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Original Article

Response of promising lines of rice in Los Palacios municipality, Cuba

Respuesta de líneas promisorias de arroz en finca del municipio Los Palacios, Cuba

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ABSTRACT: The trial was carried out on a farm belonging to the Credit and Service Cooperative Abel Santamaría in Los Palacios municipality. The analyzed plant material consisted of six rice genotypes, four new advanced lines obtained through the hybridization method and the commercial cultivars INCA LP-5 of short cycle and INCA LP-7 of medium cycle. A completely randomized design consisting of six treatments with three replicates each was used and 11 qualitative and nine quantitative traits were evaluated. The data obtained were processed by simple rank analysis of variance (ANOVA) and the means were tested by Duncan's Multiple Range Test at 5 %. For qualitative traits, the results showed that all genotypes were characterized by erect growth habit, lack of anthocyanin pigmentation, cleft-type ligule, absence of ridges, and resistance to lodging and straw color of the paddy grain. Among the new genotypes, lines 3 and 4 showed the longest leaves and panicles, a good performance of full grains per panicle and grain mass, as well as a low number of empty grains, in addition to the best values in the characters panicles per square meter and yield, respectively. Line 2 presented the shortest cycle and showed high values of panicles per square meter and yield. The systematic study of genetic diversity analysis is essential to take full advantage of the inherent variability and broaden the genetic base of rice cultivars.

Key words: Oryza sativa L., yield, panicle, plant breeding.

RESUMEN: El ensayo se llevó a cabo en una finca perteneciente a la Cooperativa de Créditos y Servicios Abel Santamaría del municipio Los Palacios. El material vegetal estudiado estuvo constituido por seis genotipos de arroz, cuatro nuevas líneas avanzadas obtenidas mediante el método de hibridaciones y los cultivares comerciales INCA LP-5, de ciclo corto e INCA LP-7, de ciclo medio. Se utilizó un diseño Completamente Aleatorizado compuesto por seis tratamientos con tres réplicas cada uno y se evaluaron 11 caracteres cualitativos y nueve cuantitativos. Los datos obtenidos fueron procesados mediante un análisis de varianza de clasificación simple (ANOVA) y se docimaron las medias con la Prueba de Rangos Múltiples de Duncan al 5 %. Para los caracteres cualitativos, los resultados mostraron que todos los genotipos se caracterizaron por tener porte erecto, carencia de pigmentación antociánica, lígulas de tipo hendida, ausencia de aristas, resistencia al acame y color paja del grano paddy. Entre los nuevos genotipos, las líneas 3 y 4 presentaron las hojas y panículas más largas, un buen comportamiento de granos llenos por panícula y masa del grano, así como un bajo número de granos vanos, además de los mejores valores en las variables panículas por metro cuadrado y rendimiento, respectivamente. La línea 2 presentó el ciclo más corto y mostró valores altos de panículas por metro cuadrado y rendimiento. El estudio sistemático de análisis de la diversidad genética es esencial para explotar lo inherente a la variabilidad y ampliar la base genética de cultivares de arroz.

Palabras clave: *Oryza sativa* L., rendimiento, panícula, mejoramiento genético.

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INTRODUCTION

Rice is consumed mainly as a staple food in Asia, Latin America and the Caribbean, and increasingly in Africa. Global consumption of rice is expected to increase by 1.1 per cent annually. As in the last decade, Asian countries accounted for 70 per cent of the projected increase. In Latin America and the Caribbean, imports and exports of cereals will account for almost 30 per cent of domestic consumption and production respectively by 2031 (1). As the world population grows, so does the demand for food. The least costly way to keep pace with population growth is to increase rice production wherever possible. The gap between current and potential yields remains very large in many rice-producing countries. Efforts should therefore be directed at identifying the root causes of this difference (2).

The world's attention and pressure on researchers is to substantially increase the rate of production in order to meet the target projected for mid-century. Ways to increase rice yield have focused on several lines, including genetic improvement, genetics and biotechnology (3).

