



# AGRONOMIC CHARACTERIZATION OF THREE RICE (*Oryza sativa* L.) CULTIVARS OBTAINED BY *In Vitro* ANTHER CULTURE

## Caracterización agronómica de tres cultivares de arroz (*Oryza sativa* L.) obtenidos mediante el cultivo *in vitro* de anteras

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**ABSTRACT.** Rice is the main source of carbohydrates in Cuban population's diet; therefore, great efforts have been dedicated to breeding studies on this crop. In this sense, a methodological procedure was developed to obtain three rice cultivars, which were planted along with their progenitors under field conditions and agronomically characterized by using 32 qualitative and 19 quantitative descriptors. Results showed a significant and positive correlation between agricultural yield and panicles per square meter and full grains per panicle, whereas a negative correlation with rice Blast effects. Thus, the use of hypocotyl length is suggested as a selection marker in early seasons. All cultivars, were able to form a large number of fertile tillers, despite showing a short-term cycle, which enabled them to achieve the highest yields, with the help of full grains per panicle and 1000-grain mass components.

**RESUMEN.** El arroz en Cuba constituye la principal fuente de carbohidratos en la alimentación de la población, razón por la cual se dedican grandes esfuerzos en los estudios de mejoramiento genético de este cultivo. En este sentido, se desarrolló un procedimiento metodológico, a través del cual se obtuvieron tres cultivares de arroz, los cuales se sembraron en campo junto a sus progenitores y caracterizados agronómicamente con la utilización de 32 descriptores cualitativos y 19 cuantitativos. Los resultados mostraron la existencia de correlación positiva y significativa del rendimiento agrícola con las panículas por metro cuadrado y los granos llenos por panícula y negativa con las afectaciones provocadas por la Piriculariosis. Se sugiere la utilización de la longitud del hipocotilo, como un marcador de selección en épocas tempranas. Todos los cultivares, a pesar de mostrar un ciclo corto, fueron capaces de formar un número elevado de hijos fértiles, que le permitieron, junto con la contribución de los componentes granos llenos por panícula y masa de 1000 granos, obtener los más altos rendimientos.

**Key words:** human nutrition, descriptors, morphology, yield

**Palabras clave:** alimentación humana, descriptores, morfología, rendimiento

## INTRODUCTION

In breeding programs of self-pollinating species, genetic variability available in local or introduced cultivars is used, but when this variability does not exist, the breeder must form new populations for selection. In Cuba, the rice breeding programs for

agro-ecosystems of waterlogging have been based mainly on the hybridizations, by which have been obtained cultivars as 'J-104', 'Amistad'82', 'Perla de Cuba' and 'INCA LP-5', all high yield potential (1) but susceptible to Pyriculariosis.

The *in vitro* anther culture is a method that has been successfully integrated in breeding programs due to its many advantages: reducing the time to obtain cultivars; no economy of financial and material resources; increases the selection efficiency, in both qualitative and quantitative characters and facilitates

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the selection of superior cultivars (2, 3, 4). Its use has allowed the release of high yielding cultivars resistant to pests and better grain quality (5).

Given this problem, a methodological procedure aimed at obtaining Cuban rice cultivars resistant to Pyriculariosis and good agronomic performance, which integrates the *in vitro* anther culture of F<sub>2</sub> plants as a method of improvement was developed, with a proper selection of parents and achieved lines<sup>A</sup>. This study aimed to characterize three rice cultivars obtained with the use of this methodology for their registration of Cuban varieties and provide information to producers and breeders crop for use both in production and in the breeding programs.

## MATERIALS AND METHODS

The methodological procedure for obtaining Cuban resistant rice cultivars to Pyriculariosis and good agronomic performance included the selection of parents in natural infection flowerbeds versus disease, in the locality "Caribe" (site *hot spot* for Cuban conditions, located in the municipality Consolacion del Sur in Pinar del Río) and cultivar identification of high productive potential for the development of crossbreeding program, followed by *in vitro* anther culture of F<sub>2</sub> plants and subsequent selection of lines obtained, which started in the field, taking into account the production potential and resistance to the pathogen; then a second evaluation was performed under controlled conditions and with artificial inoculation of virulent haplotypes identified in Cuba, and finally the evaluation against all the variability of *Pyricularia grisea* Cav. existing in the area "Caribe".

