Original article

Evaluation of rice advanced lines (*Oryza sativa* L.) obtained by hybridization in Los Palacios, Cuba

Evaluación de líneas avanzadas de arroz (*Oryza sativa* L.) obtenidas mediante hibridaciones en Los Palacios, Cuba

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ABSTRACT: The obtaining new rice cultivars more productive in a context increasingly influenced by the effects of climate change and heterogeneous growing conditions, constitutes an important challenge for breeders. This research was developed with the objective of studying the behavior of seven advanced rice lines under flooded conditions and selecting the best ones to move on to a higher phase. A completely randomized experimental design with three repetitions was used and the genotypes constituted the treatments. The obtained information was processed through univariate and multivariate analyses. The results showed strong correlations of yield with panicles per square meter, 1000 grain mass and cycle. The Principal Components Analysis explains 83 % of the total variance in its first two axes and the proposed multiple linear regression analysis model manifests more than 85 % of the yield variability. The combination of univariate and multivariate analyzes facilitated the identification of lines G/L 4, 5, 2, 3 as the most promising to include in validation studies.

Key words: cultivars, correlations, plant breeding, yield performance.

RESUMEN: La obtención de nuevos cultivares de arroz más productivos en un contexto cada vez más influenciado por los efectos del cambio climático y las heterogéneas condiciones de cultivo, constituye un desafío importante para los mejoradores. Esta investigación se desarrolló con el objetivo de estudiar el comportamiento de siete líneas avanzadas de arroz en condiciones de aniego y seleccionar las mejores para pasar a una fase superior. Se utilizó un diseño experimental completamente aleatorizado con tres repeticiones y los genotipos constituyeron los tratamientos. La información obtenida fue procesada mediante análisis univariados y multivariados. Los resultados mostraron correlaciones fuertes del rendimiento con las panículas por metro cuadrado, masa de 1000 granos y ciclo. El análisis de Componentes Principales explica el 83 % de la varianza total en sus dos primeros ejes y el modelo del análisis de regresión lineal múltiple propuesto manifiesta más del 85 % de la variabilidad en el rendimiento. La combinación de los análisis uni y multivariado facilitó la identificación de las líneas G/L 4, 5, 2 y 3 como las más promisorias para incluirlas en estudios de validación.

Palabras clave: cultivares, correlaciones, fitomejoramiento, rendimiento.

INTRODUCTION

Rice is one of the most important staple food crops in the world, on which more than half of the global population depends as a primary daily source of calories and protein (1-3). In recent years, rice production and research have faced unprecedented challenges. Various institutes and universities around the world have launched numerous research initiatives to meet the growing demand for this crop, most of which aim to address one of two fundamental challenges: (1) breaking the yield barrier or (2) improving sustainability (4).

In Cuba, rice consumption is high, making it one of the countries with the highest per capita intake (70 kg per year). However, national rice production does not meet internal demand due to low crop yields, resulting in more than half of the rice consumed being imported (5). For this reason, strategies must be developed to increase rice production, with special emphasis on the application of science, technology, and innovation.

The well-established National Rice Breeding Program is a major strength. It has generated long-, medium-, and short-cycle cultivars that form part of the country's varietal structure, such as INCA LP-5, INCA LP-7, and Ginés LP-18. There is no need to import quality seeds for planting, as all seeds used are produced domestically. The INCA LP-5 cultivar has remained in Cuban rice production for 20 years, while others such as Anays LP-14 and Roana LP-15 have not been widely adopted by state enterprises but have been well accepted within the cooperative farming system (6). Despite progress in recent years related to the development and registration of high-yield rice cultivars for various conditions, breeders continue working to develop new cultivars that combine good yield with tolerance to biotic and abiotic factors, addressing the challenges posed by climate change.

For decades, breeding strategies have included methods such as selection, hybridization, mutation induction, and more recently, genome editing and sequencing technologies, tissue culture, and transformation methodologies that significantly facilitate rice improvement (7). In Cuba, hybridization has been the main approach, through which most of the commercial cultivars currently grown have been obtained. This method allows for expanded combinatorial possibilities by crossing individuals and genotypes with different traits. Based on the background outlined above, this research aims to evaluate the performance of seven advanced rice lines obtained through hybridization under flooded conditions, and to select those with the greatest potential to advance to a higher phase for subsequent validation and dissemination.