Using "traditional" breeding methods, it takes at least 8 to 10 years to produce a new cultivar: painstaking work including laboratory experiments and a series of field trials as well as quality analysis, field testing to determine adaptability to agricultural practices and different environments, and selection for other desired characteristics. Thereafter, two to three years of official evaluations are required before the new line can be approved on the official list for cultivation and marketing. This process will be continuous as old diseases may spread and emerge in new areas, or new strains of pathogens may appear that provoke a break in varietal resistance and also environmental factors change (4).

In Cuba, the genetic breeding program has released several rice cultivars with high yield potential, adapted to adverse factors; however, causes such as poor cultural practices, deterioration of soil properties, pests, some changes in climatic variables, among others, negatively affect the productive results, with low yields still not exceeding 3.6 t ha^{-1} as a national average (5).

Several factors are weighing on the drop in grain production, including COVID-19 pandemic, rising input prices, disruption of the international supply chain and the strengthening of US government blockade measures. Calculations of the Cuban Ministry of Agriculture set production in 2023 at 538 thousand tons of rice consumption, which meant having to import about 200 thousand tons but, falling the rates of sowing and production, the deficit of grain increases. With a complex and distant reality from that existing before the pandemic, Cuban agriculture has the challenge of rescuing the crop under new strategies. The application of agro-ecological techniques, less use of chemical products and the use of seeds of national production are part of the effort in this direction and, on the science side, the strengths are highlighted, since all the cultivars used in the country are Cuban, generated by the Grain Research Institute and the National Institute of Agricultural Sciences (INCA), which contribute to the National Crop Genetic Breeding Program (6).

For all the above, this work aims to study the behavior of advanced lines of rice from the national program of genetic breeding of this crop in areas of the peasant cooperative sector for their subsequent incorporation into the national system.

MATERIALS AND METHODS

The trial was carried out on a farm belonging to the Credit and Services Cooperative (CCS) Abel Santamaría of Los Palacios municipality, on a Fluvisol soil (7).

The plant material studied was a total of six rice (*O. sativa* L.) genotypes, including four new advanced lines obtained by the hybridization method and as a result of a subsequent selection process, and included as control the commercial cultivars INCA LP-5 (short cycle) and INCA LP-7 (medium cycle) with good performance under the country's production conditions (Table 1).

Table 1. List of genotypes studied and their origin

Genotypes	Crossing
INCA LP-7	Somaclon of Amistad 82
INCA LP-5	2077 / CP1-C8
Line 1	INCA LP-5 / IR 1529
Line 2	*Oryza glaberrima* / Amistad 82
Line 3	China / INCALP-5
Line 4	INCA LP-5 / 2084 Vietnamita

A completely randomized design consisting of six treatments (genotypes) with three replicates each was used. The genotypes were transplanted in the field on plots 2 m long by 2 m wide (4 m^2) at a distance of 0.15 m between seedlings and 0.50 m between plots.

The work and phytotechnical attention (preparation of the soil, seedling, transplanting, fertilization, irrigation and phytosanitary treatments) were carried out according to the Technical Instruction for Rice Cultivation (8).

Observations were made on 10 plants randomly selected in each plot. For qualitative variables, the value of fashion was considered and, for quantitative variables, the value of the average of the measurements made.

The following qualitative and quantitative characteristics were evaluated using IRRI's Rice Standard Evaluation System as methodology (9).

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

When the variables were counted and measured, the data obtained were processed by means of a simple classification variance analysis (ANOVA) and the averages were dosed with the 5 % Duncan Multiple Range Test using the statistical program STATGRAPHICS Plus v.5.

