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Bibliographic review

Benefits of co-inoculation of Arbuscular Mycorrhizal Fungi and rhizobia in bean cultivation

Beneficios de la coinoculación de Hongos Micorrizógenos Arbusculares y rizobios en el cultivo del frijol

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ABSTRACT: The common bean (*Phaseolus vulgaris* L.) is one of the leguminous plants of high human consumption. In Cuba, it constitutes one of the indispensable dishes in the Cuban menu, being the black bean the most common in Creole food. In spite of its importance and the fact that it is a traditional crop, it is necessary to increase the productivity of plants in a sustainable way, with little amount of resources and with the best quality standards, the national production still does not satisfy the consumption demand. Biofertilizers represent a sustainable, economically attractive and ecologically acceptable means to reduce external inputs and improve the quantity and quality of agricultural products, through the use of properly selected soil microorganisms, capable of making available to plants, through their biological activity. Among the microorganisms that have been most widely used for the development of biofertilizers are bacteria of the genus *Rhizobium* (rhizobia) and arbuscular mycorrhizal fungi (AMF). Rhizobia are nitrogen-fixing bacteria and plants, the formation of the specialized nodule, which ensures the reduction of atmospheric nitrogen, takes place in the root. Mycorrhizae are mutualistic symbiotic associations this interaction fungi benefit from the supply of carbon sources from the plant and the plant benefits from increased exploration of the soil, which enhances plant growth and development. However, the benefits of rhizobium-AMF co-inoculation in legumes of agricultural interest need further studies on these issues

Key words: legumes, biofertilizers, inoculation, plant nutrition.

RESUMEN: El frijol común (*Phaseolus vulgaris* L.) constituye una de las especies de leguminosas de alto consumo humano. En Cuba, constituye uno de los platos indispensables en el menú cubano, siendo el frijol negro el más común en la comida criolla. A pesar de su importancia y el hecho de que es un cultivo tradicional, es necesario incrementar la productividad de las plantas de manera sostenible, con baja cantidad de recursos y con los mejores estándares de calidad, la producción nacional aún no satisface la demanda del consumo. Los biofertilizantes representan un medio sustentable, económicamente atractivo y ecológicamente aceptable, para reducir los insumos externos y mejorar la cantidad y calidad de los productos agrícolas, mediante la utilización de microorganismos del suelo debidamente seleccionados, capaces de poner a disposición de las plantas, mediante su actividad biológica. Entre los microorganismos que más se han utilizado para la elaboración de biofertilizantes se encuentran las bacterias del género *Rhizobium* (rizobios) y los hongos micorrizógenos arbusculares (HMA). Los rizobios son bacterias fijadoras de nitrógeno y las plantas, tiene lugar en la raíz la formación del nódulo especializado, que garantiza la reducción del nitrógeno atmosférico. Las micorrizas son asociaciones simbióticas mutualistas esta interacción los hongos se benefician con el suministro de fuentes carbonadas provenientes de la planta y ésta se beneficia por la mayor exploración del suelo, lo que aumenta el crecimiento y desarrollo de las plantas. Los beneficios de la coinoculación rizobios-HMA en las leguminosas de interés agrícola, sin embargo, es necesario proseguir los estudios en estos temas.

Palabras clave: leguminosas, biofertilizantes, inoculación, nutrición de las plantas.

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INTRODUCTION

The bean crop stands out for its socioeconomic importance and for the area used for planting and grain production. Other factors make beans have a preferential place, especially its nutritional composition, being a rich source of protein, minerals such as calcium, iron, phosphorus, magnesium and zinc and vitamins thiamine, niacin and folic acid (1).

In Cuba, it constitutes one of the indispensable dishes in the Cuban menu, being the black bean the most common in the Creole food (2). In spite of its importance and the fact that it is a traditional crop, it is necessary to increase plant productivity in a sustainable way, with a low amount of resources and with the best quality standards (3).

