EFFECTS OF BIOBRAS-16 ON RICE (*Oryza sativa* L.) YIELD AND OTHER CHARACTERS

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ABSTRACT. The experimental study was carried out in the areas of “Los Palacios” Rice Research Station, which belongs to the National Institute of Agricultural Sciences (INCA), as well as in “Cubanacán”, “Caribe”, and “Montoto” farms from “Los Palacios” Rice Agroindustrial Complex, with the objective of studying the effects of BIOBRAS-16, a commercially known brassinosteroid analog synthesized in Cuba, on rice yield. For this purpose, five treatments were studied using INCA LP-2 variety, and knapsack sprinklings were performed by hand. Concerning land extensions, J-104 commercial variety was used and the applications were carried out by plane. The results showed that panicle number per square meter was the highest influencing component on yield response. Under experimental conditions, the best BIOBRAS-16 response was provided by the treatment in which the application was performed at the beginning of heading and during the grain-filling period, using a total rate of 20 mg.ha^{-1}. There was a positive response of BIOBRAS-16 application to agricultural extensions, regardless the rates and application times used in each of them.

Key words: rice, brassinosteroids, yield

RESUMEN. El trabajo experimental se desarrolló en áreas de la Estación Experimental del Arroz “Los Palacios” perteneciente al Instituto Nacional de Ciencias Agrícolas y las extensiones agrícolas en las granjas Cubanacán, Caribe y Montoto del Complejo Agroindustrial Arrocero “Los Palacios”, con el objetivo de estudiar la influencia sobre el rendimiento del cultivo del arroz de un análogo de brasinoesteroide sintetizado en Cuba, conocido comercialmente como BIOBRAS–16. En el experimento fueron estudiados cinco tratamientos, se utilizó la variedad INCA LP-2 y las aspersiones se realizaron manualmente con una mochila; en el caso de las extensiones se empleó la variedad comercial J-104 y la aplicación se hizo con avión. En los resultados, el carácter número de panículas por metro cuadrado fue el componente que más influyó en la respuesta que se obtuvo en el rendimiento. En condiciones experimentales, la mejor respuesta del BIOBRAS-16 se obtuvo con el tratamiento donde la aplicación se realizó al inicio de la paniculación y en la etapa del llenado del grano, con dosis total de 20 mg.ha^{-1}, y con el tratamiento donde las aplicaciones se realizaron tanto en la etapa de ahijamiento activo como al inicio de paniculación, utilizando una dosis total de 50 mg.ha^{-1}. En las extensiones agrícolas hubo respuesta positiva a la aplicación de BIOBRAS-16 independientemente de las dosis y los momentos de aplicación utilizados en cada una de ellas.

Palabras clave: arroz, brasinoesteroides, rendimiento

INTRODUCTION

In the latest years, several countries have focused their efforts on investigations related to synthesis, biological activity, and practical applications of brassinosteroids, a new class of plant growth regulators. These compounds are characterized by having a strong plant growth promoting activity, in concentrations of even thousand times lower than those used with the already known phytohormones (1).

The intensive study of such phytohormones provides new prospects for their use, as substitutes for growth hormones in different biological processes. In Cuba, the synthesis of various analogues from these compounds has been achieved, at laboratory level. Among them, the formulation named BIOBRAS-16, which presents a biological activity as growth regulator, according to bioassays performed, and it has been applied to several crops as yield stimulant (2, 3, 4, 5, 6).

On the other hand, increasing rice production and yield, reducing costs, as well as fulfilling consumption demands, are common goals of growers and researchers nowadays, especially in Latin America and the Caribbean. Since 1996, Cuba is immersed in a recovering process of this grass. In this sense, great challenges are still to be faced, especially concerning yield that still presents lower values than 3.5 to 3.6 t.ha^{-1} (7).

The present work was carried out taking into account the features of brassinosteroids and the need for increasing rice yield. The main purpose was that of studying the influence of a brassinosteroid analogue formulation on rice yield, as well as determining the most appropriate doses and times of application for such product.

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MATERIALS AND METHODS

The experiment was carried out in the areas of “Los Palacios” Rice Research Station, which belongs to the National Institute of Agricultural Sciences (INCA), as well as in “Cubanacán”, “Caribe”, and “Montoto” farms from “Los Palacios” Rice Agroindustrial Complex (CAI), on a Ferruginous, Gley nodular Hydromorphic soil. Its chemical features appear in Table I.

