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Original article

Trait association in advanced rice lines treated with Biobras-16® and QuitoMax®

Asociación de caracteres en líneas avanzadas de arroz tratadas con Biobras-16® y QuitoMax®

Rogelio Morejón-Rivera[1]*, Sandra H. Díaz-Solís[1], Alfredo Miranda-Castillo[2]

[1]Unidad Científico Tecnológica de Base “Los Palacios”. Instituto Nacional de Ciencias Agrícolas (INCA), carretera San José-Tapaste, km 3½, Gaveta Postal 1, San José de las Lajas, Mayabeque, Cuba. CP 32 700

[2]Empresa Agroindustrial de Granos “Los Palacios”, calle 26 entre 19 y 21 # 1920, Los Palacios, Pinar del Río, Cuba.

ABSTRACT: This study was carried out at the farm of producer Rodolfo Miranda in Los Palacios municipality with the objective of seeking a relationship between yield, its components and other traits in rice genotypes treated with the biostimulants Biobras-16® and QuitoMax®. A completely randomized design was used with eighteen treatments (a control without application and independent applications of Biobras-16® and QuitoMax®) and three replicates each, and six quantitative traits were evaluated. The data matrix obtained was processed by means of Multivariate Principal Component Analysis, Multiple Linear Regression and Pearson correlations. The results revealed that most of the variables evaluated showed correlations, except full grains per panicle that was not interrelated with any other trait. The Principal Component analysis explained 74 % of the total variance, all the original variables contributed to the first component, except full and vain grains per panicle that contributed to the second component. Groups I and II included the cultivar INCA LP-7 and Line 4 in combination with the products under study and Line 3 with Biobras-16®. They were characterized by having the highest values for all traits, except vain grains per panicle, and the model proposed by the multiple linear regression analysis explained more than 85 % of the variability in yield, being an optimal predictor of this trait for studies under similar conditions.

Key words: *Oryza sativa* L., yield, farms, multivariate technical..

RESUMEN: En la Finca del productor Rodolfo Miranda del municipio Los Palacios se desarrolló este trabajo con el objetivo de buscar una relación entre el rendimiento, sus componentes y otros caracteres en genotipos de arroz tratados con los bioestimulantes Biobras-16® y QuitoMax®. Se utilizó un diseño completamente aleatorizado con dieciocho tratamientos (un testigo sin aplicación y aplicaciones independientes de Biobras-16® y QuitoMax®), tres réplicas cada uno y se evaluaron seis caracteres cuantitativos. La matriz de datos obtenida fue procesada mediante los Análisis Multivariados de Componentes Principales, Regresión Lineal Múltiple y las correlaciones de Pearson. Los resultados revelaron que la mayoría de las variables evaluadas mostraron correlaciones, excepto el carácter granos llenos por panícula que no se interrelacionó con ningún otro carácter; el análisis de Componentes Principales explicó el 74 % de la varianza total; a la primera componente contribuyeron todas las variables originales, excepto los granos llenos y vanos por panículas que lo hicieron a la segunda; los grupos I y II, donde se incluyeron el cultivar INCA LP-7 y la Línea 4 en combinación con los productos en estudio y la Línea 3 con Biobras-16®, estuvieron caracterizados por poseer los mayores valores en cuanto a todos los caracteres, excepto los granos vanos por panícula. El modelo propuesto por el análisis de regresión lineal múltiple explicó más de un 85 % de la variabilidad en el rendimiento, siendo un predictor óptimo de este carácter para estudios en condiciones similares.

Palabras clave: *Oryza sativa*, rendimiento, fincas, estadísticas.

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*Author for correspondence: rogelio@inca.edu.cu

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INTRODUCTION

World rice production has almost tripled in the last five decades, mainly due to increased yields and, in part, to an increase in the area planted. With approximately 154 million hectares harvested each year, rice is one of the most important cereals in the world, being the main source of caloric intake and the staple food for more than three billion people in the world (1).

In Cuba, the genetic breeding program has released for production several rice cultivars with a high potential for rice cultivars with high yield potential, adapted to adverse factors. However, causes such as bad cultural practices, deterioration of soil properties, pests, some changes in climatic variables, among others, negatively affect the productive results, with low yields that still do not exceed 3.6 t ha^{-1} as a national average (2).

The application of biostimulants of vegetal growth can be an option to this problem, an aspect of great importance within scientific research for agriculture, due to the social, economic and environmental implications that they contribute (3), especially in the current context where the blockade imposes great limitations that directly influence the country's economy and essentially in the Cuban agricultural sector. It makes think of the need to bet on a more productive, resilient and compatible agriculture with the environment.