MATERIALS AND METHODS

The trial was conducted during the low-rainfall period (February to July 2022-2023) at the Basic Scientific-Technological Unit (UCTB) Los Palacios, part of the National Institute of Agricultural Sciences, under flooded conditions and on Hydromorphic Gley Nodular Petroferric soil (8). Seven advanced rice lines (Table 1), resulting from the crop improvement program implemented by INCA, were evaluated. These lines were obtained through hybridization and selected using the pedigree method.

A completely randomized design with three replications was used, and the lines constituted the treatments. Direct seeding was carried out in the field using the drill method, in plots of 2 m², with a spacing of 15 cm between rows and 50 cm between plots. Crop management practices were performed throughout the rice cycle (land preparation, sowing, fertilization, irrigation, and phytosanitary treatments) according to the Technical Guidelines for Rice Cultivation (9).

Table 1. Studied lines and their progenitors

Treatment	Lines	Progenitors
T1	L 1	IR1529/INCA LP-5
T2	L 2	INCA LP-4/VN 2084
T3	L 3	China/INCA LP-5
T4	L 4	Somewake/8073
T5	L 5	Somewake/J-104
T6	L 6	IR1529/INCA LP-5
T7	L 7	Bolito/INCA LP-4

Prior to harvest, the following traits were evaluated based on the Standard Evaluation System for Rice of the International Rice Research Institute (10):

- Panicle length: PL (cm)
- Number of filled grains per panicle: FG
- Number of unfilled grains per panicle: UFG
- Mass of 1000 grains: Mg (g)
- Number of panicles per m²: Pm^2
- Yield: Y (t ha^{-1})
- Cycle to 50 % flowering: C (days)

Panicles per square meter were sampled once per plot using a 0.25 m² frame. Filled and unfilled grains per panicle, as well as the mass of 1000 grains, were determined from 20 central panicles randomly selected. Agricultural yield, at 14 % moisture content, was calculated over an area of 1 m².

The available data were processed using one-way analysis of variance (ANOVA) for fixed effects, and means were compared using Duncan's multiple range test. The data matrix (genotype × variable) was analyzed using multivariate principal component analysis, Pearson correlations, and multiple regression to evaluate the magnitude and direction of the relationship between yield (dependent variable) and the remaining variables, using the IBM SPSS statistical package v.22 in all cases.

RESULTS AND DISCUSSION

Significant differences were found among the lines for all traits, according to the analysis of variance (Table 2), reflecting the phenotypic variability of the evaluated germplasm. Similar results have been reported by other authors (11,12).

Line 7 exhibited the longest panicles, followed by lines 4 and 5, while line 6 showed the lowest value. The rice panicle is a fundamental reproductive structure and represents the transition from vegetative to reproductive growth in the plant. Its architecture which includes components such as the rachis, primary and secondary branches, seed number, and branch length has a profound influence on grain production (13).

However, some studies reveal a negative association between panicle size and the number of panicles per unit area, which complicates the development of genotypes with both high tillering capacity and exceptionally long panicles (14). For these reasons, panicle architecture remains the focus of numerous current investigations (11,15,16).

Table 2. Results of the analysis of variance (ANOVA) for each evaluated variable

Treatment	PL	FG	UFG	Pm²	Mg	Y	C
L 1	23.65 c	129 b	6 c	437 a	29.47 c	6.93 c	98 d
L 2	23.33 c	136 b	8 bc	437 a	30.27 b	7.83 ab	108 b
L 3	23.91 c	90 d	6 c	333 c	26.97 d	6.60 cd	91 e
L 4	27.03 b	169 a	18 a	452 a	29.37 c	7.50 b	104 c
L 5	27.12 b	128 b	11 b	448. a	31.77 a	8.17 a	117 a
L 6	22.69 d	92 d	6 c	356 c	27.30 d	6.37 d	96 d
L 7	27.74 a	116 c	21 a	408 b	26.00 e	6.93 c	103 c
MG	25.07*	122.95*	11.00*	410.14*	28.73*	7.19*	102.29*
SE	0.45	5.67	1.36	2.53	0.43	0.15	1.77
CV	8.13	21.14	56.71	11.28	6.79	9.35	7.91