The seeds of the cultivars 'A/IL-11', 'A/V-L4' and 'P1/T-L6', obtained with the use of this methodology, were planted with their parents to good agronomic characters in the field directly, to trickle in plots of 2 m long x 2 m wide (4 m<sup>2</sup>), at a distance of 15 cm between rows and a density of 100 kg ha<sup>-1</sup> seed in the Scientific and Technological Base Unit "Los Palacios" Pinar del Rio province (Table I).

**Table I. Characterized rice cultivars and crosses that originated**

Cultivars	Crossing
'A/V-L4'	Amistad '82/2077
'A/IL-11'	Amistad '82/IR 759-54-2-2
'P1/T-L6'	INCA LP-1/Tetep

<sup>A</sup> Pérez, N. Obtención de cultivares de arroz (*Oryza sativa* L.) resistentes a *Pyricularia grisea* Sacc. con buen comportamiento agronómico. [Tesis de Doctorado]. Instituto Nacional de Ciencias Agrícolas, Mayabeque, Cuba, 2012, 100 p.

The plant breeding work was performed according to the Technical Instructions for the Cultivation of rice<sup>B</sup>. A design of random blocks with four replications was used. Cultivars were characterized agronomically, following the methodology developed by the Directorate of Seed Certification, detailing the description form for the registration of commercial varieties of rice (*O. sativa*), with the use of qualitative and quantitative descriptors; coleoptile color; mesocotyl length (cm); hypocotyl length (cm); predominant growth habit; tillering capacity; color lemma and palea; color apex of the lemma and palea; pubescence of lemma and palea; glumes color; stigma color; insertion angle leaf below the flag leaf; insertion angle of the flag leaf; flag leaf length (cm); flag leaf width (cm); length of the leaf below the flag leaf (cm); blade width below the flag (cm) sheet; hairiness of the leaf blade; color of the leaf blade, ligule color; shape of the ligula; ligule length (mm); atria resistance to detachment; color atria; color leaf sheath; knot color; internode color; subnodal color ring; color at the base of the stem; cycle in days to maturity; plant height (cm); lodging resistance; response to photoperiod; size of the edges; grain color apical panicle; color bead apex of apical panicle; panicle density; panicle exertion; openings at the apex of the panicle grains; panicle fertility; sortable panicle; panicle length, grain length (mm); grain width (mm); filled grains per panicle; mass of 1000 grains (g); panicles per square meter, predominant leaf longevity, agricultural yield (t ha<sup>-1</sup>) and industrial output (% of whole grains) in samples of 1 kg of paddy rice.

Color attributes were evaluated using a color chart (6). The percentage of leaf area affected (% AFA) it was estimated by the Pyriculariosis, during the vegetative phase and in the reproductive phase, percentages of damaged necks were evaluated; both data were transformed to arcsin √%. The data obtained for the cycle, the agricultural and industrial yields, panicles per square meter, grains per panicle and mass of 1000 grains were processed using SPSS version 20 (7), by analysis of double classification variance and means compared by Tukey test at 5 % probability of error; then multiple correlation analysis was performed with all quantitative data.

<sup>B</sup> MINAG. Instructivos Técnicos para el cultivo del arroz. Instituto de Investigaciones del Arroz, La Habana, Cuba, 2008, p. 115.

## RESULTS AND DISCUSSION

In Table II qualitative characteristics evaluated to cultivars 'A/V-L4', 'A/I-L11' and 'P1/T-L6' are presented and its commercial parents 'Amistad'82' and 'INCA LP-1'. It can be seen that ten of these characters allowed the differentiation of cultivars progenitors of good agronomic performance that originated them. Among them the absence of edges in the cultivars stressed that while presenting the parents 'Amistad'82' e 'INCA LP-1' are short, their presence when they are long and in most grains, it is considered an undesirable characteristic for the effects caused in the manufacturing process. Cultivars also have compact panicles, which is an indirect measure of the amount of full grains and the performance and panicles well emerged, which helps to prevent moisture accumulation in the neck, which may be favorable for appearance and disease development.