Qualitative characteristics:		Evaluation phase	
1.	Intensity of leaf green colour (IL).	Flowering	
2.	Anthocyanin pigmentation (AP).	Flowering	
3.	Plant size (PS).	Flowering	
4.	Edges (ED).	Post-harvest	
5.	Ligula form (LF).	Flowering	
6.	Panicle excersion (PE).	Ripening	
7.	Color of paddy grain (CG).	Ripening	
8.	Resistance to lodging (RA).	Ripening	
9.	Shelling strength (SS).	Ripening	
10.	Blade senescence (BS).	Ripening	
11.	Plant vigor (V).	Seedling	
Quantitative characteristics:		**Evaluation phase**	**UM**
12.	Crop cycle at 50 % flowering (C)	Flowering	days
13.	Length of the flag blade (LFB)	Flowering	cm
14.	Width of the flag blade (WL)	Flowering	cm
15.	Yield (Y)	Ripening	t ha^{-1}
16.	Number of lobes per m^2 (Pm2).	Ripening	quantity
17.	Length of panicle (LP)	Ripening	cm
18.	Number of grains filled per panicle (Gf)	Post-harvest	quantity
19.	Number of empty grains per panicle (Ge).	Post-harvest	quantity
20.	Mass of 1000 grains paddy (Mgr).	Post-harvest	g

RESULTS AND DISCUSSION

Table 2 shows the characterization of the six genotypes evaluated according to qualitative traits, showing similarities in six of the 11 analyzed traits.

All genotypes were characterized by erect bearing, lack of anthocyanin pigmentation (not observed in any organ of the plant), cleft type ligules, absence of edges, and resistance to lodging and straw color of paddy grain. Differences were found for the rest of the characteristics. However, homogeneity in some of the evaluated characters is desirable, such as erect bearing, absence of edges and resistance to scraping.

Morfoagronomy characterization is a complementary activity that consists in describing the attributes of genotypes to determine their usefulness and identify promising for genetic selection and breeding processes. These studies are frequent and provide detailed information to establish differentiation between cultivars (10-17).

The fact that the four lines studied had an erect bearing confirms the importance which breeders attached to this character at the time of selection. Ideal architecture is a new strategy for rice cultivation with very high yields (18). Significant advances have been made in recent years in understanding how plant architecture is controlled under various growing conditions (19). It is mainly determined by the height of the plant, the size and the morphology of the panicle and contributes greatly to the grain yield of rice. Some recent research has focused on the exploration of their molecular mechanisms as they provide valuable theoretical guidance for improving rice cultivars with ideal architecture, also point out that environmental factors influence the plasticity of this and the importance of analyzing them (20).

In other rice characterization trials conducted in Cuba, no differences were found between the cultivars evaluated for the qualitative characteristics anthocyanin pigmentation and ligula shape (21). Recent studies suggest that anthocyanins are an important group of secondary metabolites believed to play an important role in plant function. They are also relevant in human nutrition. Colored rice is becoming increasingly popular with consumers because of its nutraceutical properties and, among farmers, due to its higher economic value (13).

Table 2. Results of the evaluation of qualitative characteristics

GENOTYPES	PS	IL	AP	LF	ED	PE	RA	SS	BS	CG	V
INCA LP-7	E	DG	AU	C	AB	ME	R	I	L	PJ	V
INCA LP-5	E	DG	AU	C	AB	E	R	I	L	PJ	VV
Line 1	E	DG	AU	C	AB	WE	R	I	I	PJ	VV
Line 2	E	LG	AU	C	AB	E	R	I	L	PJ	V
Line 3	E	DG	AU	C	AB	ME	R	D	L	PJ	VV
Line 4	E	DG	AU	C	AB	WE	R	I	L	PJ	V

Erect **(E),** Cleft **(C),** Emerged **(E),** Vigorous **(V),** Dark Green **(DG),** Intermediate **(I),** Well Emerged **(BE),** Light green **(LG),** Resistant **(R),** Late **(L),** Very Vigorous **(VV),** Absent **(AB),** Difficult **(D),** Straw **(PJ),** Moderately emerged **(ME)**

Also, when the genetic diversity of local rice cultivars in eastern India was analyzed, it was reported that in general, considerable variations were observed in seedling vigor, the color of the apiculus, the color of the lemma and the palea and the cover of the seed (15).