*Means followed by the same letter do not differ significantly (Duncan's multiple range test. $p \leq 0.05$)

Regarding filled and unfilled grains per panicle, line 4 showed the highest values for both traits, although no significant differences in unfilled grains were found compared to line 7. Lines 1, 2, 3, and 6 had the lowest number of unfilled grains, with no significant differences among them. The number of grains per panicle is the main trait determining grain yield in rice, and other panicle-related traits influence this number [16]. Some authors consider that the two most important panicle-related traits are the number of filled grains per panicle and their weight, as there are genotypes with long panicles but few grains. Likewise, to produce genotypes with high yield potential based on a considerable number of grains per panicle, plants must have the ability to fill those grains [14].

As for spikelet sterility, it is considered normal when ranging between 10 and 15 %. In this case, values for the unfilled grains trait fluctuated between 4.6 and 15.5 % across all evaluated lines.

Lines 1, 2, 4, and 5 had the highest number of panicles per square meter, with no significant differences among them, while lines 3 and 6 showed the lowest values for this trait. The number of spikelets per m² may be determined by genotypes [17]; these represent sink size and are considered among the most important factors determining cereal crop yield. In rice cultivation, sink size can be improved by increasing the number of panicles per m² or the number of spikelets per panicle or both. Generally, higher yield is achieved by increasing one of these traits, as increasing both is difficult due to a strong compensatory mechanism between them [18].

Line 5 had the heaviest grains, while line 7 showed the lowest grain weight, both with statistically significant differences from the rest. Rice grain weight varies approximately between 10 and 50 mg per grain and is commonly expressed as the weight of 1000 grains at 14 % moisture. It is determined by seed filling and volume, influenced by the growth of the spikelet hull [19].

Yields ranged from 6.37 to 8.17 t ha⁻¹, with line 5 standing out, although not significantly different from line 2. In contrast, the lowest values were observed in lines 3 and 6. Rice yield in Cuba is considered low, and crop management must aim to close the gap for this trait by creating conditions that allow its maximum potential to be expressed. As with other crops, rice productivity also depends on the environment, and models predict that climate change is likely to alter numerous parameters that could lead to unfavorable growing conditions. Therefore, to address these challenges, it is essential to develop cultivars with higher yield potential and resistance to multiple environmental conditions [4].

The growth cycle ranged from 91 to 117 days. The shortest materials were lines 1, 6, and 3, all under 100 days, with line 3 reaching 50 % flowering at 91 days, showing significant differences from the rest, while line 5 had the longest cycle. Rice germplasm varies widely in the total time required to reach maturity, allowing breeders to develop cultivars suited to local conditions and farming practices. Those grown in the tropics are photoperiod-insensitive, and their maturation time ranges from 90 to 160 days. Some authors report that the optimal rice growth cycle appears to be between 110 and 135 days, as cultivars maturing within this range tend to yield more than those maturing earlier or later (under most favorable agronomic conditions) [14].

Yield was strongly and positively correlated with panicles per square meter and the mass of 1000 grains (Table 3). To identify cultivars with greater productive potential in rice cultivation, research has been conducted on genetic variability and correlations between yield and its components. Several authors have reported similar results, at least for one of these two relationships [11,13,18,20-22], although negative correlations between yield and 1000-grain weight have also been reported [18].

The trait "growth cycle" showed a strong positive correlation with yield. These results are consistent with those obtained in similar studies conducted under the edaphoclimatic conditions of Los Palacios [23].

Other strong positive correlations were observed between unfilled grains and panicle length, as well as between filled grains and cycle with panicles per square meter. In this case, filled grains per panicle did not correlate with yield; however, other studies have reported correlations between these traits [16,18,23]. Trait correlations are not fixed-they may vary-and the diversity of opinions regarding the relationships between yield and its components, along with evidence that evaluations have been conducted under different conditions, suggests that this may be due to the use of different varietal compositions and environmental effects.