In this sense, biofertilizers represent a sustainable, economically attractive and ecologically acceptable. It means to reduce external inputs and improve the quantity and quality of agricultural products, using properly selected soil microorganisms, capable of making available to plants, through their biological activity, an important part of the nutrients needed for their development, as well as supplying hormonal or growth-promoting substances (4).

Among the microorganisms that have been most widely used for the elaboration of biofertilizers are bacteria of the *Rhizobium* genus (rhizobia) and arbuscular mycorrhizogenous fungi (AMF).

Rhizobia are known for their symbiotic interaction with legumes, between which an intense exchange of signals is established, where the release of isoflavonoid compounds by root stands out, which induce the synthesis of nodulation factors in the bacteria (5). As a result, the formation of the specialized nodule takes place in the root, which ensures the reduction of atmospheric nitrogen through the enzyme nitrogenase and the adequate supply of ammonium to the plant in the form of ureides and amides (6).

On the other hand, mycorrhizae are mutualistic symbiotic associations existing between certain soil fungi and roots of higher plants. In this interaction, the fungi benefit from the supply of carbon sources from the plant and the plant benefits from the increased exploration of the soil, which enhances plant growth and development (7). The use of mycorrhizae as biofertilizers does not imply that fertilization can be stopped, but it allows fertilization to be more efficient and doses to be applied can be partially reduced, by increasing the percentage of nutrient absorption by plants (8).

In *Rhizobium*-AMF-legumes co-inoculation, it has been reported that symbiotic relationships provide a greater exchange between symbionts and superior effects to plants. In this case, the *Rhizobium*-legume symbiosis provides N_2 and the mycorrhizae increase the uptake of other elements, including P, which is very important to ensure adequate biological N fixation, increase the number, dry weight in nodules and plant growth (9).

In the present bibliographic review, some results obtained in the studies of the inoculation of Arbuscular Mycorrhizal Fungi and *Rhizobium* in the cultivation of beans are summarized.

Importance of bean cultivation

The common bean (*Phaseolus vulgaris* L.) is the most consumed legume in the world; currently, around 19 million tons are produced annually, in environments as diverse as Latin America, North and Central Africa, China, USA, Europe and Canada. Among these, Latin America is the largest producer and consumer, led by Brazil, Mexico and Central America and the Caribbean (10). The common bean is the most important edible legume in the world and provides an important source of protein (22 %), vitamins, and minerals (Ca, Cu, Fe, Mg, Mn, Zn) to the human diet, especially in developing countries (11,12). In the most developed countries, annual production exceeds 21 million tons and represents more than half of the world's total production of pulses for consumption (13,14).

Beans are one of the most important grains for direct human consumption (15). This legume constitutes one of the fundamental grains in Cuban people diet along with rice and viands; it is a food of preference in the daily diet in at least one of meals. In Cuba in 2018, 147 500 ha were planted with an average yield of 1.09 t ha^{-1} (16); however, national production still does not meet consumption demand (17).

Nutritional requirements of the bean crop

The nutrient requirements of a crop vary with the level of production (fertilization and management technology), soil, and agroecological conditions (18,19). Beans absorb high amounts of N, K and Ca and lesser amounts of S, Mg and P. An essential measure to determine total nutrients to be applied to a crop is to know the amounts of elements contained in the soil by chemical analysis in a laboratory. The supply of nutrients to the plant in recommended amounts is a fundamental objective in fertilization programs. Table 1 shows percentages of nutrients in the dry matter of the plant upper leaves and the summary of the amount of nutrients extracted by crops expressed in kg of nutrients per ton of grain (20).

