Cultivos Tropicales, 2018, vol. 39, no. 1, pp. 108-112 January-March

ISSN impreso: 0258-5936
ISSN digital: 1819-4087

Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas
http://ediciones.inca.edu.cu

INFLUENCE OF BIOBRAS-16® AND QUITOMAX® ON BEAN PLANT BIOLOGICAL ASPECTS

Influencia del Biobras-16® y el Quitomax® en aspectos de la biología de plantas de frijol

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ABSTRACT. Common bean is the most important legume in human consumption worldwide and in Cuba, it is necessary to increase crop production, since bean and rice are the nutritional basis of the people. Biobras-16® and QuitoMax® are bioactive products which are able not only enhancing plant growth and increasing crop yield but also they have anti-stress effects. This paper aims to determine the effect of the seed spray with QuitoMax® and Biobras-16® on bean plant growth and some biochemical indicators. Bean seeds of cv. Cuba C-25-9-N were sprayed with Biobras-16® 0,05 mg L^{-1} and QuitoMax® 500 mg L^{-1}, one day before sowing. At the time of sowing, each seed was inoculated with 1 x 10^{-3} L of Azofert® and they were placed in pots containing eutric agrogenic Lixiviated Red Ferralitic soil with a low dose of mineral fertilizer (2,78 g of complete formula, NPK, 9-13-17). At 42 days after sowing, some growth indicators and chlorophyll, total soluble carbohydrate and protein concentrations were determined in the leaves. Results demonstrated that Biobras-16® significantly stimulated aerial part growth and leaf total soluble carbohydrates while QuitoMax® favoured only the last indicator.

Key words: biochemistry, brassinosteroids, growth, *Phaseolus vulgaris*, chitosane

RESUMEN. El frijol común es la leguminosa de mayor importancia para el consumo humano a nivel mundial y en Cuba, se necesita incrementar la producción de este cultivo, ya que junto al arroz es la base alimenticia de la población. El Biobras-16® y el QuitoMax® son productos bioactivos capaces de estimular el crecimiento de las plantas e incrementar los rendimientos, además de que poseen efectos antiestrés. El objetivo de este trabajo fue determinar el efecto que la aspersión a las semillas con QuitoMax® y Biobras-16® ejercen en el crecimiento y algunos indicadores bioquímicos de plantas de frijol. Semillas de frijol cv. Cuba C-25-9-N se asperjaron con Biobras-16® 0,05 mg L^{-1} y QuitoMax® 500 mg L^{-1}, el día anterior a la siembra. En el momento de la siembra, se inoculó cada semilla con 1 x 10^{-3} L de Azofert® y las mismas se colocaron en macetas que contenían suelo Ferralítico Rojo Lixiviado agrogénico éutrico con una dosis baja de fertilizante mineral (2,78 g de fórmula completa, NPK, 9-13-17). A los 42 días después de la siembra, se evaluaron los indicadores del crecimiento y se determinaron en las hojas las concentraciones de clorofilas, carbohidratos y proteínas solubles totales. Los resultados demostraron que, mientras que el Biobras-16® estimuló significativamente el crecimiento de la parte aérea y la concentración de carbohidratos solubles de las hojas, el QuitoMax® solamente favoreció este último indicador.

Palabras clave: bioquímica, brasinoesteroides, crecimiento, *Phaseolus vulgaris*, quitosano

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is among the legumes that have seeds, one of the most important. Considered the most important legume in human consumption worldwide, it is grown mainly in developing countries (1).

The low production of the crop in many cases is due to the limited availability of quality seeds, the damage caused by the incidence of pests and diseases, high prices on inputs, incidences of climate and low water availability, among others. Currently, increasing the productivity of plants grown in a sustainable manner, with low quantity of inputs with the best quality standards, is an immediate need (2).

In agriculture there is a range of biostimulant products with the capacity to promote growth and increase crop yield, in addition to having anti-stress effects (3-5).