All the techniques used follow the Analytical Technique Handbook (9). The study was carried out during two seasons, which correspond to 1999-2000 poorly rainy season (winter) and 2000 rainy season (spring). Sowings were performed on December 15, and July 22, respectively, using INCA LP-2 variety which was sown in a randomized complete design with three replications, in 3x3 (9 m^2) plots.

A favorable climate behavior was recorded for crop development during the two seasons studied. Chemical fertilization, as well as the remaining crop cultivation, for both seasons were performed following Rice Technical Patterns (10). BIOBRAS-16 formulation was used, which contains a brassinosteroid analogue as active ingredient that is produced at the “Centro de Estudios de Productos Naturales” belonging to the Chemistry Faculty from the University of Havana. Such formulation presents a concentration of 1 $mg.mL^{-1}$. The five tested treatments appear in Table II.

Sprinklings were performed by hand, using a SUPERAGRO knapsack, with a 16-liter capacity and cone nozzle at steady pressure, during the period between 9:00 and 10:00 a.m., sprinkling foliage until it was fully wet.

The following characters were evaluated at harvest time:

- ★ Final height (cm)
- ★ Panicle length (cm)
- ★ Panicles/m^2
- ★ Weight of 1000 grains (g)
- ★ Full grains/panicle
- ★ Empty grains/panicle
- ★ Agricultural yield ($t.ha^{-1}$).

Standard Evaluation System for Rice (11) and Varietal Description Formulary (12) were the methodologies used for evaluating final height and panicle length.

To determine the agricultural yield and its components, the traditional crop system was used (13, 14). Panicles per m^2 were sampled once per plot, in a frame of 0.1 m^2. The remaining components (full grains/ panicle and weight of 1000 grains) were determined in 20 central panicles selected at random and crop agricultural yield was estimated in an area of 1 m^2/plot.

A variance analysis of simple classification was applied to all data and means were compared following Duncan’s multiple range test.

Agricultural extensions were made at three farms from “Los Palacios” Rice Agroindustrial Complex, together with the two experiments, having the purpose of performing a large-scale assessment. In this case, J-104 commercial variety was used and plane sprinklings were carried out early in the morning, wetting foliage homogeneously. Table III shows the doses and times of application.

Table I. Soil properties

Determinations	Values	Methods
Organic matter	3.29	Walkley-Black
Available phosphorus P (ppm)	63.0	Oniani (extraction by H_2SO_4)1N
Available potassium K ($Cmol.kg^{-1}$)	0.21	Oniani (extraction by H_2SO_4)1N
Exchangeable calcium Ca ($Cmol.kg^{-1}$)	18.3	Maslova (CH_3COONH_4) pH 7.1N
Exchangeable magnesium Mg ($Cmol.kg^{-1}$)	2.7	Maslova (CH_3COONH_4) pH 7.1N
pH (H_2O)	6.2	Potenciometric

Table II Studied treatments

Treatment	BIOBRAS-16 Dose ($mg.ha^{-1}$)	Time I	Dose ($mg.ha^{-1}$)	Time II
1 (Control)	0	----	0	----
2	10	Active tillering	10	Beginning of heading
3	25	Active tillering	25	Beginning of heading
4	10	Beginning of heading	10	Grain filling
5	25	Beginning of heading	25	Grain filling

Table III. Agricultural extensions at farms from “Los Palacios” Rice Agroindustrial Complex

Farms	Times and doses of application	Season	Applied area (ha)	Control area (ha)
Caribe	10 $mg.ha^{-1}$ in the beginning of heading	1999/2000 Winter	88.6	67.1
Cubanacán	10 $mg.ha^{-1}$ in active tillering +10 $mg.ha^{-1}$ in the beginning of heading	1999/2000 Winter	40.3	47.0
Montoto	10 $mg.ha^{-1}$ in active tillering + 10 $mg.ha^{-1}$ in the beginning of heading	2000 Spring	60.4	53.7

At harvest time, the same characters as in the experimental part were evaluated. Sampling was performed in four 1 m^2 points, selected at random, in fields with product application and without it. For measuring panicles/m^2, a 0,1m^2 frame was used. Twenty central panicles were selected at random, with the purpose of determining full and empty grains per panicle, as well as weight of 1000 grains. For data statistical processing, T-Student test was used.

