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Original article

Effect of Bioenraiz® inoculation and its final fermentation on the growth and physiology of rice (*Oryza sativa* L.), cultivar ¨Selección 1¨

Efecto de la inoculación de Bioenraiz® y su fermentado final en el crecimiento y fisiología de arroz (*Oryza sativa* L.) cultivar Selección 1

Ionel Hernández Forte[1*], Claudia Pérez Arabí[1], Anisley Barrios Hernández[2], Vivianne Machado Brito[1], Lisbel Travieso Hernández[1], Betty L. González Pérez[1], Vivian León Fernández[2], Daisy Dopico Ramírez[2]

[1]Instituto Nacional de Ciencias Agrícolas. Km 3 y ½ Carretera a Tapaste, San José de Las Lajas, Mayabeque, Cuba. Gaveta Postal No. 1. C. P. 32700.

[2]Unidad Empresarial de Base Bioprocesos Cuba 10. Instituto Cubano de Investigaciones de los Derivados de la caña de azúcar. Calle 4 e/3 y 5, Pablo Noriega, Quivicán, Mayabeque, Cuba.

ABSTRACT: A little evidence in Cuba addresses the biostimulant effect of Bioenraiz® in agriculture which has high concentration of metabolites with auxinic activity. The objective of the work was determine the effect of Bioenraiz® and the final product resulting from the fermentation with the strain *Rhizobium* sp. on the growth and some physiological parameters of the rice cultivar “Selección 1”. Seedlings roots of rice cultivar “Selección 1” were soaked in both bioproducts for twenty minutes, and later they were transplanted into pots with Red Ferralitic soil. The plants were maintained in semi-controlled conditions for 60 days and then the plant height, root length, dry weight of the aerial and root parts, the total content of chlorophylls a, b and total chlorophylls and the total content of carotenoids were determined. Bioenraiz® produced positive effects on the root length and root dry weight of rice plants. No effect of both products was seen on the chlorophyll and carotenoid content. This is the first report in Cuba when a beneficial effect of Bioenraiz® was showed in rice crop.

Key words: biostimulation, grass, rhizobia, indolacetic acid.

RESUMEN: Pocas evidencias en Cuba abordan el efecto bioestimulador de Bioenraiz® en la agricultura, el cual tiene altas concentraciones de metabolitos con actividad auxínica. El objetivo de este trabajo fue: Determinar el efecto de Bioenraiz® y el producto final de la fermentación con presencia de la cepa *Rhizobium* sp. en el crecimiento y algunos parámetros fisiológicos del cultivar de arroz Selección 1. Las raíces de plántulas de arroz cultivar Selección 1 se embebieron en ambos bioproductos durante veinte minutos y posteriormente se trasplantaron en macetas con suelo Ferralítico Rojo. Las plantas se mantuvieron en condiciones semicontroladas durante 60 días y se determinó la altura, la longitud de la raíz, la masa seca de la parte aérea y de raíz, el contenido de clorofilas a, b y total y el contenido de carotenoides totales. El empleo de Bioenraiz® produjo efectos positivos en el largo y masa seca de las raíces de las plantas. No se apreció efecto de ambos productos en el contenido total de clorofilas y carotenoides. Esta es la primera investigación en Cuba donde se evidencia un efecto positivo de Bioenraiz® en el cultivo del arroz.

Palabras clave: Bioestimulación, gramínea, rizobio, ácido indol acético.

*Author for correspondence: ionel.hdez09@gmail.com

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INTRODUCTION

The application of mineral fertilizers supplies part of the nutritional needs of economically important crops in Cuba, such as rice (*Oryza sativa* L.) (1). However, the irresponsible use of these products has a negative impact on ecosystems, as it affects the balance established by biogeochemical cycles. This leads to the acidification of arable soils and water, both surface and deep, and to an increase in the emission of greenhouse gases such as nitrous oxide into the atmosphere (2). This environmental impact is associated with serious consequences for human health and a considerable increase in production costs (3).

On the other hand, rhizobia are bacteria that have traditionally been studied for their ability to establish a symbiotic relationship with leguminous plants (4). However, some studies confirm the positive effect of their inoculation on other non-leguminous crops such as lettuce (*Lactuca sativa*), wheat (*Triticum* spp.) and corn (*Zea mays* L.) (5-7). These evidences have allowed the classification of rhizobia as Plant Growth Promoting Bacteria (PGPB), since they produce a beneficial effect on plant growth and development, due to their biofertilizing, biostimulant and biocontrolling activity (8).