Most of the lines and markers showed a dark green color, and only in line 2 light green color was observed. Similar results were found by other authors in analogous works (22). Also a morfoagronomic characterization study of three accessions of aromatic pigmented rice, revealed that these cultivars produce long, broad and dark green leaves with anthocyanin coloration on the tips, middle nerves and margins. The color of the basal leaves' pods is dark green with a slightly violet hue, while the intensity of the leaves' green color is high with anthocyanin coloration (13).

There were no edges in all genotypes. In this sense it is known that, for the most part, rice cultivars do not have grains with edges or only a few show small edges, so that this character rarely constitutes a problem in breeding. Similar results were obtained when evaluating rice germplasm, where only 22 % presented grain edges (23).

The genotypes presented well-emergent, moderately emergent and emergent panicles, while none showed partially enclosed or included panicles. Panicles must fully emerge from the flag leaf sheath and it is accepted that the completely emerging panicle character is dominant over the partially enclosed or enclosed panicle, although the air temperature and, possibly, the shade that the plant receives significantly changes the expression of the character. In many lines and cultivars, panicles stand out completely if the weather is warm after their initiation, but if it is a little cold, the emergence of the panicles is incomplete (24).

Panicle extraction is an important agronomic variable to consider in the selection process, those that have the ability to emerge completely from the leaf flag avoid sterility (bad filling of the spigot), pathogen attack at the base of the panicle and harvest difficulty. Studies in the Philippines on hybrid rice indicate that the degree of panicle exertion may be influenced by the application of gibberellic acid. The best results were achieved when higher doses were applied, obtaining a greater number of panicles, thus suggesting that the application of gibberellic acid is directly related to the extraction of the panicle (25).

It is important to note that the four new lines had a similar behavior to witnesses, being resistant to lodging. This variable is influenced by the intrinsic genetic characteristics of the cultivar. It is a complex process that expresses the response of the plant to the effects of biotic and abiotic factors, often irreversible losses occur due to reasons of objective and subjective nature. These include violations in crop technology such as unbalanced mineral nutrition, high planting density or improper herbicide treatment. Also during the growing season, weather conditions such as rain, wind, hail or extreme temperatures can cause lodging (26).

In relation to ginning, only line 3 was classified as difficult to ginning with a few detached grains, while lines 1, 2 and 4 behaved as intermediates, similar to the control cultivars. There were no genotypes of easy granulation (50 % of grains detached), although one of the lines shared among its parents *Oryza glaberrima*, which confirms the effectiveness in the selection process. Seed shelling is an important trait related to crop efficiency. Although a number of genes have been identified to control seed shelling in rice, the extensive variation in the genetic architecture of shelling is not yet clear (27). In recent years, several key genetic factors have been identified that regulate shelling in crops, and the selection of desirable mutations has promoted domestication and crop improvement by reducing shelling (28).

In three lines, the senescence was late (2, 3 and 4), similar to cultivars INCA LP-5 and INCA LP-7; only line 1 behaved as intermediate and there was no early senescence. Premature leaf senescence negatively affects grain yield in rice; due to this, several investigations related to this issue have been developed (29-31).

Leaf senescence, the final stage of plant development, in which the change from the carbon capture phase to the nitrogen remobilization phase as the ultimate source of nutrient supply to developing grains occurs, is a highly regulated molecular mechanism. Functional green permanence has been identified as a promising feature that will be introduced to improve the yield potential of rice. Several studies have been carried out on this subject, revealing that the functional green permanence trait of some cultivars seems to come not only from genetic control preventing chlorophyll degradation but also from the greater capacity to absorb nitrogen from the soil due to the strong sustained activity of the roots during period of grain filling (32-34).

The lines were classified as vigorous and very vigorous with similar behavior to the two control cultivars. Early establishment of seedlings in rice is an important character that helps in competition for light, air and water and thus improves tolerance to various abiotic stresses which, consequently, can favorably affect the yield. However, there is not much research on this subject and previous studies focus only on assessing the character itself (35).