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Particularly in Cuba, some biostimulants have been used that are innocuous for the environment, among which are Quitomax® and Biobras-16®, liquid formulations containing as active ingredients polymers of chitosan and spironogenic analogues of brassinosteroids, respectively. Both formulations have proven to be effective, applied as foliar sprays, in stimulating the growth and yield of different crops (6,7); however, there is very little information available about the effect of these formulations on the growth of bean plants when they are sprinkled on the seeds prior to sowing. It is expected, then, that the use of these products, sprinkled on the seeds prior to the inoculation with Azofert®, could be a way to promote the growth of the bean plants.

Due to the above, the main objective of this work was to determine the effect exerted by spraying the seeds with Biobras-16® or Quitomax®, prior to inoculation with Azofert®, on the growth of bean plants.

MATERIALS AND METHODS

The experiment was developed in the central area of the National Institute of Agricultural Sciences. For this, bean seeds were sprayed cv. Cuba C-25-9-N, with Biobras-16® 0,05 mg L^{-1} or QuitoMax® 500 mg L^{-1}, concentrations associated with the characteristics of each of the compounds and which were the most effective in an experiment previous conducted under controlled conditions (8). At the end of the spraying, the seeds were kept in the dark, at an ambient temperature of 26 ± 1 °C and a relative humidity of 70 % for 24 hours. After that time, all the seeds, treated or not, were inoculated with 1 x 10-3 L of Azofert®, at a concentration of 5,4 x 108 CFU mL^{-1}. Subsequently, the seeds inoculated were planted in plastic pots of 10 L capacity (five seeds per pot and 10 pots per treatment), which contained Ferralitic Red soil leached agrogenic eutric (9). To each pot, at the time of sowing, 2,78 g of the complete formula (NPK, 9-13-17) was added, which represents 25 % of the dose of 400 kg ha^{-1} (10). The treatments were then shaped as follows:

T1- Control
T2- Quitomax® 500 mg L^{-1}
T3- Biobras-16® 0,05 mg L^{-1}

The plants grew in open conditions, where the average temperature in the experimental period was 21,8 ºC and the relative humidity of 79,7 %, according to the data provided by the Meteorological Station of Tapaste, Mayabeque, located approximately 500 m of the area where the experiment was executed. The plants were irrigated daily.

At 42 days after sowing, the following evaluations were made to ten plants per treatment: length of the roots and stem (evaluated with a graduated ruler and expressed in cm), dry masses of roots, leaves and stems (dried materials in an oven at 70 ºC to constant weight which was determined on a Sartorius analytical balance with 0,0001 g of precision and expressed in mg $plant^{-1}$) and total leaf area per plant (cm^2) evaluated by a leaf surface Integrator model MS 300 The beam of the third trifoliate leaf of each plant was also selected in the morning hours to estimate the total chlorophylls by using the SPAD-502 equipment.

Three samples of leaves of 0,25 g each were also taken, by treatment and the concentrations of total soluble carbohydrates (mg g^{-1} fresh mass) and total soluble proteins (µg g^{-1} fresh mass) were determined by the anthrone (11) and microLowry (12) techniques, respectively. Two readings (n = 6) were made on each sample in a Genesys-6 UV-visible spectrophotometer.

The data were processed by simple classification variance analysis according to the STATGRAPHICS Plus 5.1 program. The Duncan multiple range comparison test was used for $p \leq 0.05$, with the objective of discriminating differences between the means.

RESULTS AND DISCUSSION

Table 1 shows the results of stem and root length behavior, the dry mass of stems, leaves and roots; as well as the foliar area of the bean seedlings cv. Cuba C- 25-9-N.

As can be seen in Table 1, the biostimulants studied did not significantly influence the radical growth of bean plants cv. Cuba C-25-9-N, 42 days after sowing.

There are authors who have demonstrated the positive effect of brassinosteroids on the growth of the roots of some plant species (13), but it has also been reported that these compounds can inhibit the growth of this organ in wheat, mung bean and corn postures (14). This apparent contradiction may be associated with the way in which the product is applied, since it is suggested that inhibition of root growth may occur when brassinosteroids are applied directly and continuously to the roots (15). In this work, the Biobras-16® was sprinkled on the seeds before planting.

