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BIOSTIMULANT EFFECT ON YIELD OF TWO BIOFERTILIZED BEAN (*Phaseolus vulgaris* L.) CULTIVARS

Efecto de bioestimulantes en el rendimiento de dos cultivares de frijol (*Phaseolus vulgaris* L.) Biofertilizados

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ABSTRACT. Beans, inside legumes, stands out for the high human consumption and, in Cuba, this legume is part of the population basic diet. Hence, to look for alternatives that allow stimulating grain production and therefore, increase the satisfaction of consumer demand, constitute a necessity. The aim of this paper was to determine whether seed application of QuitoMax® or Biobras-16® products, increases the plant yield of two biofertilized beans cultivars. The experiment was performed at Unit of Basis Science and Technology "Los Palacios". Seed sprayings with QuitoMax®, Qm (500 mg L^{-1}) or Biobras-16®, BB-16 (0,05 mg L^{-1}), before inoculation with Azofert® biofertilizer, Az, were made. Seeds were placed to Hydromorfic Gley Nodule Ferruginose Petroferric soil and four treatments (100 % N, control, Az+30 % N, Qm+Az+30 % N y BB-16+Az+30 % N) by cultivar were constituted. At the harvest time, the following evaluations: shoot, legume and grain dry weights, legume and grain number per plant, grain number per legume, fresh weight of 1 000 grains and practice yield, were made. Results showed Azofert® inoculation substituted 70 % of nitrogen fertilizer used without affecting crop yield. Besides, seed sprayings with Biobras-16®, before Azofert® inoculation, increased significantly crop yield, in both cultivars, compared to control treatment with 100 % nitrogen fertilizer.

Key words: brassinosteroids, legumes, production, chitosan, Rhizobium

RESUMEN. El frijol, dentro de las leguminosas, se destaca por su alto consumo humano, en Cuba, forma parte de la alimentación básica de la población. De ahí, la necesidad de buscar alternativas que posibiliten estimular la producción de sus granos y por ende, incrementar la satisfacción de la demanda de consumo. El objetivo del presente trabajo fue determinar si la aplicación de los productos QuitoMax® o Biobras-16® a las semillas, estimulaba el rendimiento de plantas de dos cultivares de frijol biofertilizadas. Para ésto se ejecutó un experimento en la Unidad de Ciencia y Tecnología de Base "Los Palacios" donde se realizaron aspersiones a las semillas con QuitoMax®, Qm (500 mg L^{-1}) ó Biobras-16®, BB-16 (0,05 mg L^{-1}), previo a la inoculación con el biofertilizante Azofert®, Az. Las semillas se depositaron en un suelo Hidromórfico Gley Nodular Ferruginoso Petroférrico y se conformaron cuatro tratamientos por cultivar (100 % N, control, Az+30 % N, Qm+Az+30 % N y BB-16+Az+30 % N). En el momento de la cosecha se realizaron las siguientes evaluaciones: masa seca de tallos, vainas y granos, número de vainas y granos por planta, número de granos por vaina, masa fresca de 1000 granos y el rendimiento práctico. Los resultados demostraron que la inoculación con Azofert® fue capaz de sustituir el 70 % de la fertilización nitrogenada que se utiliza en el cultivo, sin afectar el rendimiento agrícola. Además, la aspersión de las semillas con Biobras-16®, previo a la inoculación con Azofert®, estimuló el rendimiento de las plantas, superando significativamente al tratamiento control con el 100 % de la fertilización nitrogenada en ambos cultivares.

Palabras clave: brasinoesteroides, leguminosas, producción, quitosana, Rhizobium

INTRODUCTION

Bean is one of the leguminous species that has a preferential place, due to its nutritional composition as a source rich in proteins, minerals such as calcium, iron, phosphorus, magnesium and zinc and the vitamins thiamine, niacin and folic acid (1) .