The statistical analysis showed significant differences for all quantitative traits at a significance level of 95 % (Table 3). Similar results were obtained in investigations of the same type, where the descriptive statistics of eleven quantitative traits revealed that all these showed a significant variation within the collection of germplasm evaluated (15). Also, in genetic diversity studies of rice accessions, using morphological traits, highly significant differences were observed for all the quantitative characters analyzed, which indicated variability among the 87 accessions studied (10). On the other hand, when morphological variables were analyzed, a significant variation was found for all qualitative and quantitative parameters among nine wild rice species (36).

In this case the cultivar INCA LP-7 showed the highest values of leaf length character followed by lines 3 and 4. Genotype INCA LP-5 and lines 1 and 2 presented the smallest leaves. The leaf width showed its best performance in INCA genotype LP-7 and line 1. Values for this character fluctuated between 1.82 and 1.52 cm, with the thinnest leaves

Table 3. Results of one-way analysis of variance (ANOVA) for quantitative traits

No.	Genotypes	LL	WL	PL	GF	Ge	Pm2	Mgr	Y	C
1	INCA LP-7	27.70 a	1.82 a	27.09 a	116.85 e	11.55 c	701.00 a	30.40 a	6.53 a	121 a
2	INCA LP-5	23.11 e	1.52 e	21.42 d	131.45 d	14.30 a	477.50 e	29.10 c	3.90 e	105 b
3	Line 1	23.17 e	1.77 b	22.15 c	167.50 a	13.50 b	507.00 d	29.05 c	3.92 e	103 b
4	Line 2	23.94 d	1.60 d	22.25 c	148.35 c	10.10 d	589.50 b	27.45 d	5.23 c	97 c
5	Line 3	26.92 b	1.71 c	23.66 b	157.75 b	8.75 f	559.50 c	29.75 b	4.21 d	110 b
6	Line 4	26.25 c	1.52 e	22.32 c	155.25 b	9.80 e	470.00 e	30.90 a	5.99 b	106 b
	General Mean	25.18	1.66	21.48	146.19	11.33	550.75	29.44	4.96	107.00
	Standard Error	0.2542	0.0178	0.3824	2.008	0.1987	8.8962	0.1247	0.1635	0.532
	C. of Variation (%)	8.56	9.02	11.19	9.56	18.89	13.41	3.99	21.22	3.73

Means with equal letters in a column do not differ from each other (Duncan's Multiple Range Test, p≤0.05)

being found in the cultivar INCA LP-5 and line 4. In other investigations, variability had also been detected for both descriptors in the genotypes evaluated (13,23).

Increasing the yield potential of rice is the main objective of breeders and growers involved in crop breeding programs. In this regard, recent research suggests that rice with erect loins is generally high yielding and the loins remain uncurved until ripening. Also, cultivars of erect panicles with short flag leaves may show a higher photosynthetic rate in the ripening stage and therefore this trait could be a potential phenotypic marker for high yield of erect-panicle rice (37).

Panicle length was in the range of 21.42 to 27.09 and longer panicles characterized cultivars INCA LP-7 and lines 3 and 4. By contrast, INCA LP-5 showed more discrete values for this character. In studies on the morphoagronomic characterization of rice germplasm, ranges between 22.84 and 28.28 cm and between 26 and 30 cm of well-emerged lobes are reported and it is suggested that this character contributes positively but is not the only factor responsible for high grain yield (13,23).

The number of grains filled per panicle fluctuated between 116 and 167, with lines 1, 3 and 4 showing the best performance. Cultivars INCA LP-7 and INCA LP-5 were found to have the lowest values for this trait. The number of grains per panicle is known to be one of the components of rice yield and is mainly determined by panicle architecture and branch differentiation (38,39). Some authors suggest that this trait is the critical trait for increasing grain yield in breeding practice (40-42). In a study of yield characteristics covering 200 varieties of japonica rice grown in central China, a significant increase in grain per panicle was observed over the last 30 years, and many associated genes were subjected to artificial selection during the breeding process (43).

