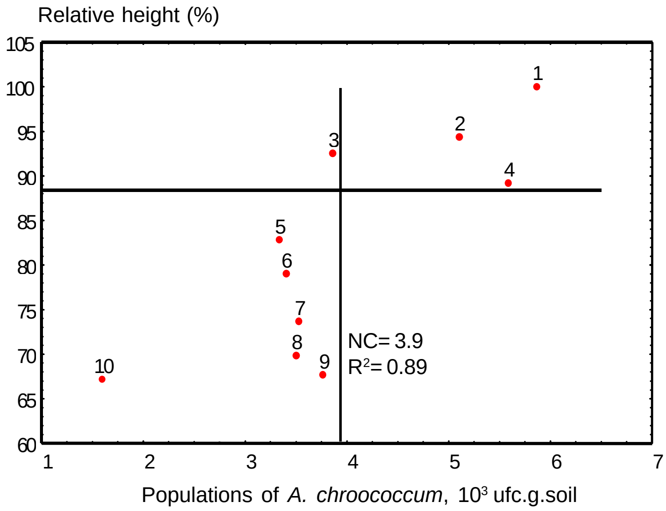
Figure 5. Critical level of *A. brasilense* populations for tomato seedlings, cultivated in compacted Ferralitic Red soil

Onion. Critical level was determined to this horticultural species for *A. chrooococcum* populations (Figures 7, 8). Such level was 3,9 x 10^3 ufc.g sr^{-1} and estimated taking into account the two morphological indicators used. This PGPR population, which was higher than the determined level, was achieved both, through independent inoculation of this rhizobacterium and coinoculated with AMF. By means of these treatments, the highest values of height and root length were attained.



1: *G. fasciculatum + A. Brasilense;* 2: *A. Brasilense;* 3: *A. chroococcum;* 4: *G. clarum + A. Brasilense;* 5: *G. clarum + A. chroococcum;* 6: *G. fasciculatum;* 7: *G. fasciculatum + B. cepacia* 8: *G. fasciculatum + A. chroococcum;* 9: Production check; 10: *B. cepacia;* 11: *G. clarum;* 12: *G. clarum + B. cepacia;* 13: Absolute check

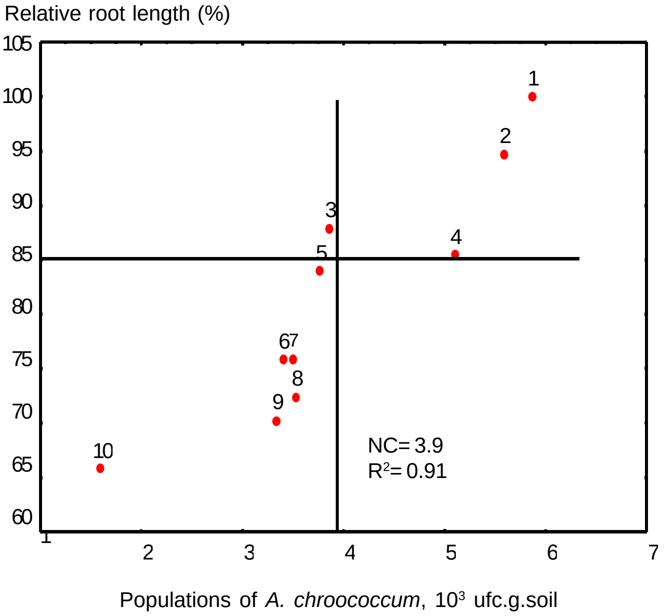
Figure 6. Critical level of *A. brasilense* populations for tomato seedlings cultivated in a compacted Red Ferralitic soil



1: *G. clarum + A. chroococcum*; 2: *A. chroococcum*; 3: Production check; 4: *G. fasciculatum + A. chroococcum*; 5: *A. brasilense*; 6: *G. clarum*; 7: *G. fasciculatum*; 8: *G. fasciculatum + A. brasilense*; 9: *G. clarum + A. brasilense*; 10: Absolute check

Figure 7. Critical level of *A. chroococcum* populations for onion seedlings cultivated in a compacted Red Ferralitic soil

Concerning the variant where mineral fertilization was applied (production check), by means of which seedlings were achieved with similar height and root length to those obtained by the formerly mentioned treatments, *A. chroococcum* population also increased up to near values to the established critical level. This is probably motivated by root exudation, performed by plants, which contain plenty of organic products that are similar to the rhizobacteria as such.