In recent years, multiple researches in Cuba have focused on the study of the interaction of rhizobia with rice. Rice is of great economic importance for the country with a consumption of more than 70 kg per capita per year (9). The positive effect of these bacteria in promoting growth, nutrition and yield of important rice cultivars for the country such as INCA LP-5 and INCA LP-7 crop has been widely demonstrated (10-12). This suggests that the development and use of rhizobia-based bioproducts constitutes an alternative to mineral fertilization of rice. The rice cultivar Selección 1 is one of the eleven cultivars designated for planting in the main Cuban rice producing stations. It has an average yield of 5.8 to 7.1 t h^{-1} and excellent milling quality. In 2020, about 47 thousand ha were planted throughout the country (1). However, there are no studies that reflect the effect of biological products based on PGPB on the growth of this cultivar.

Bioenraiz® is a Cuban biostimulant for agricultural use that is produced by submerged fermentation of a *Rhizobium* sp strain. The last stage of its production process consists of removing the bacterial cells from the final fermentation, leaving as active ingredient the auxinic metabolites produced by the bacteria during fermentation (13). In Cuba, there is very little information published in scientific magazines that demonstrate the effect of Bioenraiz® in crops. Only one research, in the cultivation of coffee (*Coffea arabica* L.) summarizes the biostimulatory effect of the product as it increases seed germination (14). Thus, it is of interest to know the benefits of Bioenraiz®, especially in economically important crops such as rice. The objective of this study was to determine the effect of Bioenraiz® and the final fermentation product with the presence of the *Rhizobium* sp. strain on the growth and some physiological parameters of the rice cultivar Selección 1.

MATERIALS AND METHODS

Two certified biological products with optimal quality control criteria were used: the biostimulant Bioenraiz® and the final fermentation product with the presence of the *Rhizobium* sp. strain (final fermentation product). Both came from the Bioprocesses Base Business Unit "Cuba 10" from Quivicán Municipality, Mayabeque Province; currently responsible for the production of these biopreparations.

Certified rice seeds of the Selección 1 cultivar were pre-germinated. For this purpose, they were placed in Petri dishes on sterile filter paper, which was moistened with 10 mL of sterile distilled water. Twenty-five seeds per plate were placed and incubated at 28 ºC, in darkness for 72 h to promote germination.

Pre-germinated rice seeds with 0.5-1.0 cm long radicles were placed in three 1.2 kg capacity pots at the rate of 50 seeds per pot. These contained the same volume of a mixture of Ferrallitic Red soil and organic matter (3:1). Seven days after sowing, the seedlings were transplanted into similar pots at the rate of two seedlings per pot.

At the time of transplanting, the roots of the seedlings were soaked in both bioproducts for 20 min. Three treatments were established, with 24 plants each and two plants per pot. The treatments were: (i) uninoculated seedlings (control of the trial), (ii) seedlings inoculated with Bioenraiz®, (iii) seedlings inoculated with the final fermentation. Plants were grown under semi-controlled conditions in a greenhouse and watered every other day with tap water.

At 60 days after transplanting, plant height (cm), root length (cm), and aerial and root dry mass (g) were evaluated. Chlorophyll a, b and total chlorophyll content was also determined, the latter by summing the values of the first two, as well as total carotenoid content by spectrophotometric methods (15). For this purpose, 0.04 g of the flag leaf of the plants were weighed, immersed in 10 mL of ethanol (95 % v/v) and incubated in the dark for 24 h at room temperature. Subsequently, absorbance was read at 470, 664 and 649 nm for chlorophylls a, b and carotenes; respectively. The concentration of each pigment was expressed in μg g^{-1} of fresh leaf mass, according to the following equations:

$$C_a = 13{,}36 \quad A_{664{,}2} - 5{,}19 \quad A_{648{,}6}$$

$$C_a = 13{,}36 \quad A_{664{,}2} - 5{,}19 \quad A_{648{,}6}$$

$$Carotenoids = \frac{1000 \quad A_{470} - 2{,}13 \quad C_a - 97{,}64 \quad C_b}{209}$$

Where:

Ca, Cb: chlorophyll a and b, respectively.

A470, A649, A664: absorbance at 440, 649 and 665 nm, respectively

Statistical analysis

An analysis of variance (ANOVA) was performed to determine differences between the means of each treatment. Previously, normality was tested by Bartlett's test and homogeneity of variance by the Kormogorov Smirnov test. Statgraphic Plus version 5.0 was used for statistical processing of the data and Microsoft Excel 2016 for their representation.

RESULTS AND DISCUSSION

Although there are some references of the biostimulatory effect of Bioenraiz® on important crops in Cuba, only one research on coffee cultivation constitutes registered scientific evidence of the positive effect of this product on crops (14). The present research constitutes the first evidence in Cuba that demonstrates the biostimulatory effect of Bioenraiz® on the rice crop.

