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USE *Pyricularia grisea* Sacc. HAPLOTYPES ISOLATED ON CUBA FOR SELECTION OF RICE CULTIVARS RESISTANT TO THE PIRICULARIOSIS

Utilización de haplotipos de *Pyricularia grisea* Sacc. aislados en Cuba para la selección de cultivares de arroz resistentes a la Piriculariosis

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ABSTRACT. Piriculariosis (Blast disease) is the main phytopathologic problem that causes low rice yields in Cuba. For this reason, three *P. grisea* haplotypes isolated in Cuba from rice plants in two production areas, were evaluated in controlled conditions and with artificial inoculation. Later they were used in breeding rice varieties to blast resistant. The obtained results suggest the selection of haplotypes A 18 and B 6 for rice varieties evaluation under control conditions and, in this way, two lineages of blast are represented, as well as the broadly distributed and aggressive lineage. Seven resistant lines to the evaluated haplotypes were selected. They constitute an important germoplasm basement to obtain resistant rice varieties.

Key words: *Oryza sativa*, anther culture, virulence

RESUMEN. El principal problema fitopatológico que provoca los bajos rendimientos del cultivo del arroz en Cuba es la Piriculariosi, por tal motivo se evaluaron, en condiciones controladas y con inoculación artificial, tres haplotipos de *P. grisea* aislados en Cuba de plantas de arroz en dos zonas de producción. Estos fueron posteriormente utilizados en la selección de cultivares de arroz resistentes a la enfermedad. Los resultados permitieron la selección de los haplotipos A 18 y B 6 para la evaluación de cultivares en condiciones semicontroladas y, de esta forma, quedan representados dos linajes del patógeno y, entre ellos, el más agresivo y ampliamente distribuido. Se seleccionaron siete líneas resistentes frente a los haplotipos evaluados, las que constituyen una importante base germoplásmica para la obtención de cultivares resistentes.

Palabras clave: *Oryza sativa*, cultivo de anteras, virulencia

INTRODUCTION

Among the important pests in rice cultivation stands out fungus *Magnaporthe grisea* Barr (*Pyricularia grisea* Sacc) that causes Piriculariosis, considered the most devastating disease worldwide due to its wide distribution (1). It affects leaves, stems, plant knots and different parts of panicles and grains, resulting in a significant agricultural yield decrease and sometimes the complete crop failure; thus, this disease is a serious limitation for crop production, which has become the main phytopathological problem of Cuba.

Piriculariosis is considered a complex disease, because of its pathogenic variability (2). In Cuba, the genetic diversity of this fungus and the structural characteristics of populations (lineages and haplotypes) were determined, which can contribute significantly to the establishment of breeding strategies based on the theory of lineage exclusion, taking into account that in each rice growing region there are groups of different lineages; therefore, the selection of parents that provide resistant genes to such groups is very important (3).

Moreover, in every breeding program for disease resistance, it is necessary to have the source for genetic resistance, to establish suitable conditions for pathogen infection, in order to ensure an effective selection, and to choose the adequate breeding method.

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Among them, the *in vitro* anther culture of F_2 plants has many advantages, since the population from this combination of breeding methods (hybridizations and *in vitro* cultivation) represents the genetic variability of F_2 population and the *in vitro* plants obtained are genetically homozygous. This reduces the time for obtaining new cultivars, saves financial and material resources and increases the efficiency of selection, which makes easy the identification of superior individuals (4, 5, 6, 7)[A].

Knowing these problems, this work was conceived to evaluate the pathogenicity of three haplotypes of *P. grisea* fungus identified in Cuba for its further use in the selection of disease resistant cultivars.

MATERIALS AND METHODS

Evaluation of *P. grisea* haplotypes isolated in Cuba for its use in selecting disease resistant cultivars

Three *P. grisea* haplotypes, which have been preserved in the myco collection of the Center for Technological Applications and Nuclear Development (CEADEN) since 1998, were reactivated[B]; they were isolated from rice plants into two production areas (Table I) and belong to two lineages of greater variability.