On the other hand, in the past, empirical estimates of crop yields were based on the practical knowledge of farmers or on sampling procedures in representative areas, but these methods are generally misleading. In recent years, a priori yield information is generally produced from models, either with information on crop management, climate and soil data, among others (4).

Based on the above, the objective of this work is to search for a relationship between yield, its components and other traits in rice genotypes treated with the biostimulants Biobras-16® and QuitoMax® to provide useful information to specialists in the development of genetic breeding programs for this crop.

MATERIALS AND METHODS

This work was carried out on the farm of the producer Rodolfo Miranda, belonging to the Cooperative of Credits and Services (CCS) "Abel Santamaría", in Los Palacios municipality, on a Fluvisol soil (5).

Six rice genotypes (*Oryza sativa* L.) constituted the plant material studied, integrated by four new advanced lines obtained through the hybridization method (Line 1, Line 2, Line 3 and Line 4) and the commercial cultivars INCA LP-5 of short cycle and INCA LP-7 of medium cycle.

A completely randomized design was used with eighteen treatments (a control with no application and independent applications of Biobras-16® and QuitoMax®) and three replicates each (Table 1). In the field, the genotypes were transplanted in plots 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 (soil preparation, seedbed, transplanting, and fertilization, irrigation and phytosanitary treatments) were carried out according to the Technical Instructions for Rice Cultivation (6).

Two biostimulants were applied: Biobras-16® has a brassinosteroid analog as active ingredient and a formulation was used at a concentration of 1 mg mL^{-1} and QuitoMax®, a liquid formulation based on chitosan polymers, at a concentration of 4 g L^{-1}.

The spraying was done manually, using a CareSpray knapsack with a capacity of eight liters and 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.

The following quantitative traits were evaluated, using the IRRI Standard Evaluation System for Rice (7) as methodology.

- Full grains per panicle, **Gll** (number).
- Empty grains per panicle, **Gv** (number).
- Panicle per m^2, **Pm** ^{2} (number).
- Mass of 1000 grains, **Mg** (g).
- Agricultural yield, **Y** (t ha^{-1}).
- Crop cycle at 50 % flowering, **C** (days).

The characters filled grains per panicle, empty grains per panicle, and 1000-grain mass were determined on 20 randomly taken central panicles and cycle as the number of days from germination until 50 % of plant panicles had emerged. Panicles per square meter were sampled once per plot, in a 0.25 m^2 frame and the agricultural yield of the crop was calculated on a 1 m^2 area.

Table 1. Treatments used

No.	Treatments	No.	Treatment	No.	Treatment
1	INCA LP-7	7	INCA LP-7+Biobras-16®	13	INCA LP-7+QuitoMax®
2	INCA LP-5	8	INCA LP-5+Biobras-16®	14	INCA LP-5+QuitoMax®
3	Line 1	9	Line 1+Biobras-16®	15	Line 1+QuitoMax®
4	Line 2	10	Line 2+Biobras-16®	16	Line 2+QuitoMax®
5	Line 3	11	Line 3+Biobras-16®	17	Line 3+QuitoMax®
6	Line 4	12	Line 4+Biobras-16®	18	Line 4+QuitoMax®

The data matrix obtained was processed using Multivariate Principal Component Analysis (using Euclidean distance squared), Multiple Linear Regression (considering yield as the dependent variable and the rest as independent) and Pearson correlations, all with the help of the statistical package Statgraphics Plus v.5.

RESULTS AND DISCUSSION

From the statistical analysis, Table 2 shows the phenotypic correlations (Pearson correlations) between the variables analyzed.

The correlation coefficient is a statistical tool that generally shows relationships between independent traits, and the degree of linear relationship between those traits. In plant breeding research, measurements of several characters or variables are taken usually from the same plant (8). This can have a value between 0 and ±1, the sign indicating the dependence or relationship that exists. The closer the value is to one, the greater the correlation dependence between the two indices.

Yield was correlated strongly and positively with 1000-grain mass, panicles per square meter and cycle, while it was negatively correlated with empty grains per panicle. The degree of association between traits is an important factor when dealing with a variable as complex as yield that is controlled by many genes and is influenced strongly by the environment (9). Selection for yield may not be effective unless the other components of yield that directly or indirectly influence it are taken into consideration. When selection pressure is exerted to improve any variable highly associated with yield, it simultaneously affects many other correlated traits. Therefore, knowledge about the association of the trait with yield and with each other provides guidance to the breeder, giving a clear understanding of the contribution with respect to the establishment of the association by genetic and non-genetic factors (10). In this sense, coincidence has been found in some works (10-16) and non-coincidence in another (9) where the relationships between these characters were studied.

In addition, a strong and positive correlation was found between the cycle and the characters mass of 1000 grains and panicles per square meter. Similar results were reported in other studies on genetic diversity in rice germplasm (9,10).