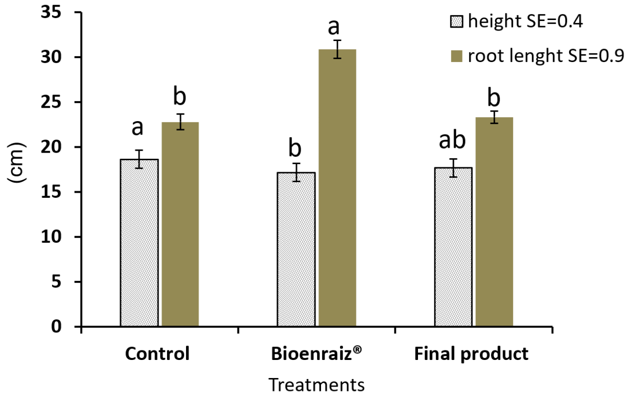
Rice was used for the study because of its importance in the diet of Cubans and because the Bioenraiz® effect on this grass is unknown. The results showed that Bioenraiz® increased the length of the roots of rice plants cultivar Selección 1, 60 days after the application of the product (Figure 1).

Significant differences were observed in the height of the plants inoculated with Bioenraiz® and the control treatment, in favor of the latter. However, there were no differences in this variable between plants inoculated with this bioproduct and those treated with the final fermentation product (Figure 1).

The results also showed a positive effect on the root growth of rice plants when inoculated with both products (Figure 2 and 3).

Plants inoculated with Bioenraiz® showed higher aerial dry mass than those treated with the final fermentation product. In addition, no differences were observed between control plants and those inoculated with both products in this variable (Figure 3).

Bioenraiz® is a biostimulant with high concentrations (>150 mg L^{-1}) of metabolites with auxinic activity, mainly



The data reported are the means + the standard errors of the mean from 24 sample replicates. Different letters means significant differences between treatments (Tukey HSD $p < 0.05$, n = 24). SEx, standar error ANOVA

Figure 1. Effect of Bioenraíz® inoculation and final product on height and root length of rice plants cultivar Selección 1, at 60 days after inoculation under greenhouse conditions. Plants without inoculation were the control treatment

indole acetic acid (IAA), which is its main active ingredient. This acid is released to the medium during the fermentation of the Rhizobium sp. strain (14). Phytostimulation, from the production of indole compounds such as IAA, is recognized as one of the most important mechanisms used by rhizobia to promote the growth of grasses (16). This would explain the biostimulatory effect of Bioenraiz® and of the final fermentation product in increasing root dry mass. Plants with a more developed root system can explore a larger soil surface and absorb more nutrients.

Multiple studies have proven the biostimulatory effect of IAA produced by different bacterial genera recognized as PGPB. Such is the case of Pseudomonas and Bacillus, which enhance root initiation, cell elongation and root hair formation (17-19). Previous research on grasses such as corn and sugarcane (*Saccharum officinarum*) reaffirms the biostimulant activity of IAA from *Azospirillum* and *Bacillus* (20-22). *Rhizobium* also exerts a phytostimulant effect on

Figure 2. Effect of inoculation of Bioenraíz® and final product on growth of rice plants cultivar Selección 1 at 60 days after inoculation under greenhouse condition

sugarcane 30 and 75 days after transplanting, since cell-free supernatants of the bacteria contain metabolites with auxinic activity such as IAA and gibberellins (23).

In Cuba, there are some works with the genus *Azospirillum* in gramineae crops, mainly rice, which have demonstrated its phytostimulatory effect for being a group of bacteria highly producers of IAA. The use of *Azospirillum*-based inoculants increased the vegetative development of the crop, with effectiveness rates of up to 21.77 % in height and 102.06 % in dry mass of the aerial part, with respect to the control without inoculation. Similar works have shown the possibility of reducing the nitrogen fertilization of the rice crop by 33%, based on the phytostimulant activity of this bacterial genus, starting from the production of IAA (24).