The light green color of the leaf and stem base distinguished the cultivar 'A/I-L11' of parent 'Amistad'82' e 'INCA LP-1' and of the cultivars 'A/V-L4' and 'P1/T-L6', and the ability of high tillering transmitted by the parent 'INCA LP-1' to the cultivar 'P1/T-L6' distinguished it from 'A/V-L4' and 'A/I-L11'. They are also included within these ten characters, which brought differences between parents and lines, the predominant growth habit, pubescence of the lemma and palea, the angle of the flag leaf insertion and the knot color. In summary, the processes of recombination and *in vitro* culture differences caused favorable for some cultivars tested qualitative characteristics, present in the parents 'Amistad'82' e 'INCA LP-1' and 23 characters were common to parental cultivars and good agronomic characters.

**Table II. Qualitative characteristics evaluated to three commercial cultivars and their parents, 'Amistad'82' and 'INCA LP-1'**

No.	Characters	Classification				
		A/V-L4	A/I-L11	Amistad'82	P1/T-L6	INCA LP-1
1	Color of coleoptile	Light green	Light green	Light green	Light green	Light green
2	Predominant growth habit	Erect	Erect	Erect	Erect	Intermed
3	Tillering capacity	Middle	Middle	Very high	Very high	Very high
4	Color of lemma and palea	Straw	Straw	Straw	Straw	Straw
5	Color the apex of the lemma and palea	Straw	Straw	Straw	Straw	Straw
6	Pubescence of lemma and palea	At the apex	At the apex	Partial or total	At the apex	At the apex
7	Color glumes	Straw	Straw	Straw	Straw	Straw
8	Color stigma	Whitish	Whitish	Whitish	Whitish	Whitish
9	Insertion leaf below the flag leaf	Erect	Erect	Erect	Erect	Erect
10	Insertion angle of the flag leaf	Erect	Erect	Semi rrect	Erect	Erect
11	Hairiness of the leaf blade	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate
12	Color of the blade leaf	Green	Light green	Green	Green	Light green
13	Color of ligule	White	White	White	White	White
14	Shape of the ligule	Grub	Grub	Grub	Grub	Grub
15	Resistance to detachment of the atria	Eciduous	Eciduous	Eciduous	Eciduous	Eciduous
16	Color of the atria	Light green	Light green	Light green	Light green	Light green
17	Leaf sheath color	Green	Green	Green	Green	Green
18	Node color	Yellowish	Yellowish	Green yellowish	Yellowish	Yellowish
19	Internode color	Light green	Light green	Light green	Light green	Light green
20	Subnodal ring color	Light green	Light green	Light green	Light green	Light green
21	Color in the stem base	Beige	Light green	Withish	Beige	Green yellowish
22	Lodging resistance	Resistant	Resistant	Resistant	Resistant	Resistant
23	Response to photoperiod	Insensitive	Insensitive	Insensitive	Insensitive	Insensitive
24	Size of the edges	Absence	Absence	Short	Absence	Short
25	Grain panicle apical color	Straw	Straw	Straw	Straw	Straw
26	Apex color of panicle apical grain	Straw	Straw	Straw	Straw	Straw
27	Panicle density	Compact	Compact	Intermed	Compact	Intermed
28	Panicle exsertion	Well landmass	Well landmass	Well landmass	Well landmass	Well landmass
29	Empty grains at the apex of the panicle	1	1	1	1	0
30	Panicle fertility	Very fertile	Very fertile	Very fertile	Very fertile	Very fertile
31	Shelling panicle	Resistant	Resistant	Resistant	Resistant	Resistant
32	predominant leaf longevity	Late	Late	Late	Late	Late

To characterize 13 cultivars of Venezuelan rice, a group of authors found that 13 qualitative characters allowed the differentiation among them, which they attribute to the fact that their genetic base is narrow (8). In this sense, when analyzing the genetic diversity of Cuban rice cultivars, based on morphological characters, their genealogy and DNA polymorphism, has emphasized the need to diversify the parents in breeding programs, aiming to broaden the base crop genetics (9). Table III the association between evaluated quantitative characters is shown. It is a significant positive correlation between performance and panicles per square meter and performance and filled grains per panicle is appreciated. These components, the influence on performance, are considered markers for selection in early generations of high yield cultivars (10, 11).