To offer to the crop the optimal conditions and obtain adequate yields, it is important to know its development and the stages of higher demand of nutrients (21). In this

Table 1. Percentage of nutrients in the dry matter of the upper leaves of the common bean plant and amount of nutrients extracted by the bean, expressed in kg of nutrients per ton of grain

Part of the plant	N	P	K
Mature upper leaves (% dry mass)	> 3	0,4	2-4
Seed extraction (kg t^{-1} grain)	31-129	8-21	25-68

regard, in a greenhouse experiment it was found that the maximum rate of absorption of nutrients of beans corresponds to 50 days after planting for nitrogen (N), potassium (K) and calcium (Ca) and 60 days for magnesium (Mg) and sulfur (S) (22). The absorption and accumulation of nutrients by the bean, allows to know the amount and intensity of nutrient absorption throughout the vegetative cycle (23). Through patterns of accumulation and absorption of nutrients will be obtained basic information on the most appropriate time for fertilization (24).

Bean is a plant that presents moderate nutrient extractions in relation to other crops. However, when a nutrient is deficient, this deficiency is expressed in reduced growth or possible death of plant tissues (25).

In trials carried out in Brazil, it was determined that the maximum extraction of elements occurs at the end of the phenological cycle of the bean crop, approximately between 65 and 80 days, N is located in first order, followed by K and Ca, in almost similar quantities, and finally Mg and P (23).

Bean fertilization

Bean fertilization is an extremely important task for bean production. Adequate fertilization provides the necessary nutrients for good growth, development and production of the crop. Traditionally, in recent years the crop has been fertilized to meet the requirements of nitrogen (N), phosphorus (P) and potassium (K), and in many cases only for the first two (26,27).

Soils for common bean cultivation have very variable physical and chemical conditions. There are soils whose nutritional deficiencies can affect crop development and yield. In different varieties and populations, the average nutrient uptake to achieve high agricultural yields ranges from 0.133-0.016-0.116 t ha^{-1} and an average extraction and export of 0.0322-0.054-0.0172 t of nitrogen, phosphorus and potassium, respectively per t of seeds. Nutrient applications are recommended at the bottom of the furrow (28).

In irrigated monoculture areas, two applications of fertilizers should be made. The first at the moment of sowing with a complete formula, where 1/3 of the nitrogen is deposited with all the phosphorus and potassium, which can be adjusted with the planter, as long as it guarantees that the fertilizer is located below the seed to avoid direct contact with it. The second application, covering the remaining 2/3 of nitrogen, will be 20-25 days after germination of the grain (stages V4-R5) with a nitrogen fertilizer, the same will be done in lateral bands, separated from the sowing thread between 10-12 cm. This operation should be carried out with adequate soil moisture.

Urea can be applied advantageously both by introducing it to the soil and through the leaves; it has been proven that absorption through the latter route is fast and by foliar spraying a convenient nitrogen concentration can be maintained in leaves. Foliar spraying makes available to plants much smaller amounts of nitrogen than those achieved because of the application of urea to the soil (29).

When very high amounts of nitrogen fertilizers are applied to crops, of which only a part is used by plants, the other part is lost by volatilization and washing (the latter contaminates aquifers) with the consequent economic and ecological loss (30).

Because of this, it is advisable to apply fertilizers according to the needs of the crop or to reduce their doses, combining them with the use of biofertilizers or green and organic fertilizers, which help to a better use of nutrients in the soil, being less expensive and ecological.

Biofertilization of beans

Biofertilizers are products based on microorganisms that normally live in the soil and have the capacity to make available to plants, through their biological activity, an important part of the nutrients they need for their development (31).

The use of plant growth promoting microorganisms is a strategy in sustainable agriculture worldwide (32). Their use is focused on increasing plant nutrition, with the corresponding substitution of chemical fertilizers and the search for protection against diseases and pests. Microorganisms intervene in a series of processes such as decomposition, mineralization of organic compounds and mobilization of nutrients in the soil-plant interaction (32).

The long-term sustainability of agricultural systems should promote the effective use and management of the internal resources of agroecosystems. In this sense, biofertilizers are a vital component of sustainable systems, since they constitute an economically attractive and ecologically acceptable means (33). The need to obtain high agricultural yields while preserving the environment is linked to the widespread use of these products (34).