RESULTS AND DISCUSSION

Results from 1999-2000 winter season. There is a remarkable influence on yield, of all the treatments where BIOBRAS-16 (BB-16) was applied. Treatments 3, 4, and 5 stood out without considerable differences among them; achieving increases of 31.38, 30.29 and 31.38, respectively, with regard to the control without application (Figure 1).

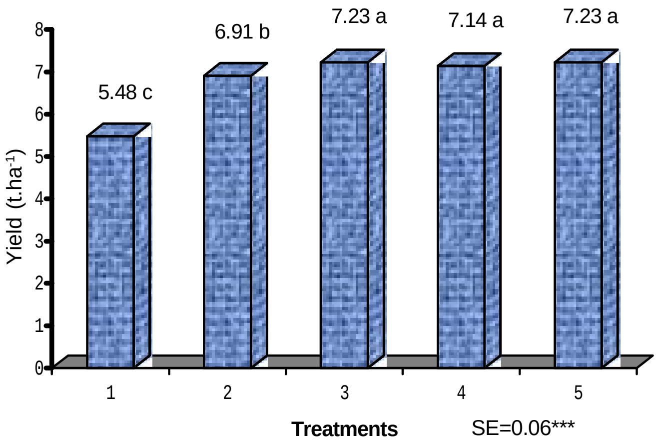

Figure 1. Behavior of agricultural yield

In this sense, it has been proved that Brasinolide increases rice grain volume, as well as the percentage of ripe grains. This issue has been attributed to a higher synthesis and translocation of photosynthetic product (15).

As to number of panicles/m^2, the highest influence was achieved by treatments where BB-16 was applied at the beginning of heading and during grain filling stage, regardless the dose used (Table IV). Concerning yield components, this was the most influencing character, since the others did not show statistically significant differences.

Other authors (16) have informed that, also in rice, a positive response in the increase of agricultural yield was found, when BIOBRAS-16 dose between 10 and 100 $mg.ha^{-1}$ were applied to some crop phenophases (tillering, primordium change, or beginning of heading). It is possible due to a higher number of full grains per panicle. It was also noticed that primordium change is the best application moment for both varieties and seasons studied.

Regarding final plant height, treatments with the best results were those in which applications were performed during active tillering and at the beginning of heading, no matter the dose used. In both cases, the best height increase stimulation occurs when applications are performed at the vegetative phase (Table IV). It could be highlighted that, in every case where BB-16 was applied, final height was significantly superior to that of the control. According to some authors, the use of brassinosteroids in crops is effective, in relation to growth and yield improvement, issue that evidences their vegetable growth promoting effect (1, 17).

This way, it was proved that the best yield responses for this season were obtained by treatments where BB-16 was applied at the beginning of heading and at grain filling stage, regardless the dose used. The same occurred by using the total dose of 50 $mg.ha^{-1}$ splitted, during active tillering and beginning of heading stages. Number of panicles/m^2 was the most influencing component on such response.

By the same token, increases in rice agricultural yield were achieved in China, by means of a study using brassinosteroid analogue TS 303. It was proved that such analogue presents a higher biological activity and its promoting effects were superior to those of epibrassinolide and homobrassinolide, under field conditions.

Results from 2000 spring season. Referring to yield, the most promising results correspond to treatments 2, 3 and 4, which were those provided with a total dose of 20 $mg.ha^{-1}$, independently of the application moment, as well as with 50 $mg.ha^{-1}$ during active tillering and the beginning of heading (Figure 2). Similar results were recorded during winter season, because treatments 3 and 4 showed superiority again, regarding plant response to the product applied.

Several investigations have showed the effectiveness of such compounds. In India, for instance, the influence of 28-homobrassinolide (HBR) leaf sprinkling was studied on rice productivity and the best response was obtained

Table IV. Influence of BIOBRAS-16 on yield and other variables in cv. INCA LP2 rice plants (winter season)

Treatments	Panicle length	Final height	Panicles/m^2	Full grains/panicle	Empty grains/panicle	Weight of 1000 grains
1	23.58	69.27 d	259.10 c	96.00	7.33	27.00
2	23.45	75.10 a	348.43 b	104.67	13.00	27.77
3	23.48	75.30 a	342.83 b	97.67	14.00	28.23
4	23.03	70.80 c	411.73 a	91.67	10.67	28.47
5	23.55	73.50 b	407.20 a	96.33	13.00	28.73
EE	0.37 ns	0.37***	11.65***	2.87 ns	2.79 ns	0.60 n.s