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In Cuba, this legume has great importance; however, domestic production accounts for only 3 % of consumer demand, so it is necessary to import about 110 000 tons of grain each year (2). For this reason, one of the priorities of Cuban agriculture, at present, is to increase the production of this crop using technologies that respond to the environment.

The common bean is able to carry out the biological nitrogen fixation process (BNF), through symbiosis with soil bacteria commonly known as rhizobia (3). Nitrogen biologically fixed by these microorganisms in symbiosis guarantees a direct source of this element to be used by the plant (4) and to replace, in part, the importation of mineral fertilizer. Azofert® is an inoculant composed of bacteria of the genus *Rhizobium* and essential metabolites in this interaction (5).

On the other hand, there is a range of biostimulant products (FitoMas-E®, Biobras-16® and QuitoMax®) that have been successfully used in agriculture (6-8) and their application in combination with biofertilizers is a priority strategy in the search to improve and preserve the physical, chemical and biological conditions of soils, increase the potential for agroproduction and substitute imports (9, 10). However, in most cases, biostimulants have been sprayed on the plants and there are hardly any references to the use of these biostimulants prior to inoculation, which may also be a viable alternative to increase production in the case of beans. For this reason, the main objective of this study was to determine whether seed treatment with QuitoMax® or Biobras-16®, prior to inoculation with Azofert®, stimulated the yield of two bean cultivars.

MATERIALS AND METHODS

The experiment was carried out in January 2015, in areas of the Basic Technological Science Unit, Los Palacios, Pinar del Río, belonging to the National Institute of Agricultural Sciences (INCA). For this, two cultivars of beans (Cuba C-25-9-N and CUL 156) were used, from the Grain Research Institute of the Ministry of Agriculture. The seeds were sprayed ($3x10^{-3}$ L per 50 seeds) with Biobras-16®, BB-16 (0,05 mg L^{-1}) or QuitoMax®, Qm (500 mg L^{-1}), dried at room temperature and on the following day, at the time of sowing, they were inoculated with Azofert® at a rate of $200x10^{-3}$ L of inoculum per 46 kg of seed.

The seeds were deposited in a Gley Nodular Ferruginous Petroferric Hydromorphic soil (11), using direct manual seeding technology at a distance of 0,70 m between rows and 0,07 m between plants with a standard of 50 kg ha^{-1} of seeds.

The phytotechnical activities were carried out as recommended by the Technical Guide for Bean Cultivation (12), except fertilization, which was carried out by the application, at the time of planting, of triple superphosphate (46 % P_2O_5) and chloride of potassium (60 % K_2O), at 75 and 65 kg ha^{-1}, respectively. At 15 days after the germination of the crop, nitrogen fertilization with urea (46 % of N) was carried out, at a rate of 70 kg ha^{-1}, in the case of treatments where 100 % were applied (controls); while in the rest only 30 % of the dose was applied.

The treatments studied in each of the cultivars were: 100 % N (Control), Azofert®+30 % N, Qm+Azofert®+30 % N and BB-16+Azofert®+30 % N, which makes a total of eight treatments and a random block design with four replicates was used. The experimental plots consisted of eight rows of 5 m each.

At the time of harvesting, ten plants were selected at random from the two central rows of each experimental plot to evaluate: dry mass of stems, pods and grains; the number of pods and grains per plant; the number of grains per pod and the fresh mass of 1 000 grains. For the agricultural yield, 8 m^2 of the center were harvested in each experimental plot, the plants were threshed and the grains were dried to 14 % moisture.

A double-rank variance analysis was performed for each of the variables studied and the means of the treatments were compared according to Duncan's Multiple Range Test at $p \leq 0,05$ (13). The Statistical Program used was Statgraphics plus 5.1 (14).

RESULTADS AND DISCUSSION

The dry mass of the stems, pods and grains of the plants that received the bacterial inoculation with the addition of 30 % of the nitrogen fertilization did not differ significantly from those that received 100 % of the fertilization in neither of the two cultivars studied (Table I). This means that the inoculation may be exerting an effect similar to 70 % of the remaining N.