Table 1. Effect of sprinkling seeds with QuitoMax® or Biobras-16® on some growth indicators in young bean plants cv. Cuba C-25-9N biofertilized with Azofert® and with a low dose of mineral fertilizer, 42 days after sowing

Treatment	Length (cm)		Dry mass (g planta^{-1})			Plant leaf area (cm^2)
	Stems	Roots	Stems	Leaves	Roots	
Control	28,4	36,2	1,91 b	2,60 b	1,76	1 013,2 b
Quitomax® 500 mg L^{-1}	28,5	44,4	2,59 ab	2,71 b	2,08	1 367,8 ab
Biobras-16® 0,05 mg L^{-1}	27,4	43,0	2,81 a	4,47 a	2,17	1 633,8 a
E,S,x	1,37 NS	4,53 NS	0,24*	0,19*	0,21 NS	112,2*

NS, * mean no significant difference and difference at p≤0.05 according to analysis of variance, respectively. Equal letters mean means that do not differ significantly according to Duncan's multiple range test at p≤0.05

On the other hand, it has been found that the coating of wheat seeds with chitosan stimulated germination and some indicators of growth such as fresh mass and length of roots (16); however, in this work the concentration used did not stimulate the growth of the roots.

From this it is inferred that there is no consistent response to the radical growth to the application of Biobras-16® and QuitoMax®, so it is necessary to continue deepening the effects that these products exert on the growth of this organ of plants of bean.

However, sprinkling the seeds with Biobras-16® significantly stimulated the dry mass of the aerial part of the plants (Table 1). Studies carried out by other authors (17) have shown that the use of 24-epibrasinolide in the cultivation of pigeonpea beans (*Cajanus Cajan* (L) Mill), increased the fresh and dry mass of the plant, the foliar area, the content of water from leaves and roots, as well as photosynthetic pigments, sugar concentration, the rate of photosynthesis and the efficiency of water use.

By foliar application of 10 -6 M homobrasinolide to plants of the *Satureja khuzestanica Jamzad* species, foliar biomass and photosynthesis were favored. Growth promotion was also associated with higher chlorophyll content and increased carbohydrate accumulation (18).

One of the first bioassays used to determine the biological activity of brassinosteroids was the bioassay of the second bean internode (19), in which with the application of these compounds not only elongation was demonstrated, but also curvature, thickening and splitting of the internode . Hence, the stimulation in the dry mass of the stems that was observed with the application of Biobras-16® can be explained.

As for the foliar area of the plants, it can be seen that the sprinkling of the seeds with Biobras-16® also increased this variable. These results confirm those obtained previously (20), where it was demonstrated that the sprinkling of bean seeds cv. Bronco with epibrasinolide (EBL) (5 x 10-6 M), significantly increased the leaf area per plant, as well as the dry mass of the leaves.

Regarding QuitoMax®, no statistically significant stimulation of this product was found in the growth of the aerial part of the plants at 42 days after sowing; which may be associated with the concentration and form of application used in this experiment.

As can be seen in Table II, the total chlorophylls in the leaves estimated with Spad-502, at 42 days after sowing, showed similar values in all the treatments evaluated, which indicates that the biostimulants did not influence the chlorophyll levels presented by the leaves of the bean plants of this cultivar.

In the international literature there is evidence of the positive effect of 24-epibrasinolide at 0,5 mg L^{-1} (21) and oligomers of chitosan at 60 mg L^{-1} (22) in the concentration of pigments in the leaves of plants of pepper and coffee, respectively. However, other authors have reported that chitosan oligosaccharides did not modify the chlorophyll a, b, and carotenoid concentrations in the leaves of bean plants (23) and that the foliar spray with 24-epibrasinolide 10^{-7} M and 10^{-12} M it did not modify the concentration of total chlorophylls in the leaves of soybean and corn plants, respectively (24,25).

