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Original article

Influence of Biobras-16® and QuitoMax® biostimulants on two rice genotypes

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ABSTRACT

The study was carried out in Los Palacios municipality, with the objective of evaluating the effect of Biobras-16® and QuitoMax® biostimulants on the yield and its components of an advanced line of rice and the commercial cultivar INCA LP-5, as a strategy to increase yields under these soil and climatic conditions. The two genotypes were evaluated with biostimulants independently and the combination of both in two stages of the crop, using doses of 25 mg ha^{-1} of Biobras-16® and 50 mg ha^{-1} of QuitoMax®. A completely randomized design with a bifactorial arrangement was used, with eight treatments of three replicates each, and five quantitative traits were evaluated. The data obtained were subjected to univariate statistical analysis. Agricultural yield was higher when genotypes were treated, confirming the potential of Biobras-16® and QuitoMax® to be used as stimulators of this trait in rice cultivation. The best results were achieved with the combinations of Line 1 with QuitoMax® and the mixture of Biobras-16® plus QuitoMax® with this same genotype.

Key words: *Oryza sativa*, yields, farms

INTRODUCTION

The demand for agricultural products is expected to grow significantly by about 70 % by 2050, according to a calculation based on i) guaranteeing a moderate and adequate diet for all inhabitants by that date; ii) maintaining the level of consumption in those countries with richer diets; and iii) limiting the use of food for biofuels. The growing demands must be met with proportional increases in production, while at the same time meeting the need to reduce the environmental impact associated with agricultural activity [(1)].

An alternative to this problem is the application of plant growth biostimulants, an aspect of great importance in scientific research for agriculture, due to the social, economic and environmental implications they provide. It also constitutes a prioritized strategy to improve and preserve the physical, chemical and biological conditions of soils, increase the agro-productive potential and substitute imports [(2)].

In Cuba, QuitoMax®, a liquid biostimulant based on chitosan polymers, developed by the National Institute of Agricultural Sciences, and Biobras-16®, a brassinosteroid analog obtained at the University of Havana, are among the products that have been used as yield stimulators in several crops [(3,4)]. These products not only reduce the farmer's production costs, but also provide greater respect for the environment and generate benefit to crops, eliminating residues and producing healthy food. They can complement and sometimes even replace or reduce fertilizer use [(5)].

Considering the above, the present work was carried out with the objective of evaluating Biobras-16® and QuitoMax® effect on the yield and its components of an advanced line of rice and the commercial cultivar INCA LP-5.

MATERIALS AND METHODS

The work was carried out in the farm of the producer Rodolfo Miranda, belonging to the Credit and Service Cooperative (CCS) "Abel Santamaría", in Los Palacios municipality, on a Fluvisol soil [(6).]

The plant material studied consisted of two rice genotypes (*Oryza sativa* L.), including a new advanced line obtained through the hybridization method (Line 1) and the commercial cultivar INCA LP-5.

A completely randomized design with bifactorial arrangement was used, a "Genotype" factor composed of two levels and another factor "Product" with four levels, in total eight treatments with three replications. The genotypes were transplanted in the field in plots

of 2 meters long by 2 meters wide (4 m^2) at a distance of 15 cm between seedlings and 50 cm between plots.

The phytotechnical work and care carried out in the trial (soil preparation, seedbed, transplanting, fertilization, and irrigation and phytosanitary treatments) were carried out according to the Technical Instructions for Rice Cultivation [7].

Two biostimulants were used:

1. Biobras-16® (BB-16), has as active ingredient a brassinosteroid analogue, it is produced at the Center for the Study of Natural Products of Chemistry Faculty from University of Havana and a formulation at a concentration of 1 mg mL^{-1} was used.

2. QuitoMax® (Q), a liquid formulation based on chitosan polymers, is produced at the Bioactive Products Laboratory of the National Institute of Agricultural Sciences and a concentration of 4 g L^{-1} was used.

The treatments included a control (no application), independent applications of Biobras-16® and QuitoMax® and a fourth treatment where both products were combined.

Spraying was done manually, using an eight-liter CareSpray knapsack with a cone nozzle at constant pressure between 9 and 10 am, spraying the foliage until it was well wetted.

The doses used were 25 mg ha^{-1} and 50 mg ha^{-1} for Biobras-16® and QuitoMax®, respectively, at the beginning of paniculation and grain filling stages for both biostimulants.