However, seed spraying with Biobras-16® or QuitoMax® influenced differently in each organ and for each cultivar. For example, they had no influence on the dry mass of the stems in any of the two cultivars studied; while seed spraying with QuitoMax® and Biobras-16® significantly increased the dry mass of the pods of the cultivars Cuba C-25-9-N and CUL 156, respectively, reaching values higher than those obtained with fertilization mineral.

Table I. Effect of seed spraying with QuitoMax® and Biobras-16® on the dry mass of stems, pods and plant grains of Cuba C-25-9-N and CUL 156 bean cultivars, biofertilized with Azofert® and with a low dose of nitrogen fertilization

Treatments	Dry mass (g plant^{-1})		
	Stems	Pods	Grains
Cuba C-25-9-N			
100 % N	3,67 ab	4,40 bc	10,05 b
Az + 30 % N	2,67 b	4,49 bc	9,79 bc
Qm + Az + 30 % N	3,56 ab	5,54 a	10,93 b
BB-16 + Az + 30 % N	4,42 a	5,09 ab	13,14 a
CUL 156			
100 % N	3,06 b	3,75 cd	9,99 bc
Az + 30 % N	2,63 b	3,65 cd	8,84 cd
Qm + Az + 30 % N	2,62 b	3,32 d	8,55 d
BB-16 + Az + 30 % N	3,36 b	4,95 ab	12,90 a
E.S.x	0,21*	0,19*	0,23*

Equal letters do not differ statistically according to the Duncan Multiple Rank Test for p≤0,05
Az-Azofert®, BB-16-Biobras-16®, Qm- QuitoMax®

In the case of the dry mass of the grains, the treatment with Biobras-16® was the best in both cultivars, surpassing significantly the rest of the treatments studied.

The number of pods per plant (Table II) showed a different behavior in each of the cultivars under study, since while the substitution of 70 % of nitrogen fertilization by bacterial inoculation did not affect this indicator in the case of cv. Cuba C-25-9-N, did cause a significant decrease in cv. CUL 156. However, bacterial inoculation significantly increased the number of grains per pod of the plants of both cultivars, so that the number of grains per plant was not modified, especially in cultivar CUL 156.

As for the effect of biostimulants, it should be noted that in the cultivar Cuba C-25-9-N, the treatment with Biobras-16® only increased significantly the number of grains per plant; while in the cv. CUL 156; in addition, this treatment increased the number of pods per plant. On the other hand, seed spraying with QuitoMax® did not influence these indicators in any of the two evaluated cultivars.

The treatments did not modify the mass of 1000 grains, indicating that the influence on crop yield should be associated to the effect that the treatments had on the number of grains per plant in each of the cultivars studied.

As for the practical yield of the crop, which is presented in the Figure, it is highlighted that the substitution of 70 % of the nitrogen fertilization by the bacterial inoculation did not cause a significant decrease of the yield in any of the two cultivars under study. Moreover, it should be noted that only spraying seeds with Biobras-16®, prior to inoculation with Azofert®, stimulated plant yield of both cultivars, so that significantly exceeded (29-30 %) to that obtained in the control treatment plants (100 % N).

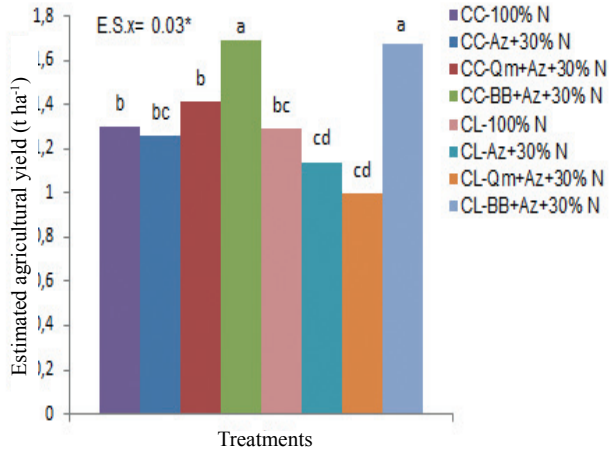
It should be specified, that in the case of cv. Cuba C-25-9-N, this increase in yield was associated with a significant increase in the number of grains per plant; while in CUL 156 it was associated with an increase in the number of pods and grains per plant.