Table 3. Phenotypic Correlation Matrix

	FG	UFG	Pm2	Mgr	Yield	C
PL	0.466	0.893**	0.474	0.073	0.451	0.543
FG		0.501	0.887**	0.568	0.672	0.543
UFG			0.411	-0.186	0.257	0.356
Pm2				0.726	0.802*	0.765*
Mgr					0.848*	0.747
Yield						0.922**

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

Understanding the relative contribution of individual traits to yield can be achieved through correlation studies. Therefore, to increase grain yield in rice, direct selection of genotypes based on positively correlated traits will be more fruitful when planning any breeding program for this crop (20).

Eigenvalues, contribution percentages, and cumulative variance of the first two principal components from the principal component analysis are shown in Table 4, based on the criterion that components with eigenvalues explaining more than 70 % of the total variance are considered acceptable. This approach helps reduce the dimensionality of the dataset by creating significant principal components that contribute to the maximum variability among genotypes (11).

Table 4. Eigenvalues, percentage of contribution and cumulative variance of the components, and correlations with the original variables

	C1	C2
Eigenvalues	4.34	1.46
% contribution	0.62	0.21
% cumulative	0.62	0.83
Panicle length	-0.53	0.59
Filled grains	-0.85	0.21
Unfilled grains	-0.44	0.78
Panicles/m^2	-0.97	0.09
Grain mass	-0.79	-0.57
Yield	-0.91	-0.30
Cycle	-0.87	-0.22

The first principal component contributed 62 % of the total explained variance, and correlations with the original variables show that panicles per square meter, yield, and filled grains per panicle, 1000-grain weight, and growth cycle were among the traits that contributed most each negatively. Meanwhile, unfilled grains per panicle and panicle length contributed positively to the second component, which explained 21 % of the total variance.

Similar results to those of this trial have been reported in various studies conducted in India using principal component analysis, although the percentage contributions to total variation among genotypes differ. Two components explained 69 % (29), three compiled 72.1 % (16), and five accounted for 74.4 % (28). It is important to note that the first component should explain the largest portion of total variance; in this study, it explained 62 %, and the first two components together explained 83 % both values considerably higher than those previously reported.

The graphical positioning of the lines and original variables on the plane formed by the two components is shown in Figure 1. The seven evaluated lines are distributed into four groups, and the degree of association among variables can also be observed, determined by the angular separation of their projections and their distances from the origin. The leftward projection on the first component of traits such as panicles per square meter, yield, filled grains per panicle, 1000-grain weight, and growth cycle indicates that the lines located at this end (lines 4 and 5), which form Group I, exhibit the highest values for these variables.

The mean values for each trait and the cultivars belonging to each group are presented in Table 5. Group I showed the best results for yield, 1000-grain weight, panicles per square meter, and filled grains per panicle that is, for yield and its components as well as the longest growth cycle.

It is known that rice grain yield is mainly determined by three visible morphological traits: grain weight, number of grains per panicle, and effective number of panicles. These traits are influenced by a series of "invisible" physiological factors, including nutrient use efficiency and photosynthetic efficiency, which cannot be directly observed and therefore limit the advancement of rice yield improvement. Understanding the molecular basis underlying these physiological traits will be of great help in further enhancing this character (31).

Group II (lines 1 and 2) followed Group I in terms of yield, filled grains per panicle, and 1000-grain weight, and was also characterized by having the lowest values of unfilled grains after Group IV.

Group III had the longest panicles, the highest number of unfilled grains, and the lowest 1000-grain weight. Meanwhile, Group IV, which included lines 3 and 6, exhibited the shortest growth cycle, the lowest number of filled and unfilled grains, the shortest panicles, and the most modest results in terms of yield and panicles per square meter. However, despite being earlier-maturing, these lines achieved yield values that doubled the national average. Moreover, these materials offer advantages such as better use of the planting calendar, reduced demand for water, fertilizers, and phytosanitary products, and greater chances of escaping adverse climatic events.

The results of the multiple linear regression analysis are shown in Table 6, where the dependent variable is yield, and the independent variables include panicle length, filled and unfilled grains per panicle, 1000-grain weight, growth cycle, and number of panicles per square meter.