Biofertilizers include all biological resources that help or stimulate the development of agricultural crops through transformations of elements or compounds found in the soil in unusable forms, so that they are converted into forms that can be used through the action of microorganisms or microorganism-plant associations (35).

Among the biofertilizers that have been most widely used in Cuba, the following stand out: Dimargon®, based on *Azotobacter chroococcum*; AzoFert® and Biofer®, composed of rhizobia as the active principle; Fosforina® and Nitrofix®, based on *Pseudomonas fluorescens* and *Azospirillum* sp. respectively and EcoMic®, composed of arbuscular mycorrhizal fungi.

The quality of an inoculant is measured by its capacity to maintain a high population of the microorganism for several months after inoculation. The number of cells above 10^8 per gram or mL of inoculant, or around 10^6 per seed, ensures the best benefits from the interaction (36).

Biological N fixation by legumes

Biological nitrogen fixation (BNF) is one of the main components of agricultural sustainability. A limited number of prokaryotic species, generally bacteria and blue - green algae (*Cyanophyceae*), carries it out and it is manifested in diverse processes, both symbiotic and non-symbiotic. Within the group of prokaryotic microorganisms that establish symbiosis with plants belonging to the *Fabaceae* family are the bacteria of the *Rhizobiaceae* family, mainly the genera *Rhizobium*, *Bradyrhizobium* and *Azorhizobium*, which infect and nodulate plant roots, and among the free-living microorganisms that establish non-symbiotic N fixation are bacteria of the genera *Azotobacter*, *Beijerinckia*, *Pseudomonas*, *Azospirillum*, *Clostridium* and others (37,38).

Bacteria of the genus *Rhizobium* are natural inhabitants of the soil, although their population depends on various factors such as pH, temperature, humidity and energy sources (39). The rhizosphere of legumes stimulates the proliferation of *Rhizobium* genera, because they find a more favorable environment there than in the rest of the soil.

In addition, biological specificity must be considered, which is manifested when the host and macrosymbiont interact with some degree of selectivity to give rise to nodular infection and GNF, so it is necessary that the *Rhizobium* strain effective for the legume be found in the rhizosphere of the plant (40).

However, indigenous rhizobia are not always found in sufficient numbers or compatible with the specific legume crop to stimulate biological nitrogen fixation and increase yields. Inoculation of legumes with rhizobia is an important option to improve biological nitrogen fixation in crop production systems (41).

Nitrogen biologically fixed by some microorganisms in symbiosis with leguminous plants guarantees a direct source of this element to be utilized by the plant, and in this way, the element becomes less susceptible to natural processes such as volatilization, denitrification and leaching. The process of biological nitrogen fixation contributes to plant nutrition and development, mainly in soils deficient in this element (42).

Biological nitrogen fixation (BNF) has been widely used instead of nitrogen fertilizers in legume production, because of its economic efficiency in sustainable agroecosystems (43). The use of native rhizobial strains as biofertilizers contributes to the maintenance of soil biodiversity by decreasing the negative effects of mineral fertilizers (44).

Thus, the positive increase in agricultural yield with the inoculation of rhizobial species suggests that they can be used as agricultural yield stimulants in sustainable agriculture (45). Likewise, it has been demonstrated that the weight of 100 seeds of plants that were inoculated with rhizobial isolates were similar to the values of plants in which nitrogen fertilizer was applied (46).

In summary, an adequate inoculation in leguminous plants, with effective strains, increases symbiotic fixation and consequently increases the dry mass of the plants and increases the supply of nutrients (47).

Nodule formation in leguminous plants

The formation of nodules in roots of leguminous plants is one of the stages of fundamental importance in the process of biological nitrogen fixation due to the perfect relationship of symbiosis that exists between the plant (legume) and the bacteria. It consists in that the microorganism delivers nitrogen to the plant in an assimilable form for the plant, and in turn, the plant supplies the nutritious substances that the *Rhizobium* needs to fulfill its vital functions (48).