The filled grains trait showed no correlation with any other trait evaluated. In studies carried out in China there was a similar behavior among japonica cultivars (15), however, in other investigations this trait expressed a strong and direct relationship with yield (11,12,15,16).

Table 2. Matrix of phenotypic correlations

	GII	Gv	Pm^2	Mgr	Y
Gv	-0.31				
Pm ^{2}	-0.26	0.08			
Mgr	0.45	-0.26	0.27		
R	0.38	-0.51*	0.54*	0.57*	
C	-0.19	-0.02	0.80**	0.63**	0.47*

*The correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level

To reduce the data dimensionality and to explain the process variability sources, Table 3 shows some issues of the first two components of the Principal Component Analysis. It is following the criterion of considering acceptable the components whose eigenvalues explain 70 % or more of the total variance.

The first component contributed 46 % of the total variance explained and the correlations with the original variables indicate that the characters panicles per square meter, mass of 1000 grains, yield and cycle are among those that contributed most positively. Full and empty kernels contributed positively and negatively, respectively, to the second component, explaining 28 % of the total variance.

Figure 1 shows the graphic location of the treatments and the original variables on the plane formed by components 1 and 2; the position occupied by the 18 treatments evaluated allowed the formation of four groups. The means by variables and the treatments belonging to each group are presented in Table 4.

Principal Component Analysis is a useful tool for analyzing the data generated from the characterization and preliminary evaluation of germplasm. It allows knowing the relationship between the quantitative variables considered and the similarity between the accessions. In the first case, in order to know which variables are or are not associated, which characterize in the same sense or in the opposite sense; and in the second, to know how the accessions are distributed, which ones are similar and which ones are not. It also makes it possible to select the most discriminating quantitative variables to limit the number of measurements in subsequent characterizations (17). This method can contribute to the decision making of the plant breeder in the selection of valuable genotypes, through many morphological characteristics, since in most trials the characteristics involved in yield and some of its components are considered with greater emphasis. In research on genetic diversity of Korean rice germplasm for adoption in Nigeria, this technique showed that the first four components explained 73.59 % of the total variation,

Table 3. Eigenvalues, percentage contribution and cumulative contribution of the components and correlations with the original variables

	C1	C2
Eigenvalues	2.78	1.66
contribution %	0.46	0.28
cumulative %	0.46	0.74
GII	0.08	0.66
Gv	-0.25	-0.44
Pm ^{2}	0.44	-0.40
Mgr	0.47	0.24
Y	0.50	0.12
C	0.49	-0.33

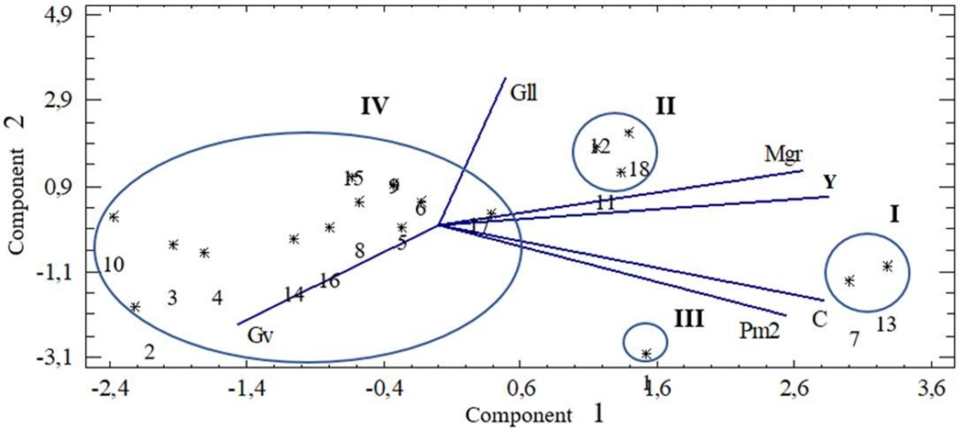

Figure 1. Distribution of genotypes and original variables in the first two components

Table 4. Distribution of genotypes and means by classes, according to Principal Component Analysis

Groups	GII	Gv	Pm²	Mgr	Y	C
I	156.25	10.27	750.25	31.50	8.455	121.00
II	178.64	7.93	530.50	31.39	7.61	107.00
III	116.85	11.55	701.00	30.40	6.53	121.00
IV	159.96	10.80	519.50	29.56	4.98	103.67
Groups			**Genotypes**			**Effectives**
I			INCA LP-7+Biobras-16®. INCA LP-7+QuitoMax®			2
II			Line 3+Biobras-16®. Line 4+ Biobras-16®. Line 4+QuitoMax®			3
III			INCA LP-7			1
IV			INCA LP-5. Line 1. Line 2. Line 3. Line 4. INCA LP-5+ Biobras-16®. Line 1+Biobras-16®. Line 2+Biobras-16®. INCA LP-5+QuitoMax®. Line 1+QuitoMax®. Line 2+QuitoMax®. Line 3+QuitoMax®			12

suggesting the presence of a great genetic variability, which is important, since it gives a wide spectrum of selection to breeders (9). In another study on genotype-environment relationship, where this multivariate technique was also used in coffee cultivation, its effectiveness was demonstrated (18).