Means with common letters do not differ significantly at $p<0.05$

when performing the application in two moments: at active tillering and at the beginning of heading, with a dose of 0.1 mg.ha^{-1} HBR. Besides increasing yield considerably, a higher number of panicle per square meter was achieved. It is known that HBR and other brassinosteroids have positive effects on rice yield and productivity, stimulation of plant metabolism, growth, and grain yield increment. Such increase in grain yield has been attributed to HBR capacity for increasing the number of grains per panicle, improving spikelet fertility, as well as grain size, due to stimulation produced during photosynthate translocation to grains (19).

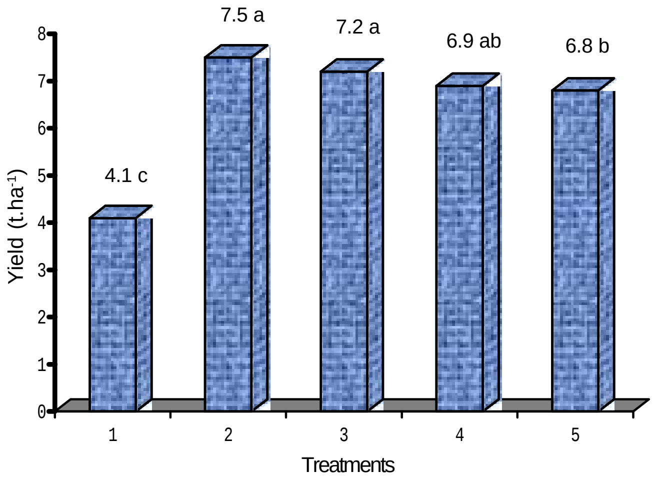

Figure 2. Yield agricultural behavior

In this case, different from the winter season, statistical analysis detected considerable differences for all evaluated characters, except for panicle length and final height (Table V).

As to full grain number per panicle, all the treatments where BB-16 was applied are considerably superior to the control, without statistical differences among them. By the same token, an increase in the number of empty grains per panicle was recorded during this period; however, it is important to highlight that the worst behavior was presented by the control. A similar tendency for these characters was confirmed also during spring season, in the agricultural extension from Montoto farm.

The highest influence in number of panicles/m^2 was obtained by treatments where BIOBRAS-16 was applied at a total dose of 20 mg.ha^{-1}, independently of the application moment. In the same way, the formerly mentioned treatments and that of a total dose of 50 mg.ha^{-1}, applied during active tillering and at the beginning of heading, showed the best response regarding weight of 1000 grains character. In this sense, grain weight is a very stable character under good cultural conditions, which mainly depends on the variety (20). Thus, it is possible to improve yield by increasing grain weight, since varieties presenting big grains accumulate starch in a more efficient way, during the ripening time (21).

On the other hand, also different from the former season, no influence of BIOBRAS-16 was seen in final plant height. This issue can be attributed to the fact that, during winter season, a delay in growth occurs due to the action of low temperatures and, under such adverse conditions, vegetable growth is more strongly stimulated by brassinosteroids. Different from this, the weather is more favorable during spring season and high temperatures promote plant growth; therefore, the BIOBRAS-16 applied influenced yield components to a higher extent.

Responses to brassinosteroids include effects on elongation, cell division, reproductive and vascular development, membrane polarization and proton pumping, source-sink relationships, as well as stress modulation. They also interact with environmental signs and can affect the development of insects and fungi (22).

BIOBRAS-16 has been also applied to different crops in other countries like Colombia, Venezuela; and Chile, achieving good results. For instance, in Venezuela (where the product is known as *BIOCRECE*), crops like cotton, maize, rice, coffee, and sorghum, have been sprinkled in amounts that range between 10 and 20 mg.ha^{-1}, obtaining yield increases between 7 and 40 % (23).

This way, the best response for this season was obtained by the treatment where BB-16 was applied at the beginning of heading and at grain filling stage, with a total dose of 20 mg.ha^{-1}, as well as by treatments where applications were performed both, at active tillering stage and at the beginning of heading, independently of the dose used.

Results from agricultural extensions belonging to "Los Palacios" Rice Agroindustrial Complex. Results from three agricultural extensions, in the same number of farms belonging to "Los Palacios", are shown in Table VI.