Five quantitative traits were evaluated during the crop development cycle, using the methodologies: Standard Rice Evaluation System, CIAT Varietal Descriptors and Varietal Description Form for Rice.

- Full grains per panicle, **Gll**.
- Empty grains per panicle, **Gv**.
- Panicle per m^2, **Pm^2**.
- Mass of 1000 grains, **Mg** (g).
- Agricultural yield, **Y** (t ha^{-1}).

Observations were made on 10 randomly selected plants in each plot. Panicles per square meter were sampled once per plot, in a 0 25 m^2 frame. The remaining components (filled grains/panicle and mass of 1000 grains) were determined on 20 randomly taken central panicles and the agricultural yield of the crop was calculated on an area of 1 m^2.

The data obtained were processed by simple rank analysis of variance (ANOVA) with bifactorial arrangement and means were denoted by Duncan's 5 % Multiple Range Test, using the STATGRAPHICS Plus v.5 statistical software.

RESULTS AND DISCUSSION

Statistical analysis showed significant differences for the interaction between the factors studied for all traits at a significance level of 95 % (Table 1).

Table 1. Results of simple rank analysis of variance (ANOVA) for yield components of rice genotypes subjected to biostimulant application

Treatments		Gll	Gv	Pm^2	Mg
Genotypes	**Product**				
INCA LP-5	Control	131,45 f	14,30 a	477,50 e	29,10 b
Line 1	Control	148,35 d	10,10 d	589,50 b	27,45 e
INCA LP-5	Biobras-16®	171,80 a	9,50 e	545,00 c	29,95 a
Line 1	Biobras-16®	145,55 d	10,35 d	422,00 f	28,25 cd
INCA LP-5	QuitoMax®	156,05 b	11,30 c	523,50 d	29,99 a
Line 1	QuitoMax®	151,95 c	8,94 f	585,50 b	28,05 d
INCA LP-5	BB-16+Q	141,00 e	6,40 g	543,00 c	29,55 b
Line 1	BB-16+Q	148,90 d	12,81 b	610,00 a	28,51 c
Overall average		149,38*	10,46*	537,00*	28,86*
Standard error		2,0003	1,0951	10,3879	0,1325
C. of Variation (%)		9,36	18,21	14,28	3,54

Means with equal letters do not differ from each other (Duncan's Multiple Range Test, $p \leq 0.05$)

For the number of full grains per panicle, the best yielding combinations were INCA LP-5 with Biobras-16®, this same cultivar with QuitoMax® and Line 1 with QuitoMax®, in that order. Treated INCA LP-5 always outperformed the control. Other authors also found significant differences with respect to the control in the number of full grains per panicle when QuitoMax® was applied, and these increased independently of the treatments used [(3)]. Likewise, in research with Biobras-16® it was found that the enhanced yield components were different. In the cold season, this product only significantly increased the number of panicles per square meter, while, in the spring, it increased, in addition to the number of panicles per square meter, the number of full grains per panicle and the mass of 1000 grains. This could be the reason for the higher yield increases obtained in the spring season compared to the cold season [(8)]. In addition, results obtained in beans and corn show the positive effect of Biobras-16® and QuitoMax® on the number of grains [(2,9)].

The best combinations for the trait of empty grains per panicle were INCA LP-5 when Biobras-16® + QuitoMax® were applied, and Line 1 when QuitoMax® was used. Several causes are known to affect rice grain shattering, those related to plant health (different causal agents, use of hormonal herbicides at the fertilization and grain filling stage), but there are also agrochemical (insufficient or excess nitrogen, micronutrient deficiency), genetic (non-total emersion of panicles and fertilization capacity) and climatic (relative humidity, strong and dry winds, drought and temperature) causes. On the other hand, it has been suggested that the use of biostimulants protects plants against different stress situations, improves fruit and grain set or increases crop quality, which could explain the behavior for this trait [4,10,11].

In panicles per square meter, Line 1, statistically, showed the best value, in combination with Biobras-16® + QuitoMax®. It is suggested that panicles per square meter is the most variable component and has been the main cause that has limited agricultural yield under Cuban conditions. Its values are closely related to the quality of soil preparation and planting, planting standards, tillering capacity of cultivars, water management and nitrogen fertilization. Other researchers obtained similar results when they found significant differences in QuitoMax® application, obtaining the maximum number of panicles in the treatment where the product was applied to the seed and two foliar sprays were made at different times; while the lowest number of panicles was achieved in the treatments where QuitoMax® was not applied [3].