Table I. Origin of *P. grisea* haplotypes isolated from different varieties and two rice areas of Cuba

Lineage	Haplotype	Cultivar	Collecting location
A	18	IR 837	"Los Palacios"
A	69	IACuba - 20	"Sur del Jíbaro"
B	6	IACuba - 25	"Los Palacios"

(Fuentes, 1998)

Small portions of haplotype mycelia were seeded in Petri dishes containing Agar Water (Table II); after 72 hours, monospore cultures were made in SA medium (rice bran) and incubated for seven days at 28 ± 2 ^{0}C and darkness. From pure cultures of the haplotypes studied, mycelial discs of 5 mm diameter were taken and planted separately in the PDA culture medium with pH 5.6 made of natural potato extract of variety 'Desiree', harvested in the central area of the National Institute of Agricultural Sciences (INCA).

Table II. Composition of culture media used for growth and sporulation of *P. grisea* haplotypes evaluated

Media	Ingredients	Quantity (g L^{-1})
Agar Water	Agar	40,0
SA	Rice bran of rice	20,0
	Sucrose	5,0
	Agar	20,0
PDA	Natural potato extract	4,0
	Dextrose	20,0
	Agar	15,0

To evaluate haplotypes, seeds of cultivar 'J-104' were disinfected with sodium hypochlorite at 1.5% for one minute then rinsed several times with distilled water. Subsequently, these were sown in pots containing a mixture of Gley Nodular Ferruginous petroferric soil (8) and cured filter cake in 3:1 ratio. The soil was previously sterilized by autoclaving for one hour at 121 ^{0}C during three consecutive days.

The seeding density was 20 seeds per pot, using five pots (replicates) per each haplotype evaluated. At 21 days of plant germination, artificial inoculations were performed with a hand sprayer containing pathogen spore suspensions adjusted to a concentration of 10^6 conidia per milliliter and a dose of 2 mL per pot (9). Then, high humidity and favorable temperature led to disease development.

The incubation period, size of lesions (mm) and leaf area affected were assessed in 10 plants by isolation. The most aggressive haplotype caused higher percentage of leaf area and higher lesions. Data of affected leaf area were transformed to arcsin √% and both this and the size of lesions were processed by Single Classification Variance Analysis. Means were compared according to Tukey test at 5 % probability of error. *P. grisea* haplotypes were selected for its use in the selection of disease resistant cultivars.

Selection of isogenic lines resistant to Piriculariosis under semi-controlled conditions with artificial inoculation

To perform the assay, 1.5 g of healthy seeds from isogenic lines were used (Table III), selected for their resistance to Piriculariosis with natural infection in the field (10), which came from *in vitro* anther culture of F_2 plants from crosses between four resistant cultivars to Piriculariosis (2077, IR 759-54-2-2, Moroberekan and Tetep) and five cultivars of good agronomic performance (Amistad'82, INCA LP-1, INCA LP-6, 6066 and IR1529-430), which were seeded in metal trays, replicated twice, on sterile filter paper, distributed in rows 0.5 m (200 kg ha^{-1})

[A]Blasco, T., Marqués, L. y Sales, E. Caracterización de una familia de líneas doble-haploides de arroz para componentes del rendimiento [Trabajo de Diploma segundo ciclo]. Universidad de Zaragoza, Escuela Politécnica Superior (Huesca), Departamento de Agricultura y Economía Agraria, Área de Producción Vegetal. 2012. 100 pp.

[B]Fuentes, J. L. Estructura y diversidad genética de poblaciones cubanas del hongo *Pyricularia grísea*. [Tesis de Maestría]. Mención Biotecnología Vegetal, CIAT, Colombia y CEADEN, Cuba, 1998. 64 pp.

Table III. Isogenic lines evaluated per crossing

Crossing	Identification	Lines evaluated
Amistad´82 / 2077	A/V	A/V-L4
Amistad´82 / IR 759-54-2-2	A/I	A/I-L11 y A/I-L15
Moroberekan / Amistad´82	M/A	M/A-L6
INCA LP-1 / Tetep	P1/T	P1/T-L6
INCA LP-6 / Moroberekan	P6/M	P6/M-L10
Tetep / INCA LP-6	T/P6	T/P6-L6
IR75954-2-2 / 6066	I/S	I/S-L16
IR 1529-430 / IR 759-54-2-2	IR/I	IR/I-L6

After 21 days of germination, plants were inoculated separately with pathogen haplotype 18 from lineage A and haplotype 6 from lineage B, following a similar procedure and concentration of conidia to that developed for haplotype evaluation. The dose used was 5 mL per row (9). Also, cultivars '2077', 'IR 759-54-2-2', 'Moroberekan' and 'Tetep' were used as resistant rootstocks whereas 'J-104' as a susceptible cultivar.