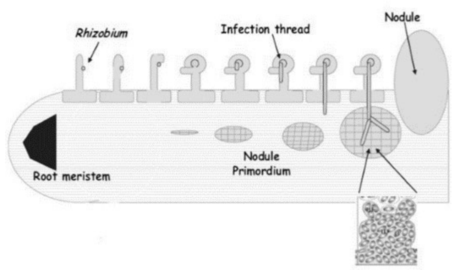

Figure 1. Schematic diagram of nodule development

The formation of root nodules in leguminous plants is a complex process that results in GNF and requires harmonious interaction between the host plant and bacteria of the genus Rhizobium in the rhizosphere (Figure 1). Many bacterial and plant species are extremely specific; however, some *Rhizobium* species interact with a large number of legumes (49). Nodulation takes place only when suitable legume and rhizobial species establish contact (50). The excretion of specific compounds and the induction of nod gene activity in the microsymbiont is the main role of the host plant in the process.

After receipt of flavonoid signals from the root exudate, the bacteria adhere to the surface of root hairs. The production of the *Nod* factor by the bacteria initiates the sequence of events (left to right), which at the morphological level includes the thickening, deformation and curling of the root hair. The formation of the infection thread arising from the encapsulated bacterial cells, the growth of the infection thread towards the nodule primordium with the release of the bacteria through infection droplets (51).

The exchange of signals between the two symbionts is the first step in nodule formation (52,53). The plant root releases exudates (flavonoids, organic acids, and amino acids) that attract the bacteria to a particular site on the plant root and activate the secretion of the *nod* factor by the bacteria (54). After recognition of the nod factor by the plant, the rhizobia attach to the root hairs and promote their deformation and cortical division in the root cortex (55).

The bacteria are then trapped in the coiled root hairs and penetrate the plant via the infection thread. Once inside, the bacteria multiply rapidly in plant cells and they are transformed into bacteroids, surrounded by the plant membranes to form symbiosomes (56). In this structure, the bacteria initiate the conversion of N_2 to NH_3, which is the result of the nitrogen fixation process.

The internal color of the nodules varies from red to white and is a characteristic used to measure the effectiveness of fixation. When the symbiosis is working effectively, they have a pink to red coloration due to the presence of the pigment leghemoglobin, whose function is to regulate O_2

levels and supply O_2 to the bacteria. Light-colored nodules generally indicate little or no N_2 fixation efficiency (48).

Nodule fixation activity is affected by soil moisture conditions (57). A thin layer of water around the nodule decreases fixation, because there is a reduced O_2 supply, as well as a slight decrease in nodule water content leads to a strong inhibition of respiration and fixation.

Arbuscular Mycorrhizal Fungi

Mycorrhizal symbiosis is a mutually beneficial association established between plants and certain soil fungi. It is the oldest association on the planet and is thought to have been present 400 million years ago (58).

Three types of mycorrhizal associations have been defined, taking into consideration their morphoanatomical and ultrastructural characteristics: Ectomycorrhizae, Ectendomycorrhizae and Endomycorrhizae. The endomycorrhizae are not detected with the naked eye, they form an external network of hyphae and penetrate the interior of the cortical cells without colonizing the endoderm. It is the most widespread group on the planet and is divided into several subtypes, of which the most representative is the arbuscular, which is the most important in tropical ecosystems.

AMF tolerate a wide pH range from 5 to 8 (59). Despite this, some species are not adapted to pH conditions different from the soil from which they were isolated (60), although in general, AMF have the widest geographic distribution of all biofertilizing microorganisms.