The “intensification” system of rice cultivation, which applies in several Asian countries, is based on the development of vigorous plants, using management practices that reduce competition among plants at the growth beginning. These plants have abundant and deep roots, produce 30 to 100 panicles per plant (many effective) and panicles develop many grains. There are reports that this system allows to obtain yields greater than 8 t ha<sup>-1</sup> in poor soils and without applying chemical fertilizers (12). The cycle was not correlated with performance, unlike other studies that report a high and positive correlation (13). A longer cycle allows the plant using nitrogen, higher dry matter accumulation and generally higher yields; hence the cultivars that prevailed in Cuba for several decades, such as IR 880 and J-104 were mid-cycle. However, the development of early germplasm is one of the fundamental objectives of breeding programs by the advantages that these cultivars represent better use of the planting schedule, use less fertilizer and consume less water.

**Table III. Matrix phenotypic correlations of quantitative traits evaluated commercial cultivars and their parents, 'Amistad'82' and 'INCA LP-1'**

	RI*	PM	GLI	PG	C	Alt	LP	LG	AG	Lhb	Ahb	LH	AH	Lig	Me	Hp	AFA	CA	
RA	0,24	0,89*	0,90*	0,49	-0,06	0,77	0,70	-0,27	0,00	-0,03	-0,24	-0,50	0,02	-0,03	-0,45	0,52	-0,96*	-0,97**	
RI		0,63	0,59	-0,06	-0,82	0,24	0,34	-0,31	0,12	0,60	0,81	0,28	0,37	-0,23	-0,14	0,07	-0,01	-0,38	
PM			0,98**	0,23	-0,40	0,61	0,62	-0,49	0,11	0,15	0,16	-0,38	0,13	-0,27	-0,42	0,31	-0,74	-0,95*	
GLI				0,32	-0,26	0,66	0,56	-0,37	0,27	0,07	0,20	-0,32	0,31	-0,25	-0,24	0,33	-0,73	-0,91*	
PG					0,14	0,90*	0,69	0,70	-0,19	0,35	-0,14	0,33	0,33	0,78	-0,02	0,92*	-0,57	-0,30	
C						-0,14	-0,47	0,32	0,43	-0,81	-0,53	-0,25	0,15	-0,01	0,53	-0,16	-0,03	0,25	
Alt							0,89*	0,33	-0,22	0,42	-0,07	0,11	0,24	0,56	-0,29	0,92*	-0,78	-0,66	
LP								0,11	-0,58	0,63	-0,12	0,02	-0,14	0,55	-0,67	0,89*	-0,75	-0,69	
LG									-0,09	0,28	0,04	0,74	0,43	0,82	0,45	0,53	0,17	0,48	
AG										-0,57	0,42	-0,03	0,74	-0,60	0,79	-0,51	0,21	0,06	
Lhb											0,45	0,64	0,03	0,57	-0,36	0,58	0,03	-0,04	
Ahb												0,64	0,69	-0,20	0,42	-0,18	0,48	0,17	
LH													0,55	0,56	0,45	0,27	0,53	0,56	
AH														-0,01	0,78	0,05	0,18	0,10	
Lig															-0,13	0,83	-0,15	0,17	
Me																-0,37	0,56	0,58	
Hp																	-0,63	-0,41	
AFA																			0,90*

RA \* - Agricultural yield (t ha<sup>-1</sup>), RI - Industrial yield (% whole grains), PM- panicles per square meter, GLL-filled grains per panicle, PG-Mass of 1000 grains (g), C-Cycle ( days)  
 Alt-Plant height (cm), LP-length panicle (cm), LG-length grain (mm), AG-width grain (mm), Lhb-length of leaf flag(cm), AHb -width of flag leaf(cm), LH-length of leaf below the flag leaf (cm), width  
 AH-leaf below the flag leaf (cm), Lig-length ligule (mm), ME mesocotyl -length (cm), Hp-hypocotyl length (cm), AFA-leaf area affected by Pyriculariosis, CA- Percentage of damaged panicles in the  
 neck by the Pyriculariosis. Significant correlations from 0,87 to p <0,05 and 0,95 to p<0,01