At the vegetative phase, fungus incubation period (days), size of lesions (mm) and the percentage of leaf area affected were evaluated in 10 plants per line and haplotype. Leaf area data were transformed to arcsin √% and both this and the size of lesions were processed by Single Classification Variance Analysis. Means were compared according to Tukey test at 5 % probability of error. Isogenic line selection was made taking into account the resistance degrees shown (0 to 3) according to the scale proposed by IRRI (11) against *P. grisea* haplotypes employed.

RESULTS AND DISCUSSION

Evaluation of *P. grisea* haplotypes isolated in Cuba for its use in selecting disease resistant cultivars

When assessing the performance of J-104 cultivar against *P. grisea* haplotypes isolated in Cuba (Table IV), the first symptoms were observed six days after the initial pathogen contact with leaf surface for all haplotypes.

The first spots were brown, like small coffee spots and, between 9 and 14 days, they had a diamond-shaped with whitish center and yellow edges. The description of symptoms reported in this work coincided with those of other authors, who used the same cultivar (J-104) and reported an incubation period of seven days as well as maximum development of lesions at 14 days (12).

When analyzing the average size value of lesions and the percentage of leaf area affected, it was observed that the cultivar 'J-104' was susceptible to all haplotypes tested, according to the scale proposed by IRRI (11). Haplotype A18 was the most aggressive, since it caused higher percentage of leaf area affected and larger spindle-shaped or diamond spots. Moreover, the lineage B haplotype was less aggressive than both haplotypes grouped in lineage A.

When studying the pathogen population present in "Los Palacios" and "South of Jibaro" Rice Agroindustrial Enterprise, haplotypes were classified into four genetic lineages and highlighted the lineage A as the most widely distributed[B].

The susceptibility of cultivar 'J-104' against *P. grisea* previously mentioned in works developed in Cuba is confirmed, both in production and under controlled conditions (12, 13).

In short, haplotype A18 was selected for cultivar evaluation under semi-controlled conditions with artificial inoculation; however, haplotype B6 will be taken into account in any case, since it belongs to another pathogen lineage.

The use of these haplotypes for evaluating rice cultivars would be useful in gene selection for resistance to pathogenic populations of *P. grisea* present in Cuba. From there, genes conferring resistance to different lineages could make a pyramid and thus achieve longer durability against the attacks of that pathogen.

Selection of isogenic lines resistant to Piriculariosis under semi-controlled conditions with artificial inoculation

The lines selected for its resistance to Piriculariosis in naturally infected field with good agronomic performance (coming from *in vitro* anther culture of F_2 plants from crosses between disease resistant cultivars and cultivars of good agronomic performance), when evaluated against A18 and B6 haplotypes under semi-controlled conditions(10), showed their first symptoms six days after inoculation, like in the pathogenicity assay (Table V).

Early lesions observed were brown, like small coffee spots and, between nine and 14 days, they became diamond-shaped with whitish center and yellowish edges around it. Also in this case, haplotype A18 turned out to be more aggressive with higher percentage of leaf area affected and larger lesions in the susceptible cultivar J-104.

Table IV. Performance of rice plants cultivar J-104 against P. grisea haplotypes isolated in Cuba

Haplotypes	Incubation period (days)	Lesion size (mm)	Leaf area affected		Degree of scale
			Original data (%)	Transformed data	
A 18	6	6,0 a	43,2	0,9 a	7
A 69	6	4,6 b	19,2	1,1 b	6
B 6	6	3,0 c	9,2	1,3 c	5
ESx		0,4*		0,1*	

Medias con letras en común por columna, no difieren significativamente para p≤0,05 (*) según Prueba de Tukey.