Seed spraying with Biobras-16®, prior to inoculation with Azofert®, apparently triggered a series of physiological and biochemical events in the plants that had an effect on increased grain yield of both cultivars, regardless of the differences in the duration of the crop cycle.

Table II. Influence of spray on seeds with QuitoMax® and Biobras-16® in some components of the yield of Cuba C-25-9-N and CUL 156 bean cultivars, biofertilized with Azofert® and with a low fertilization dose Nitrogen

Treatments	Nu. pods plant^{-1}	Nu. grain plant^{-1}	Nu. grains pod^{-1}	Mass of 1 000 grains
Cuba C-25-9-N				
100 % N	17,4 b	60,1 de	3,52 c	168,04
Az + 30 % N	16,4 b	63,7 cd	4,36 a	153,39
Qm + Az + 30 % N	14,9 bc	68,0 bc	4,58 a	168,73
BB-16 + Az + 30 % N	17,3 b	71,5 b	4,18ab	185,22
CUL 156				
100 % N	16,9 b	56,2 ef	3,46 c	176,63
Az + 30 % N	12,8 cd	53,4 fg	4,22 ab	169,51
Qm + Az + 30 % N	10,8 d	50,1 g	4,63 a	173,29
BB-16 + Az + 30 % N	21,0 a	78,9 a	3,78 bc	175,58
E.S.x	0,58*	0,91*	0,18*	7,33 N.S.

Equal letters do not differ statistically according to the Duncan Multiple Rank Test for p≤0,05
Az- Azofert® BB-16-Biobras-16® Qm- QuitoMax®



Equal letters do not differ statistically according to the Duncan Multiple Rank Test for p≤0,05
Az- Azofert® BB-16-Biobras-16® Qm- QuitoMax®

Figure. Effect of seed spraying with QuitoMax® and Biobras-16® on the practical yield (based on 14 % moisture) of plants of the bean, Cuba-C-25-9-N (CC) and CUL 156 (CL) cultivars, biofertilized with Azofert® and with the addition of 30 % of nitrogen fertilization

The biological fertilization effectiveness in this crop, in our country, has been studied previously. Thus, in a study of three genotypes of common bean (BAT-304, Velasco Largo and BAT-93), the effect of mineral fertilization and biofertilization on plant yield was studied. It was found that the biological fertilization exceeded the mineral in 10,4 and 6,0 %, in the BAT-304 and Velasco genotypes, respectively. However, in the BAT-93 genotype, mineral fertilization increased yield by 18,45 %, relative to biofertilization (15). This means that the response depends not only on the microorganism, but also on the cultivar and its ability to associate in symbiosis.

In San Luis de Potosí, Mexico (16), it was demonstrated that biofertilization with *Rhizobium* or arbuscular mycorrhizal fungi, as well as the application of brassinosteroids, yielded bean crop yields similar to those obtained with the mineral fertilization recommended for cultivation. Conditions where the study was conducted.