1: *G. clarum* + *A. chroococcum*; 2: *G. fasciculatum* + *A. chroococcum*; 3: Production check; 4: *A. chroococcum*; 5: *G. fasciculatum* + *A. brasilense*; 6: *G. clarum*; 7: *G. clarum* + *A. brasilense*; 8: *G. fasciculatum*; 9: *A. brasilense*; 10: Absolute check

Figure 8. Critical level of *A. chroococcum* populations for onion seedlings cultivated in a compacted Red Ferralitic soil

Nutrient extraction

Table II presents N, P, and K extractions by tomato and onion seedlings after seedbed stage.

Nitrogen. It was noticed that, in tomato, treatments with *A. brasilense* inoculated independently and combined with *G. clarum* and *G. fasciculatum* AMF species, achieved superior N extraction to the remaining treatments. This behavior could be explained by the occurrence of processes, which foster the effect of these microorganisms, such as the production of phytohormones favoring root development and nutrient absorption. At the same time, the former issue has a positive influence on vegetable growth promotion (23), together with a possible biological N fixation, due to the capacity of PGPR in this sense, which redounds to a better nitrogen nutritional status of plants.

In onion, the highest N extractions were achieved with the joined presence of *A. chroococcum* and both AMF species. This can be attributed to the characteristics of this microbial genus, which has great affinity with the crop (22), where its capacities for biological N fixation are stimulated. Differences between those treatments and *G. clarum* + *A. brasilense* were not found and all of them were superior to the production check.

In both crops, coinoculated treatments extracted superior or similar N to that achieved by treatments inoculated independently, except *G. fasciculatum* + *B. cepacia* for tomato, and *G. fasciculatum* + *A. brasilense* for onion. Thus, in tomato, single inoculation of *A. brasilense*, as well as combined with both AMF species was superior to the remaining treatments. In the case of onion, increases in N extractions stood out when promoted by coinoculations of *A. chroococcum* and both AMF species, together with the joined presence of *G. clarum* + *A. brasilense* in seedlings. It has to be highlighted that this last treatment was common for both horticultural species; this could find a cause in the fact that chemotactic mechanisms of both vegetable species favor *A. brasilense* colonization, giving this PGPR a more versatile character.

Phosphorus. In tomato, as well as in onion, the highest extractions were achieved with *G. fasciculatum* + *A. brasilense*, and *G. clarum* + *A. brasilense* treatments. In onion, no difference was found with respect to *G. fasciculatum*, which was inoculated independently. Coinoculation using *A. brasilense* with *G. clarum* AMF was efficient, as it was in N extraction, causing statistically superior or equal phosphorus extractions to those made by treatments which achieved the highest nutrient extractions in each crop. This issue coincides with other authors (24), who explained that mycorrhization through its physical effect of widening plant absorption system,

Table II. Values of N, P, and K (kg.ha^{-1}) extractions made by seedlings of both crops after seedbed stage

Treatments	Tomato N	Tomato P	Tomato K	Onion N	Onion P	Onion K
Absolute check	24.4 d	3.37 def	26.98 f	25.72 e	5.40 g	15.96 f
Production check	40.8 b	4.97 bc	39.05 c	54.73 c	15.53 bc	36.15 bc
Glomus clarum	39.2 b	4.52 c	37.74 cd	42.51 d	14.67 e	25.50 e
Glomus fasciculatum	31.5 c	3.64 d	33.73 de	45.28 d	15.81 ab	29.69 d
Azotobacter chroococcum	29.2 c	2.86 f	34.21 de	60.86 b	12.77 f	39.91 a
Burkholderia cepacia	31.4 c	5.28 b	33.31 e	(ne)	(ne)	(ne)
Azospirillum brasilense	47.7 a	3.45 de	37.41 cde	53.19 c	12.67 f	34.35 c
G. clarum + *A. chroococcum*	39.7 b	4.79 c	34.21 de	65.52 a	15.04 de	39.30 a
G. clarum + *B. cepacia*	42.7 b	3.04 ef	33.51 de	(ne)	(ne)	(ne)
G. clarum + *A. brasilense*	47.6 a	5.84 b	44.10 ab	62.90 ab	16.14 a	37.74 ab
G. fasciculatum + *A. chroococcum*	43.1 b	4.67 c	43.25 b	64.50 a	15.21 cd	40.31 a
G. fasciculatum + *B. cepacia*	23.0 d	5.00 bc	47.61 a	(ne)	(ne)	(ne)
G. fasciculatum + *A. brasilense*	46.0 a	6.34 a	43.58 ab	45.58 d	16.10 a	30.69 d
SE	1.23***	0.18***	1.32***	1.05***	0.13***	1.01***