Table V. Performance of new lines obtained and rootstocks (resistant and susceptible cultivars) against *P. grisea* haplotypes A18 and B6

Cultivars and lines	A 18				B 6			
	Lesion size (mm)	Leaf area affected		Degree of scale	Lesion size (mm)	Leaf area affected		Degree of scale
		DO (%)	DT			DO (%)	DT	
A/V-L4	1,8 bc	1,2	1,48 a	3	1,1 bc	0,9	1,48 ab	2
A/I-L11	1,8 bc	0,7	1,50 a	2	1,0 bc	0,4	1,52 ab	2
A/I-L15	6,2 a	43,2	0,86 b	7	3,2 a	11,2	1,23 c	6
M/A-L6	1,9 bc	1,7	1,45 a	3	1,1 bc	0,9	1,49 ab	2
P1/T-L6	2,0 bc	1,9	1,45 a	3	1,3 bc	1,0	1,48 ab	2
P6/M-L10	1,9 bc	1,5	1,47 a	3	1,1 bc	0,9	1,49 ab	2
T/P6-L6	1,9 bc	2,8	1,43 a	3	1,4 bc	1,7	1,45 ab	2
I/S-L16	2,1 bc	1,7	1,46 a	3	1,4 bc	1,0	1,49 ab	2
IR/I-L6	6,0 a	32,0	0,98 b	7	3,5 a	10,0	1,26 c	5
IR 759-54-2-2 (R)	2,6 b	3,0	1,41 a	4	1,8 b	2,0	1,44 b	3
Moroberekan (R)	1,7 bc	2,1	1,44 a	3	0,9 bc	1,3	1,46 ab	2
Tetep (R)	0,6 c	0,4	1,53 a	1	0,4 c	0,3	1,54 a	1
2077 (R)	1,7 bc	0,8	1,48 a	2	1,0 bc	0,5	1,51 ab	2
J-104 (S)	6,5 a	44,8	0,84 b	7	3,3 a	10,4	1,25 c	6
X	2,8	9,8	1,34		1,6	3,0	1,44	
ESx	0,4*		0,04*		0,3*		0,02*	

Means with common letters per column do not differ significantly for p≤0,05 (*) according to Tukey test
DO- Originales data, DT- Transformed data, A/V- Amistad´82 / 2077, A/I- Amistad´82 / IR 759-54-2-2, M/A- Moroberekan / Amistad´82, P1/T- INCA LP-1 / Tetep, P6/M- INCA LP-6 / Moroberekan, T/P6- Tetep / INCA LP-6, I/S-IR75954-2-2 / 6066, IR/I- IR 1529-430 / IR 759-54 2-2, R- Resistant checks, C- Susceptible check

The lines 'A/I-L15' and 'IR/I-L6' have shown resistance in the previous assessment (10); however, they were susceptible in this trial, with larger spots than 3 mm and more than 10% percentages of leaf area affected, without significant statistical differences to the susceptible check 'J-104', thus occupying degrees five to seven of the scale. The other lines showed resistance with similar values to rootstocks. Fuentes (1998)[B] found that only one Cuban cultivar showed intermediate resistance to the pathogen under natural conditions, and the greatest number of haplotypes was isolated from 'J-104'; then, it is important to obtain new resistant lines against this pathogen attack.

The presence of the disease in different crop phenological phases is closely related to the occurrence of optimal environmental conditions, but its development depends on the genetic characteristics of cultivars and pathogen variability[C]; consequently, it is necessary to evaluate the lines obtained when the disease causes severe problems at the location selected as "hot spot": "Caribe" Agricultural Base Business Unit belonging to "Los Palacios" Rice Agro-industrial Complex, which according to the results of various authors, is considered ideal for this disease evaluation in Cuba (14, 15, 16)[C].

The lines 'A/V-L4', 'A/I-L11', 'M/A-L6', 'P1/T-L6', 'P6/M-L10', 'T/P6-L6' and 'I/S-L16', which were selected as resistant to the haplotypes evaluated, constitute an important basis for obtaining resistant cultivars. It is also necessary to check its resistance under natural infection conditions, as this ensures the development of durable resistant cultivars against the disease caused by *P. grisea* fungus.

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