Compared with other legumes, common beans are the legumes with the lowest capacity to fix nitrogen, although there are some varieties of *Phaseolus vulgaris* L. and strains of rhizobia that exhibit high fixation ranges (17). New research attempts to increase this capacity (18). These new intentions to improve the biological nitrogen fixation in beans include from the use of more competitive and efficient strains (19), to the development of more complex inoculants and formulations. In this work, Azofert®, a biofertilizer characterized by its quality, was used to induce in the bacteria the synthesis and excretion of metabolites of special interest and function in this interaction (5). In spite of the above, the use of biofertilizers in legumes is a promising alternative to mineral fertilizers. In this way, we have been working not only in the inoculation of rhizobia but also in the joint inoculations of rhizobia with other microorganisms such as mycorrhizal fungi, achieving stimulation of growth and increases in yield of crops (20-23). In this work it was demonstrated that the inoculation with Azofert® succeeded in replacing 70 % of the nitrogen fertilization; a result of great interest for the production of this crop in our country.

In soybeans, it has been demonstrated that it is possible to use simple inoculations with *Bradyrhizobium elkanii* and co-inoculation with arbuscular mycorrhizal fungi to meet nutrient needs, mainly nitrogen and phosphorus, allowing similar yields to be obtained when used alone The mineral fertilizer (24).

As for the increase in the yield obtained with the spraying of Biobras-16® to the seeds prior to inoculation with the biofertilizer, it can be stated that, several investigations have demonstrated the effect that the application of the analogs of brassinosteroids has on the yield of plants that have been biofertilized. Thus, in soybean (25), the effectiveness of foliar spraying of Biobras-6® in the flowering phase was demonstrated in plants whose seeds were inoculated with *Bradyrhizobium elkanii* and arbuscular mycorrhizal fungi (AMF). On the other hand, it has been reported that the co-inoculation of bean seeds with Azofert® and EcoMic® (AMF) and foliar spraying of plants with biostimulants such as FitoMas®, Biobras-16® or QuitoMax® significantly stimulates crop yield[A]

In vegetables such as lettuce, green beans and tomato, increases in yield have also been reported with AMF inoculation in combination with Biobras-16® foliar spraying (26).

Although these results have a preliminary character, a novel aspect of this work lies in the application of Biobras-16®, since in this case

[A] Rivera, R.; Calderón, A.; Nápoles, M. C.; Falcón, A.; Martin, J. V.; Marrero, Y.; Lara, D.; Calaña, J. M.; Mederos, J. D.; Coll, Y. y Núñez, M. *La factibilidad de la aplicación conjunta de biofertilizantes y bioestimiulantes en el cultivo del frijol*. Impacto ambiental de bioproductos en la agricultura, Inst. Instituto Nacional de Ciencias Agrícolas - Ministerio de Educación Superior, 2015, Cuba, 62 p., Premio MES al Resultado ya aplicado de Mayor Contribución a la protección del Medio Ambiente en Cuba.

the product was sprinkled to the seeds prior to inoculation with Azofert® and was not sprinkled leaf, as has generally been applied. It should be noted that a preliminary study carried out in a production area in Villa Clara province showed that seed spraying, prior to planting, significantly stimulated the average grain mass per plant of the cultivar "Tomeguín 93" (27).

This demonstrates that as a strategy to increase bean production, so important in the consumption of the diet of Cubans and other countries, the use of biofertilizers and biostimulants could be a rational and sustainable alternative, demonstrating that in this experiment with a 70 % less nitrogen fertilizer higher yields can be achieved with the same quality as when 100 % of the mineral fertilizer recommended for this crop is used. In addition, it should be noted that the Biobras-16® not only increases the production of the crop but also makes it environmentally friendly, so this result should be confirmed later.

As for QuitoMax®, it is necessary to continue to deepen this application method, since it is possible that the dose used is not suitable for this purpose or, considering the antimicrobial properties of chitosan, it is not convenient to apply it before inoculation with Azofert®.

CONCLUSION

Seed inoculation with Azofert® replaced 70 % of the nitrogen fertilization to be applied in the bean crop, without affecting yields, and seed spray with Biobras-16® (0,05 mg L^{-1}). Prior to inoculation, significantly stimulated the yield of Cuba C-25-9-N and CUL 156 bean cultivars compared to control plants receiving 100 % nitrogen fertilizer.

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