Numerous biotic and abiotic factors influence the establishment and functioning of the mycorrhizal symbiosis, including the interaction of AMF with microbial populations in the rhizosphere, which, in a reciprocal manner, also influences the soil microbiota (61). In general, competition for nutrients generates microbial interactions in the rhizosphere zone in accordance with plant metabolism, due to the release of secretions, gases, and mucilage (62). The association of mycorrhizal fungi and rhizospheric nitrogen-fixing bacteria benefits plants agronomically by increasing growth, production and tolerance to biotic and abiotic stresses. Some bacterial groups and AMF can interact synergistically to mobilize PO_4^{3-} from the soil to plant roots through solubilization or mineralization. Occasionally, specific rhizobacteria affect the pre-symbiotic state of mycorrhizal development (32).

It has been observed that with very high nutrient availability, the least effects of inoculation with efficient strains are obtained and the greatest effectiveness is achieved with medium availability. If availability is low or null, the symbiosis does not function adequately and plants with lower growth and low effectiveness of inoculation are obtained (9,63).

Mycorrhizal dependence is an intrinsic property of plants, according to which they can be grouped into: obligate mycorrhizal plants, which show very reduced growth in the absence of AMF symbiosis and colonization rates are higher than 60 %. Facultative mycorrhizal plants have a more profuse and developed root system, although under adverse edaphic conditions they respond to mycorrhization and colonization rates are lower than 50 %. Non-mycorrhizal plants are those that do not form an association (64).

Characteristics and benefits of Arbuscular Mycorrhizal Fungi

Mycorrhizal effectiveness is the ability of an endophyte to positively influence plant growth, increase propagule number, and enhance nutrient transfer as a result of physiological interaction between symbionts (64).

Arbuscular mycorrhizal fungi (AMF) form a mutualistic root symbiosis with approximately 80 % of vascular plants (65). AMF provide the plant with mineral nutrients and water, due to the exploration capacity of the fungal hyphae, which allows access to resources distant from the root system. The AMF extraradical hyphae contribute up to 80 % of P, 10 % of K, 25 % of Zn, 60 % of Cu and 25 % of N to the plant (66).

In this symbiotic association, both symbionts benefit each other. AMF receive carbon sources from the plant, while the plant increases soil exploration capacity, mineral nutrient uptake, and growth and development (67).

With the application of AMF in annual crops, increases in crop growth and yields have been observed, as well as improvements in the physical state of the soil, through the production of glomalin, which is a protein that acts as an adherent and binder of soil particles to form more stable aggregates. On the other hand, by increasing the capacity to absorb water and nutrients, AMF allows the host to better resist adverse soil and climatic conditions (68).

The results obtained with the EcoMic® biofertilizer produced from AMF developed by the National Institute of Agricultural Sciences (INCA) and its technological management, through seed coating, have been evaluated as successful in high and low input agricultural systems. All this is in different crops such as coffee (*Coffea arabica* L.), soybean (*Glycine max* L.), rice (*Oryza sativa* L.), corn (*Zea maíz* L.), and beans, among others of economic importance (69).

Beans inoculated with EcoMic®, in addition to increasing crop yield, can efficiently mycorrhizate succession crops, if no more than 40 days elapse between bean harvest and the sowing or planting of the successor crop (70). This permanence effect is very convenient for succession crops such as sweet potato (*Ipomoea batatas* L.) and cassava (*Manihot esculenta* Crantz), which require high amounts of EcoMic® for direct inoculation (58).

The application of mycorrhizal inoculants, more than an alternative, is a model for agriculture; true value lies in achieving an effective symbiosis through the AMF role in the enhancement of plant nutrition, so that the main function of the symbiosis is to increase the possibilities of absorption of the root system (71).

Role of Arbuscular Mycorrhizal Fungi in Nutrition

Many studies have been conducted on the role of arbuscular mycorrhizal fungi in the nutrition of different crops of economic interest, mainly due to their influence on plant growth and development and the increase in agricultural yields. This effect is closely linked to the greater volume of soil explored by roots of mycorrhizal plants, which facilitates access to nutrients from less accessible mineral and organic sources in the soil (72).