The concentration of total soluble carbohydrates of the leaves was favored significantly with the spraying of QuitoMax® and Biobras-16® seeds (Table 2). It should be noted that the QuitoMax® even when it did not significantly stimulate the indicators of leaf area growth and dry mass of the leaves, did stimulate the concentration of total soluble carbohydrates in the leaves. For its part, the Biobras-16® significantly stimulated the concentration of total soluble carbohydrates, an effect similar to that found in the foliar area and the dry mass of the leaves.

Table 2. Content of total chlorophylls, carbohydrates and total soluble proteins in the leaves of bean plants, cv Cuba C-25-9-N, whose seeds were sprinkled with QuitoMax® or Biobras-16® prior to sowing

Treatments	Total Chlorophylls (unidades Spad)	Carbohidratos solubles totales (mg g^{-1} MF)	Total Soluble Proteins (µg g^{-1} MF)
Control	37,24	3,01 b	40,36
QuitoMax® 500 mg L^{-1}	35,74	3,92 a	40,45
Biobras-16® 0,05 mg L^{-1}	35,67	4,23 a	41,31
E,S,x	1,47 NS	0,146*	1,05 NS

NS, * Mean no significant difference and difference at p≤0.05 according to analysis of variance, respectively. Equal letters mean means that do not differ significantly according to Duncan's multiple range test at p≤ 0.05

This result of the Biobras-16® is of great importance, since, although this product did not influence the total chlorophyll of the leaves, the plants had a greater leaf area and a higher concentration of total soluble carbohydrates that would imply a higher content of soluble carbohydrates in the leaves. This effect could favor the transport of these towards the formation and growth of the grains, thus affecting the yield of the crop.

The concentration of total soluble proteins showed no significant differences between treatments. The values ranged between 40 and 41 µg g^{-1} fresh mass.

It is known that brassinosteroids stimulate the concentration of carbohydrates and proteins in plants. Studies carried out on the cultivation of vines (*Vitis vinifera* L.) showed that the exogenous application of 24-epibrasinolide considerably increased the content of soluble sugars in the fruits, but decreased in the bark (26). The brassinosteroids are involved in several physiological processes in plants, including the metabolism of carbohydrates.

In this way, it was possible to confirm that the foliar spraying of 24-epibrasinolide or 28-homobrasinolide, at 20, 35 and 50 days after sowing, stimulated the concentration of soluble proteins, reducing sugars and starch in the radish bulbs (27). However, plant treatment of two wheat cultivars with 0,05 mg L^{-1} of EBL has been reported to significantly increase the protein content of the grains in a variety (Sids 1); while in the other (Giza 168) it did not exert any influence. This suggests that different varieties of the same species do not respond in the same way to treatment with brassinosteroids (28).

As for chitosan, dips of tomato seeds have been made in solutions of different concentrations of chitosan (0,1; 1,0; 2,5 and 10,0 g L^{-1}) and it has been found that, 17 days after sowing, all chitosan concentrations evaluated significantly decreased the level of total soluble proteins in the leaves; however, at 24 days, the extreme concentrations (0,1 and 10,0 g L^{-1}) of the product stimulated the concentration of proteins, while the intermediate concentrations (1,0 and 2,5 g L^{-1}) they inhibited it. These authors pointed out that the inhibition of protein concentration induced by chitosan may be due to the induction of the synthesis of compounds related to defensive mechanisms (29).

CONCLUSIONS

Spraying the seeds with Biobras-16® 0,05 mg L^{-1} stimulated the aerial growth (stem length, dry mass of stems and leaves and foliar area) and the concentration of total soluble sugars of the leaves of the plants of bean cv. Cuba C-25-9-N, 42 days after sowing. The positive influence of this biostimulant on the foliar area and the concentration of total soluble carbohydrates in the leaves at this time of growth, could later affect the formation and growth of the grains.

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Received: April 26th, 2017
Accepted: December 5th, 2017