The highest value of 1000-grain mass was shown by the cultivar INCA LP-5 when Biobras-16® or QuitoMax® was applied. In similar studies in rice for this trait, similar results were obtained when QuitoMax® was applied. The thousand-grain mass is not influenced by the method of crop establishment, but it is influenced by the variety. This trait is determined during grain filling and requires high radiation and good nutrient supply [12]. In other studies on bean cultivation, it is reported that treatments with Biobras-16® and QuitoMax® did not change the mass of 1000 beans [4].

Figure 1 presents the ANOVA results for the yield trait and the best responses are in the combinations of Line 1 with QuitoMax® and Line 1 with Biobras-16® + QuitoMax®. Line 1 outperformed, in any of the mixtures with the biostimulants, the cultivar INCA LP-5, including the control. This was the trait with the highest coefficient of variation, which could be attributed to the variability between treatments. Other authors state that, to achieve a high production of yield components, some of the parameters used are: 250 to 300 panicles m^2;

100 to 120 spikelets/panicle; percentage of empty grains not greater than 20 and grain mass of 25 to 30 g/1000 grains [13]. In other investigations where the effect of Biobras-16® and QuitoMax® on rice yield was evaluated, it was found that both products stimulate yield [3,8].

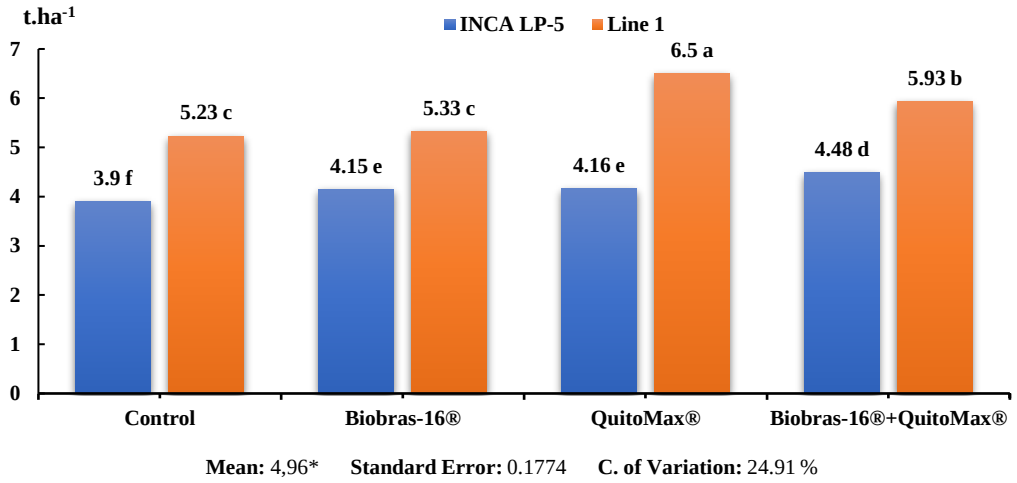

Figure 1. Results of the simple rank analysis of variance (ANOVA) for yield of rice genotypes subjected to the application of biostimulators

These results are achieved with only two applications of urea instead of the five recommended for this season, which indicates that biostimulants played an important role in the substitution of nitrogen fertilizer and, therefore, there is a saving of resources and the environment conservation is favored, coinciding with what has been stated by other specialists [14].

Previous studies suggest that the application of chitosan stimulates the physiological processes in the plant and increases cell size, which enhances the assimilation of nutrients by the plant and increases its growth and development, which brings about an increase in yields [15]. In the case of brassinosteroid analogues, investigations have been directed to evaluate the responses of growth and yield of plants to the applications of these; still pending to elucidate the physiological, biochemical and molecular mechanisms used by these compounds to exert their effects. It would allow determining if they act in a similar way to natural brassinosteroids or if they use other mechanisms of action [8]. In other countries such as Ecuador and for rice cultivation, the use of physiological activators such as BonActiv is presented as an alternative to the use of conventional fertilizers, with the advantages that this product stimulates plant development, consumes little energy, does not pollute the environment, increases soil fertility and generates elicitor action responses to phytopathogens [16].

CONCLUSIONS

- Agricultural yield is superior when genotypes are treated, confirming the potentialities of Biobras-16® and QuitoMax® to be used as yield stimulators in rice cultivation.
- The best results are achieved with the combinations of Line 1 with QuitoMax® and the mixture of Biobras-16® + QuitoMax® with this same genotype.

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