The opposite projection of the empty kernels on the first axis, in relation to panicles per square meter, mass of 1000 kernels, yield and cycle, means that the lower the number of empty grains, the higher the value of the other four characteristics. In addition, the figure shows the degree of association between the variables, which is determined by the angular separation formed by their projections and the distances of each of these from the origin, their contribution being greater the more distant they are.

According to the angular spacing, the best associations are constituted by yield and mass of 1000 grains and cycle with panicles per square meter. These four characters are the most important because they are the most distant from the origin.

Groups I and II located on the far right of component 1, which included the cultivar INCA LP-7 and Line 4 in combination with the products under study and Line 3 with Biobras-16®, were characterized by the highest values for all traits, except for empty grains per panicle. Due to its position close to these, group III, composed of the INCA LP-7 control, showed similar characteristics to group II, but its position in the lower part of component two places it as the genotype with the highest number of empty grains per panicle. It is known that biostimulants are organic substances used to enhance plant growth and development, and they have a positive influence on resistance to stress conditions and pest control, and therefore on yields. Various investigations in different crops have confirmed their effectiveness (19-23).

The genotypes contained in-group IV exhibited the discrete values of the characters studied, including commercial lines and cultivars with the application of the products and those to which no biostimulant was applied. In this sense, it is stated that biostimulants should be used in small amounts as a complement in fertilization and pest or disease control, in order to increase yield, quality and protection against adverse climatic conditions (24).

The results of the multiple linear regression analysis are shown in Table 5, where yield is the dependent variable and full and empty grains per panicle, number of panicles per square meter, mass of 1000 grains and cycle were the independent variables.

The model proposed by the multiple linear regression analysis allows, through the estimated coefficients, to express the expected change of the dependent variable yield for each unit of change of the independent variables studied. Since the p-value in the analysis of variance is less than 0.01, there is a statistically significant relationship

Table 5. Results of the Multiple Linear Regression Analysis where the dependent variable is yield

Parameter	Estimate	Standard Error	T-Statistic	P-Value
Constante	-12.9269	6.07742	-2.12705	0.0548
Gll	-0.0390683	0.0200319	-1.9503	0.0749
Gv	-0.240882	0.13377	-1.80072	0.0969
Pm^2	0.0182466	0.00524716	3.47742	0.0046
Mgr	1.49982	0.439376	3.41351	0.0051
C	-0.259498	0.098433	-2.63629	0.0217

ANALYSIS OF VARIANCE

Source	Sum of squares	GL	Mean square	F	P-Value
Modelo	32.8549	5	6.57097	8.00	0.0016
Residuo	9.85239	12	0.821033		
Total	42.7073	17			
R2	86.93				

between the variables at a 99 % confidence level. The characters full and empty grains present a p-value higher than 0.05; the highest in the independent variables, thus being the ones that contribute the least information to the model.

The equation of the fitted model is:

$$R = -12{,}9269 - 0{,}0390683 \times Gll - 0{,}240882 \times Gv + 0{,}0182466 \times Pm2 + 1{,}49982 \times Mgr - 0{,}259498 \times C$$

The R2 statistic indicates that the model explains 86.93 % of the variability in performance, determining that the linear combination of the independent variables, for studies under similar conditions, is an optimal predictor of performance. Other research has used this analysis with good results (2,11,25).

It is suggested that the success of any crop improvement program depends largely on the magnitude of genetic variability, heritability, genetic advance and the association of characters, hence the importance of this type of work for plant breeders (26).

CONCLUSIONS

- Most of the variables evaluated show correlations with each other, except full grains per panicle, which was not interrelated with any other character. Yield correlates strongly, directly with the mass of 1000 grains, panicles per square meter and cycle and inversely with empty grains per panicle.
- The Principal Component analysis explains 74 % of the total variance in the two new variables; all the original variables contribute to the first component, except full and empty grains per panicle, which contribute to the second. Groups I and II, which include the cultivar INCA LP-7 and Line 4 in combination with the products under study and Line 3 with Biobras-16 , are characterized by having the highest values for all traits, except for empty kernels per panicle.
- The model proposed by the multiple linear regression analysis explains more than 85 % of the variability in yield, being an optimal predictor of this trait for studies under similar conditions.

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