In relation to nitrogen nutrition, the absorption of nitrites (NO_2^-) and nitrates (NO_3^-) from the soil through the extraradical mycelium of AMF is known (73) and there are criteria on the possible absorption of significant amounts of organic N (74,75). This seems to be related to the presence of a high affinity NH_4^+ transporter expressed in the extraradical hyphae of *Glomus intraradices* (76).

The transfer of nutrients such as K, Ca, Mg, and S, and micronutrients such as Zn, B, Cu, and Mo through this mechanism has also been demonstrated (77). Although studies on the physiological and biochemical mechanisms that regulate the absorption processes of these elements do not abound, it has been suggested that the influence of AMF on their absorption and transfer is directly related to the increase in soil volume that fungal structures can explore (64).

The absorption of P is considered the most important benefit due to the increase of this element in the plant tissues, being one of the most studied, since, of the amounts of this element present in the soil, between 90 and 95 % are not available for the plants (78). Fundamentally, in tropical zones, this element is found in low concentrations and its low mobility is its distinctive characteristic. It is precisely under these conditions that the beneficial effect of symbiosis becomes evident, being decisive in the survival of various plant species, especially those unable to absorb less mobile forms of this element (79).

The presence of high affinity $H_2PO_4^-$ transporters determines that the absorption and transfer of P to the plant by means of the fungal hyphae is a fast and efficient process (80,81). The absorbed $H_2PO_4^-$ is rapidly transformed into polyphosphate in the extraradical mycelium (82). In conjunction with these processes, organic acids and phosphatases produced by the extraradical hyphae enable the solubilization of phosphorus fixed in the soil (83,84). When AMF interact with other microorganisms, changes occur that also influence plant nutrition. These include the supply of energy through carbon compounds from the host plant, changes in the pH of the mycorrhizosphere induced by the fungus, and the exudation of substances that stimulate or inhibit the activity of the other microorganisms (79).

In general, it has been demonstrated that high yields can be obtained using AMF, although it is necessary to apply low doses of nutrients in order to, together with the inoculation of efficient AMF strains, guarantee high yields with lower fertilization costs (69,85,86).

Coinoculation of rhizobia and arbuscualar mycorrhizal mycorrhizal fungi

The use of combined inoculants (mixture of microorganisms) in agriculture has been called "co-inoculation", which frequently increases plant growth and yield more intensely than if, the microorganisms were used separately. These combinations of microorganisms can offer plants a more balanced mineral nutrition by improving the uptake of nitrogen, phosphorus and other mineral nutrients (87). Multiple studies have shown that when co-inoculation is carried out correctly, a yield increase of between 5 and 10% can be expected (88).

In this regard, the benefits of co-inoculation of arbuscular mycorrhizal fungi and rhizobia have been demonstrated. For the most part, rhizosphere bacteria and fungi are highly dependent on associations with plants and are clearly regulated by root exudates (89).

In Brazil, several studies of *Rhizobium*-AMF-legumes co-inoculation have confirmed that nodulation and plant growth are increased and that dry mass and N and P contents are higher in mycorrhizal plants (90).

Co-inoculation enhances total soluble protein content in plants, where there is an additive effect of the fungal action, due to the time, it takes the fungus to colonize the root and produce enough external mycelium for the plant to receive the benefit (91). A higher foliar content of total soluble proteins in co-inoculated plants allows a higher metabolic activity, an effect associated to a better nutritional status and a higher foliar N content. As a result, plants with greater vigor are obtained (92).

In *Rhizobium*-AMF-legumes co-inoculation, it has been reported that symbiotic relationships provide greater exchange between symbionts and superior effects to plants than relationships based on non-symbiotic associations. In this case, the Rhizobium-legume symbiosis provides N2 and the mycorrhizae increase the uptake of other elements, including P, which is very important to ensure adequate FBN and plant growth (9). The double inoculation *Rhizobium*-AMF produces greater growth, number, dry weight in the nodules and higher contents of P and N in the plant (93).