Means in columns with equal letters do not differ at p<0.05, according to Duncan's multiple range test
(ne) - nonevaluated

as well as through its physiological effects that increase root absorbing capacities, is an important mechanism for maximizing the use of phosphate fertilizers. The presence of rhizobacteria also takes part in achieving such extractions, because they favor a higher degree mycorrhization activity.

Potassium. In tomato, the highest extractions were achieved through coinoculations of *G. fasciculatum* with the three rhizobacteria studied, together with *G. clarum* + *A. brasilense* combination, showing statistical superiority and difference with respect to the rest of the treatments. No inoculated microorganisms individually provided plants with superior potassium supply to that of the production check. In the case of onion, independent inoculation of *A. chroococcum*, as well as its combination with both AMF species, produced the highest potassium extractions, without statistical differences with the extractions made by *G. clarum* + *A. brasilense* variant, which were not different from those made by fertilized plants (production check).

The distinguished behavior of different inoculated treatments could be attributed to the variability in functions and the possibilities that they provide, as well as to the specificity of both groups of microorganisms for putting such nutrients at plant disposal.

The integral analysis on the behavior of different microorganisms studied for producing tomato and onion seedlings, confirmed their possible production with optimal quality without using mineral fertilization.

In tomato, treatments with the joined presence of *A. brasilense* and both AMF species presented the highest values of endophyte weight and colonization percentages. In the same way, those treatments extracted the highest N and are within those extracting the highest P and K. All this can explain why appropriate seedlings are obtained with these treatments.

In addition to what is formerly exposed, it can be noticed that, in onion, all inoculated treatments with both groups of microorganisms extracted higher N, P, and K than the absolute check. Therefore, they made it possible to put those nutrients at plant disposal by means of different mechanisms interacting among each other. This could have started from mineralization, N biological fixation, P solubilization, as well as the stimulation of root development and/or the secretion of growth stimulant substances, among other possible mechanisms.

Regarding inoculated treatments with *A. chroococcum*, seedlings made the highest N and K extractions. This behavior brought about the fact that, precisely the population of this rhizobacteria was the only parameter to which critical level could be determined, evidencing affinity between such rhizobacteria and exudates made by onion.

For both vegetable species, increases in the population of the respective inoculated rhizobacteria were achieved, in general, by using coinoculations. The former implies affinity between both groups of microorganisms. The need for inoculating seeds from both crops with more promising microorganisms, without mineral fertilization, was also evidenced, because of poor results of the absolute check in some evaluated indicators. This issue highlights the necessity of increasing microbial populations, which are native from soil, by means of biofertilization.