Based on the estimated coefficients, the model proposed by the multiple linear regression analysis allows the expected change in the dependent variable (yield) to be expressed for each unit change in the independent variables studied.

Since the p-value from the analysis of variance is less than 0.01, there is a statistically significant relationship among the variables at a 99 % confidence level. The variable contributing the least information to the model is 1000-grain weight, with the highest p-value among the independent variables greater than 0.05.

The R^2 statistic indicates that the model explains 85.08 % of the variability in yield, confirming that the linear combination of the independent variables, under similar conditions, serves as an optimal predictor of Yield
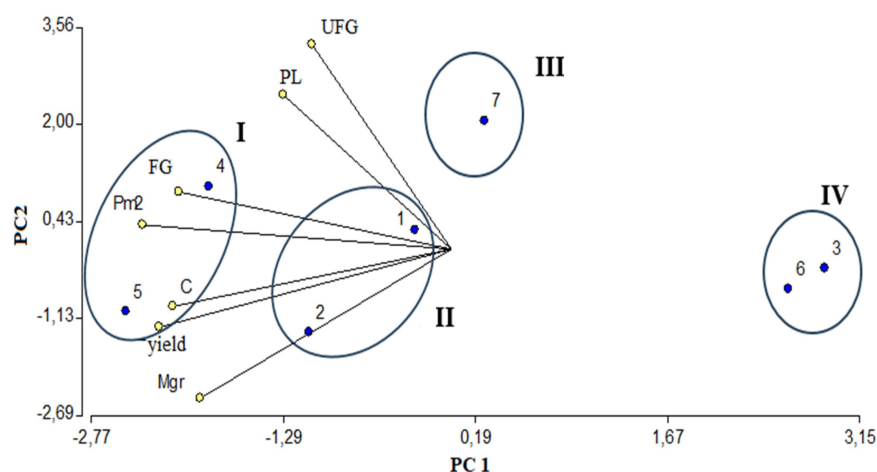

Figure 1. Distribution of genotypes and original variables according to principal component analysis

Table 5. Distribution of genotypes and class means according to principal component analysis

Groups	PL	FG	UFG	Pm2	Mgr	Yield	C
I	27.08	148.17	14.75	450.16	30.57	7.83	110.33
II	23.49	132.71	7.15	436.95	29.87	7.38	102.67
III	27.74	116.34	21.33	407.68	26.00	6.93	102.67
IV	23.30	91.26	5.92	344.52	27.13	6.48	93.67
Groups			**Genotypes**				**Effective**
I			G/L 4. G/L 5				2
II			G/L 1. G/L 2				2
III			G/L 7				1
IV			G/L 3. G/L 6				2

Table 6. Results of the multiple linear regression analysis

Parameter	Estimate	Standard Error	t-Statistic	P-Value
Intercept	-1.82363	1.67222	-1.09055	0.2939
PL	-0.00407572	0.0750251	-0.0543248	0.9574
FG	0.00526801	0.00572438	0.920276	0.3730
UFG	0.0284118	0.0320889	0.885411	0.3909
Pm2	-0.0205301	0.0146701	-1.39945	0.1834
Mgr	0.221961	0.107452	2.06569	2.06569
C	0.0379665	0.0201533	1.88389	0.0805
ANALYSIS OF VARIANCE				

Source	Sum of Squares	GL	Mean Square	F	P-Value
Model	7.70671	6	1.28445	13.31	0.0000
Residual	1.35138	14	0.0965273		
Total	9.0581	20			
R^2	85.0809				

Yield = -1.82363 + 0.0379665***C** + 0.00526801***FG** + 0.0284118***UFG** - 0.00407572***PL** + 0.221961***Mgr** - 0.0205301***Pm2**

CONCLUSIONS

The combination of univariate and multivariate analyses facilitated the identification of the most promising lines under the conditions in which the trial was conducted. Therefore, it is recommended to select lines 4 and 5, which were placed in Group I and showed the best results according to the principal component analysis, and to include lines 2 and 3 based on the analysis of variance, as they also exhibited excellent performance in terms of yield and some of its components. Additionally, the potential of line 3 for earliness should be leveraged. Thus, it is proposed that these four lines proceed to the validation phase.

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