In Cuba and the world, research carried out on other leguminous crops has also demonstrated a harmonic and ecologically compatible relationship when co-inoculating several bioproducts, for example, the bean (*Phaseolus vulgaris* L. var. Verlili), responds positively to the application alone or combined with EcoMic® and other biostimulants (94).

On the other hand, in Cuba, the combined inoculation of *Bradyrhizobium elkanii* and a strain of arbuscular mycorrhizal fungi, as well as the seed or foliar application of the plant growth biostimulator Biobras-16, increased the yield of the soybean cultivar INCAsoy-24 (95). In addition, another positive result was obtained with the co-inoculation of rhizobial isolates and AMF in the establishment of *Stylosanthes guianensis* in association with *Brachiaria decumbens* (96).

Table 2. Response of the joint application of EcoMic® and other biofertilizers during six validation campaigns in several provinces of Cuba (2010-2018)

Period	Province	Number of control fields	Control (t ha^{-1})	Ecomic® (t ha^{-1})	EcoMic®+AzoFert®	EcoMic®+AzoFert® + QuitoMax®
2010-2011	Villa Clara	9	0.87	1.25		
	S. Spíritus	3	0.73	0.98	1.15	
	C. de Ávila	1	1.3	1.6		
2011-2012	Villa Clara	4	1.02	1.23		
	Matanzas	2	1.3	1.8		
	P. del Rio	2	0.73	0.99		
	S. Spíritus	9	0.87	1.14	1.49	
	C. de Ávila	3	0.64	0.81	0.86	
	Villa Clara	1	0.9	1.02	1.5	
2012-2013	C. de Ávila	2	0.75	1.05		
	Mayabeque	9	1.10		1.52	
	S. Spíritus	4	0.93	1.15	1.28	1.47
	S. Spíritus	6	0.99	1.27	1.46	1.57
	Mayabeque	1	0.4	0.7	0.9	1.1
2013-2014	S. Spíritus	10	1.11			1.34
	Mayabeque	5	1.00			1.37
	Mayabeque	8	0.69	0.99		1.11
2014-2015	Villa Clara	7	0.88			1.23
	Mayabeque	2	1.04			1.47
	Habana	1	0.72			1.08
	C. de Ávila	4	0.86			1.18
2017-2018	Mayabeque	18	0.87	1.29		1.63

In other studies carried out in Cuba, the response of canavalia (*Canavalia ensiformis* (L.) D.C.) to co-inoculation with *Rhizobium* and arbuscular mycorrhizal fungi in two types of soil was evaluated and it was demonstrated that canavalia responded positively to co-inoculation and the most efficient strains, both of *Rhizobium* and AMF, depended on the type of soil and its properties (93).

On the other hand, under production conditions, during six validation campaigns in several provinces of Cuba. The application of the EcoMic® biofertilizer was evaluated (Table 2). It resulted in an increase in yield between 15 and 40 %, with an average increase of 31 % (0.27 t ha^{-1}). The joint application with AzoFert® and QuitoMax® (biostimulant based on chitosans), reached increases between 45 and 100 %, with average increases of 57 % (0.50 t ha^{-1}) and always in the presence of average applications of mineral fertilizers, to guarantee these increases in yield (58).

The use of biofertilizers does not mean that fertilization can be stopped, but rather that fertilization can be more efficient and the doses to be applied can be totally or partially reduced by increasing the percentage of nutrient absorption by plants (8).

CONCLUSIONS

- Due to the economic and nutritional importance of common bean, there is no doubt about the importance of adequate and balanced nutrition of this crop, through the benefits of rhizobia-AMF co-inoculation. However, it is necessary to continue studies on these issues, to not only define the best combinations of strains of each microorganism involved by type of soil and soil environment and make new isolations of promising strains, but also urgently need to extend the experimental results to the production level.
- Bringing the facilities and advantages of biofertilizer application and environmentally friendly agricultural techniques into the hands of producers is a crucial task from the agronomic, environmental, economic and social points of view.

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