According to T-Student for comparing means among control treatments and BB-16 in the agricultural extensions, considerable differences for all the characters were found, except for final height, weight of 1000 grains and panicle length in "Caribe", "Cubanacán", and "Montoto" farms, respectively.

Table V. Influence of BIOBRAS-16 on yield and other variables in rice cv. LP 2 crop (spring season)

Treatments	Panicle length	Final height	Panicles/m^2	Full grains/panicle	Empty grains/panicle	Weight of 1000 grains
1	19.53	104.07	200 d	71 b	36.67 a	25.3 c
2	20.43	103.80	320 a	80 a	25.00 b	27.7 a
3	21.00	102.70	309 b	83 a	26.00 b	27.7 a
4	20.60	102.83	318 ab	84 a	24.00 b	27.3 ab
5	20.83	102.70	278 c	80 a	26.00 b	26.8 b
EE	0.62 ns	1.00 ns	2.99***	2.53*	1.53**	0.17***

Means followed by the some letters are not significantly different at p<0.05

Table VI. Results of BIOBRAS-16 application in "Los Palacios" rice CIA

Variables		Caribe farm			Cubanacan farm			Montoto farm		
		A	C	T	B	C	T	B	C	T
X1	Panicle length	21.68	20.45	*	22.5	21.2	*	22.1	23.6	ns
X2	Final height	69.97	69.75	ns	72.3	70.0	*	88.95	81.40	*
X3	Panicles/m^2	362.5	235.0	*	351	232	*	223	187	*
X4	Full grains/panicle	80.25	71.17	*	90.1	75.3	*	81.75	70.70	*
X5	Empty grains/panicle	4.25	9.00	*	10	15	*	27.7	40.2	*
X6	Weight of 1000 grains	28.50	27.55	*	28.5	27.6	ns	29.2	27.1	*

A- 10 $mg.ha^{-1}$ in the beginning of heading
B- 10 $mg.ha^{-1}$ active tillering + 10 $mg.ha^{-1}$ in the beginning of heading
C- Control treatment
T- Signification T-Student

In this sense, it has been proved that brassinosteroids present multiple bioregulating activities and stimulate different growth and development processes. Among them are: plant growth promotion by stimulating cell division and elongation, increase of crop yield and biomass production, anti-stress effects through compensation in face of salt stress, as well as through winter, ethylene biosynthesis stimulation, interactions with the effects of auxins and abscisic acid, as well as poorly studied changes even in metabolic processes and growth, in general (24).

In the particular case of yield, Figure 3 shows the good response of such character to the product application in all the cases, regardless the dose used and moments of application. This confirms the effectiveness of this Cuban bioregulator as a promoter of rice yield.

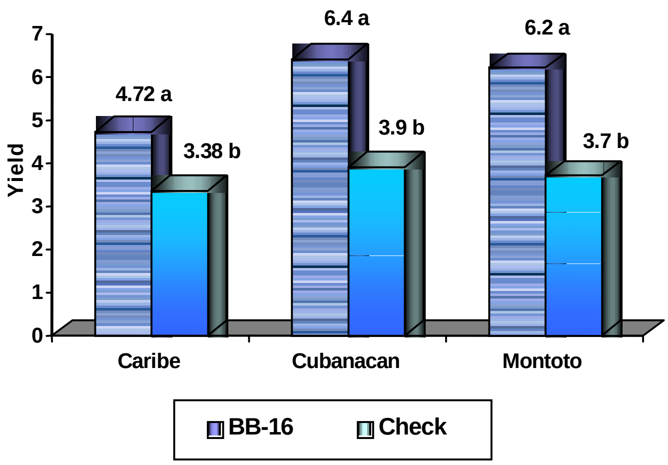

Figure 3. Yield behavior in agricultural extensions

In general and according to the discussed results, the brassinosteroid analogue BIOBRAS-16 presents great potentiality for being used as yield promoter in rice crop, independently of the application time, dose used, variety, and sowing season. Under experimental conditions, the best plant response to this product application was obtained by treatments 3 and 4, recommending their application at the beginning of heading and grain filling stages, using a total dose of 20 $mg.ha^{-1}$. By the same token, it was proved that, in agricultural extensions belonging to the three sampled farms, BIOBRAS-16 had a positive influence as agricultural yield bioregulator in rice crop.

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