REFERENCES

1. Altieri, M. A. Agroecología. Bases científicas para una agricultura sustentable. La Habana : *CLADES,* 1997.
2. Hernández, A. N.; García, D.; Hernández, A. and Heydrich, M. Determinación de algunos géneros bacterianos presentes en la rizosfera del cultivo del maíz. *Cultivos Tropicales*, 1997, vol. 18, no. 3, p. 10-14.
3. Bashan, Y. Azospirillum plant promoting strains are nonpathogenic on tomato, pepper, cotton and wheat. *Can. J. Microb*., 1998, vol. 44, no. 2, p. 166-174.
4. Azcón, R. Papel de la simbiosis micorrízica y su interacción con otros microorganismos rizosféricos en el crecimiento vegetativo y sostenibilidad agrícola. En: Ecología, Fisiología y Biotecnología de la Micorriza Arbuscular. C. México: Mundi Prensa, 2000.
5. Pulido, L. E.; Medina, N. and Cabrera, A. La biofertilización con rizobacterias y hongos micorrízicos arbusculares en la producción de posturas de tomate y cebolla. I Crecimiento vegetativo. *Cultivos Tropicales*, 2003, vol. 24, no. 1, p. 15.24.
6. Cuba. Minagri. Instituto de Suelos. Nueva versión de Clasificación Genética de los Suelos de Cuba. La Habana : Agrinfor, 1999.
7. Cuba.MINAG. Instructivo técnico del tomate. Dir. Nac. Cult. Varios, La Habana, 1983.
8. Cuba.MINAG. Instructivo técnico de la cebolla. Dir. Nac. Cult. Varios, La Habana, 1983.
9. Herrera, R. /*et al*./. Estrategia de funcionamiento de las micorrizas VA en un bosque tropical. Biodiversidad en Iberoamérica. Ecosistemas, Evolución y Procesos Socilales. En: Prog. Iberoam. Ciencia y Tecnología para el Desarrollo. Subprograma. Diversidad Biológica. Mérida. (12:Mérida), 1995.
10. Bashan, Y.; Holguín, G. and Ferrera Cerrato, R. Interacciones entre plantas y microorganismos benéficos. *Terra*, 1996, vol. 14, no. 2, p. 159-192.
11. Cate, R. B. and Nelson, L. A. A simple statistical procedure for partitioning soil test correlation data into two classes. *Soil Sci. Soc. Amer. Proc*., 1971, vol. 35, p. 658-660.
12. Bashan, Y. and Levanony, H. Current status of Azospirillum inoculation technology: Azospirillum as a challenge for agriculture. *Can. J. Microbiol*., 1990, vol. 36, p. 591-608.
13. Viñals, M. and Villar, J. Avances en la formulación y aplicación de inoculantes bacterianos de uso agrícola. *Cultivos Tropicales*, 1999, vol. 20, no. 4, p. 9-17.
14. Dibut, B.; Acosta, M. C.; Martínez, R.; Nikander, B. and Ljunggreen, H. Producción de aminoácidos y citoquininas por una cepa cubana de *Azotobacter chroococcum.*En: Seminario Científico y Taller Internacional sobre biofertilización en los trópicos (Biofertro´92).(8:1: 1992: La Habana), 1992.

15. Martínez-Viera, R. Efecto económico de las aplicaciones de biofertilizantes a base de Azotobacter en la agricultura cubana. En: Reunión Latinoamericana de Rhizobiología. Programa y Resúmenes. Taller Internacional Biofertro ¨94, La Habana. (17, 3:1994,La Habana), 1994.
16. Acosta, M.; Dibut, B.; Martínez-Viera, R.; Pérez, A.; Ljunggreu, H.; Granhall, U.; Pérez, D.; Antúnez, N. and Rodríguez, J. Cambios fenológicos en las plantas inducidas por la bioactividad del fertilizante BIOSTIN. En: Reunión Latinoamericana de Rhizobiología. Programa y Resúmenes. Taller Internacional Biofertro ¨94, La Habana. (1994:La Habana), 1994.
17. Hernández, A. Caracterización de cepas de *Pseudomonas cepacia* y *Pseudomonas fluorescens* aisladas en la rizosfera del maíz. [Tesis de Maestría], INCA, 1998.
18. Velázquez, M.; Hernández, A.; Heydrich, M and A.N. Hernández. Estudio de la interacción maíz-*Burkholderia cepacia*. *Rev. Latinoam. Microb*., 1999, vol. 41, p. 17-23.
19. Pazos, M. Aislamiento e identificación de cepas nativas, pertenecientes al género *Azospirillium*, mediante técnicas moleculares. [Tesis de Maestría]; Univ. Habana, 2000.
20. Lara, D. Evaluación de sustratos y biofertilizantes para el cultivo del tomate (*Lycopersicum esculentum* Mill) utilizando la tecnología de cepellones. [Tesis de Maestría]; INCA, 1999.
21. Ramírez, R. Biofertilización del tomate en las condiciones edafoclimáticas de suelos Pardos Mullidos sin carbonato de la provincia de Holguín. [Tesis de Maestría]; INCA, 2001.
22. Dibut, B. (2000) Obtención de un bioestimulador del crecimiento y el rendimiento para el beneficio de la cebolla (*Allium cepa*, L). [Tesis de Doctorado]; INIFAT, 2000.
23. Dommelen, A. V. Ammonium transports in *Azospirillum brasilense*. *Dissertationes de agriculture*, 1998, vol. 1, p. 1-9.
24. Siqueira, J. O. and Franco, A. A. (1988) Biotecnología de suelo: Fundamentos y perspectivas. Brasilia : MEC, 1988.

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