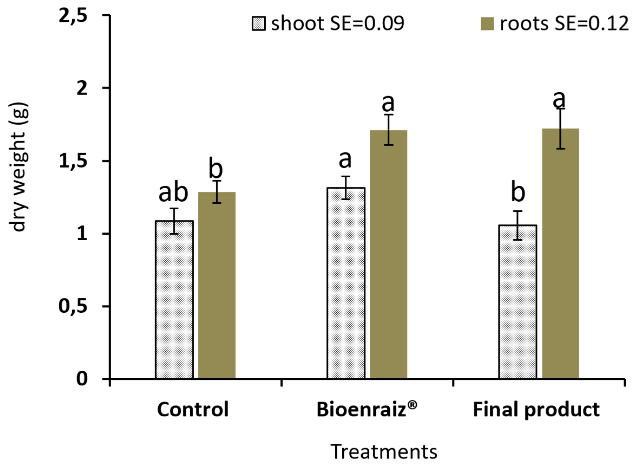
Considering that the Bioenraiz® application and the final fermentation caused positive effects on the growth of rice plants, plant physiological variables were determined to explain the effect of these products.

In addition to the IAA production, it is known that the stimulation of photosynthesis is another of the main mechanisms that explain the promotion of grass growth by rhizobia (16). This process supplies carbon compounds to the plant that contribute to its growth and is enhanced, among other indicators, by an increase in chlorophyll content. These molecules allow light to be captured, the first stage of the photosynthesis process (25). However, the results of the present investigation did not show an effect of both bioproducts in the stimulation of total chlorophyll content of rice plants cultivar Selección 1, with respect to control plants (Table 1).

In contrast, the results showed significant differences between the control plants and those treated with the final fermentation product in chlorophyll a content, while there were no statistical differences between the treatments in chlorophyll b content (Table 1).

Previous work has demonstrated the positive effect of the inoculation of bacteria of the rhizobia group on chlorophyll content; such is the case of microbial consortia in rice, *Sinorhizobium meliloti* in alfalfa plants (*Medicago sativa*) subjected to stress by high concentrations of copper and *Rhizobium leguminosarum* in *Lens culinaris* (26, 27).

On the other hand, carotenoids are pigments (yellow-orange) that protect the photosensitive apparatus by energy dissipation and extinction mechanisms. Like chlorophylls,



The data reported are the means + the standard errors of the mean from 24 sample replicates. Different letters means significant differences between treatments (Tukey HSD $p < 0.05$, n = 24). SEx, standar error ANOVA

Figure 3. Effect of inoculation of Bioenraíz® and final product on shoot dry weight and roots dry weight of rice plants cultivar Selección 1, at 60 days after inoculation under greenhouse conditions. Plants without inoculation were the control treatment

they also participate in capturing sunlight, especially in the light spectrum between 450 and 500 nm, in which chlorophylls absorb little (28). Hence the interest in determining the effect of both bioproducts on the content of these molecules. The results showed no significant differences between the treatments studied. However, previous studies revealed that the inoculation of *Bradyrhizobium* and *Enterobacter* strains produced increases in the total carotenoid content of soybean (*Glycine max*) plants under salt stress conditions (29).

Bioenraiz® is a product that, although it has been opportunely studied, has practically no published scientific results to explain its phytostimulant effect on crops. Results presented here contribute to obtain knowledge about its effect on rice cultivation and thus form part of the dissemination of the potential of this bioproduct as a plant growth stimulator. It is imperative to replace part of the mineral fertilization used in crops, especially in those of economic importance. The use of products such as Bioenraiz® could be part of this goal.

Table 1. Effect of inoculation of Bioenraíz® and final product on chlorophyll and carotenoid contents of rice plants cultivar Selección 1, at 60 days after inoculation under greenhouse conditions. Plants without inoculation were the control treatment

Treatments	chlorophyll (mg g^{-1} fresh weight)			carotenoid (µg g^{-1} fresh weight)
	a	b	totals	
Control	1.1+0.12 a	0.47+0.16 a	1.57+0.27 a	8.07+1.55 a
Bioenraiz®	1.0+0.03 ab	0.30+0.02 a	1.33+0.02 a	9.15+5.26 a
Final product	0.8+0.04 b	0.39+0.10 a	1.19+0.06 a	7.77+1.25 a
SEx	0.71	0.11	0.16	0.10

Data reported are the means + the standard errors of the mean from 24 sample replicates. Different letters means significant differences between treatments (Tukey HSD $p < 0.05$, n = 24). SEx, standar error ANOVA

CONCLUSIONS

Bioenraiz® and the final fermentation product of the *Rhizobium* sp. strain, both of which are produced domestically, are promising bioproducts for the inoculation of the rice cultivar Selección 1, one of the most widespread in the country. Both products had a positive effect on the root development of the grass. However, Bioenraiz® produced more encouraging results on root length and dry mass of the aerial part of the plants. Taking into account the limitations of the present research, it is advisable to continue with similar experiments, even extending them to other crops and field conditions, in order to have a more solid criterion when deciding on the productive process of these bioproducts and, therefore, which one would be more appropriate to use.

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