As a quantitative canonical trait, the number of grains per panicle is controlled by multiple genes and may be affected by various environmental factors (44,45). The broad heritability of this trait is relatively high, ranging from about 70 to 90 % in different studies, indicating that genetic factors are the main determinant of this trait (46,47).

The mean value for the number of empty grains per panicle was 11.33. The best results were given with lines 3 and 4, which share INCA LP-5 as parent. Several causes are known that affect the empty of rice grains, those related to plant health (different causative agents, use of hormonal herbicides in the stage of fertilization and filling of the grain), but also appear agrochemicals (insufficient or excessive nitrogen, micronutrient deficiency), genetic (non-total emergence of panicles and fertilization capacity) and climatic (relative humidity, strong and dry winds, drought and temperature).

In the number of panicles per square meter, the cultivar INCA LP-7 showed the highest value, statistically; this character behaved in a range between 470 and 701 panicles and among the new genotypes, lines 2 and 3, exhibited the best results. This is the most variable yield component and has been the main limiting factor in agricultural yields under Cuban conditions. Studies conducted in China determined the relationships between yield and agronomic characteristics of rice, finding that the number of panicles per square meter ranged from 232-271 (36). On the other hand, it has been detected that the key characteristics of Indica hybrid rice cultivars include a higher number of panicles (from 250-300 104 ha^{-1}) (48).

The mass of 1000 grains varied between 27.45 and 30.90 g. INCA cultivar LP-7 and line 4 showed the highest value. Line 2 presented the worst behavior. It is argued that the mass of 1000 grains is a stable genetic characteristic in good growing conditions. In research developed in Argentina it is stated that the weight of the grains is specific to the cultivar, although they highlight some variability within each one of them (49).

The yield reached an average of 4.96 t ha^{-1} and its values were between 3.90 and 6.53 t ha^{-1}. Cultivar INCA LP-7 and line 4 were the best performing. The most discrete values were presented by the cultivar INCA LP-5 and line 1. This was the character with the highest coefficient of variation, which could be attributed to the high variability between treatments.

Other authors have found that the phenotypic coefficient of variance was higher than the genotypic coefficient of variation for all the characters they studied, which could be due to environmental factors influencing their expression. Breeders should take this into account when establishing a selection program to improve yield. (50).

Generally, breeded cultivars have a good adoption rate due to their positive impact on production, which is negative for the conservation of local cultivars on farms, which have developed specific characteristics that allow them to adapt to agro-ecosystems. For this reason, it is necessary to protect them through on-farm or *ex situ* conservation.

Therefore, similar efforts should be made to preserve and develop local cultivars to avoid the extinction of useful genetic resources (14).

The average value for the cycle was 107 days, with line 2 being the most precocious; on the contrary, the cultivar INCA LP-7 had the longest cycle, with an average of 121 days and the highest yields as reported above, This confirms that a longer cycle allows the plant to use more nutrients, greater accumulation of dry matter and, generally, higher yields, hence the cultivars that have predominated in Cuba are those of medium cycle. However, the development of early germplasm is one of the fundamental objectives of breeding programs because of the advantages that these cultivars represent by making better use of the planting schedule, using less fertilizers and consuming less water; and they are also less exposed to pests and diseases (51). Precocity as a breeding goal is suitable to escape causes of environmental stress, such as drought or low temperature during the reproductive phase. In addition, a short vegetative period allows more efficient use of irrigation water. Combining in a cultivar the character of precocity with optimal levels of breeding, vigor and yield capacity is a fascinating challenge for plant breeders (24).

CONCLUSIONS

1. Systematic study of genetic diversity analysis is essential to exploit the inherent variability and broaden the genetic base of rice cultivars.
2. The homogeneity in some qualitative characters, specifically in the erect bearing, absence of edges and resistance to lodging, are desirable variables in the studied rice lines.
3. Lines 3 and 4 are characterized by having the most promising quantitative values among the new genotypes: number of panicles per square meter and yield, respectively, and length of panicle, filled grains and vats per panicle, in both.

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