Other characters, such as height and the flag leaf length, showed no correlations with performance, unlike the results are shown by other authors, who evaluated different cultivars included in this study and found positive correlations and highly significant (11). Significant and positive correlations between hypocotyl length and mass of 1000 grains, the plant height and the panicle length were observed. They do not appear in the literature references about it, but it would be desirable to repeat this evaluation with a greater number of cultivars, as hypocotyl length may be a marker for selection in early times of these important yield components. The damages caused by Pyriculariosis both the leaves and the panicle neck were positively correlated with each other and negatively with agricultural yields, whereas the percentages of affected necks were associated with two of its major components: panicles per square meter and filled grains per panicle. These results demonstrate the importance of genetic crop improvement aimed at obtaining disease-resistant cultivars, as susceptible cultivars planted in favorable conditions for fungal growth conditions can be completely destroyed in the vegetative phase.

Typical disease spots on the leaves of the rice plant can join, depending on environmental conditions and susceptibility to grow and produce reducing photosynthetic area, while neck panicle, the fungus colonization inhibits photosynthesized flow towards the grain formation, resulting in empty panicles and fall of these, which in both cases causes significant yield losses (14, 15). Table IV the analysis of variance made the most important agronomic traits is presented; it can be seen that the quantitative parameters related to agricultural yield and its components as well as industrial quality allow better characterization of cultivars, with significant differences among them and the parents, which coincides with the results obtained by other authors (8).

When analyzing the behavior of cultivars, 'A/I-L11', which combined the highest agricultural and industrial yields no significant differences with 'A/V-L4' and 'P1/T-L6', and upper stands all the cultivar 'Amistad'82' agricultural performance. Industrial performance A/I-L11 was higher and statistically different from the rest.

Yield components, panicles per square meter and filled grains per panicle, were higher for the cultivar 'A/I-L11', which shows that performance is associated with grain production obtained not only by an increase in panicles per square meter; They should also be considered full grains present in these panicles. The greater mass of the grains was obtained by 'P1/T-L6' no significant differences 'A/I-L11', A/V-L4 and the cultivar 'INCA LP-1'. All cultivars, despite showing a short cycle, were able to form a large number of fertile tillers, that allowed, together with the contribution of components, filled grain by panicle and mass of 1000 grains, get the highest returns, superior even to cultivate 'INCALP-1' mid-cycle. The 'A/L-11', 'A/V-L4' and 'P1/T-L6' cultivars that formed resistance to *P. grisea* with good agronomic characters were included in biodiversity fairs developed through programs PPB in "Los Palacios", "Bahia Honda" and "Mantua" in the Pinar del Rio province in "Florida", Camagüey province and the special municipality of the "Isla de la Juventud", to facilitate their dissemination producers, resulting in greater acceptance cultivar A/I-L11, which was entered in the commercial register of Cuban varieties, with the name of Anays LP-14 and currently it is planted on farms seed for extension rice production.

**Table IV. Behavior of some quantitative characteristics evaluated to commercial cultivars and their parents, 'Amistad'82' and 'INCA LP-1'**

Cultivars	Cicle (days)	Agricultural yield (t ha <sup>-1</sup> )	Panicles per m <sup>2</sup>	Grains filled per panicle	Mass of 1000 grains (g)	Industrial yield (% whole grains)
A/V-L4	127 b	7,5 ab	474 ab	105 b	28,5 ab	57,5 b
A/I-L11	128 b	8,1 a	505 a	143 a	29,0 ab	59,6 a
P1/T-L6	127 b	7,5 ab	467 abc	108 b	30,1 a	58,1 b
Amistad'82	127 b	5,1 c	425 c	71 d	28,3 b	58,2 b
INCA LP-1	142 a	6,8 b	436 bc	88 c	29,2 ab	55,5 c
X	130	7	461	103	29	57,8
Esx	1,53*	0,23*	9,9*	2,36*	0,37*	0,26*

Means with common letters per column, no differ significantly p<0,05 according Tukey test

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