Genetic Diversity Analysis of the Rice Blast Pathogen Population at Two Locations in Cuba

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ABSTRACT

The genetic structure of rice blast pathogen populations was studied using 149 and 107 monoconidial isolates from Los Palacios and Sur del Jíbaro rice production locations in the western and central regions of Cuba, respectively. These isolates were collected from 35 rice genotypes and total DNA from each isolate was digested with the EcoRI enzyme and probed with a dispersed *Magnaporthe grisea* repeated sequence (MGR586), which has been widely used in the population analysis of this fungus. MGR586 DNA fingerprinting groups were identified based on DNA restriction fragment length polymorphism similarities.

This analysis allowed the identification of 134 haplotypes that were partitioned into four highly related genetic groups. The mean similarity between groups ranged from 75 to 85 %. Group A was predominant in both locations and was composed of 84.7 % of all monoconidial isolations from 33 rice genotypes. Isolates from groups B, C and D were associated with one or a small number of very related varieties in the LP region. Genetic diversity estimates between and within the pathogen collection showed a wider diversity in pathogen populations at the western rice region. This analysis also suggested that the migration of this pathogen could have occurred from the western to central rice regions in Cuba. These results suggested that the specific features of the Cuban fungal populations would be particularly useful for selecting germplasm sources for more durable blast-resistance breeding.

Keywords: DNA Fingerprinting - *Pyricularia grisea* - Rice

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RESUMEN

Análisis de la Diversidad Genética de Poblaciones del Patógeno del Añublo del Arroz en Dos Localidades de Cuba. Fue estudiada la estructura genética de poblaciones del patógeno del añublo del arroz en dos regiones arroceras localizadas en el occidente (Los Palacios) y en el centro (Sur del Jíbaro) del país, utilizando 149 y 107 aislados monoconidiales, respectivamente. El ADN de estos aislados, los cuales se colectaron de 35 genotipos de arroz, se digirió con la enzima de restricción EcoRI y posteriormente se evaluó con la sonda de ADN, MGR586; la cual ha sido ampliamente utilizada en análisis de poblaciones del este hongo. Se identificaron grupos de dactiloscopia de ADN-MGR sobre la base de similitudes en el polimorfismo en el largo del fragmento de restricción de ADN. Este análisis permitió la identificación de 134 haplotipos distribuidos en cuatro grupos genéticos altamente relacionados. La similitud media entre grupos estuvo en un rango entre el 75 y el 85 %. El grupo A fue el predominante en ambas localidades y estuvo compuesto por el 84.7 % de todos los aislados monoconidiales, los cuales fueron obtenidos de 33 genotipos de arroz. Los aislados de los grupos B, C y D, presentes solo en la región de Los Palacios, estuvieron asociados con uno o con un pequeño número de genotipos estrechamente relacionados.

Los estimados de diversidad genética desarrollados entre colecciones del patógeno y dentro de estas, mostraron una mayor diversidad en la población del patógeno de la región arrocera de occidente. Este análisis además sugirió que pudieron ocurrir eventos de migración de la región occidental del país hacia la central. Los resultados obtenidos sugieren que la información sobre poblaciones de este patógeno en Cuba puede ser particularmente útil para el desarrollo variedades mejoradas con resistencia más duradera al añublo.

Palabras claves: Dactiloscopía de ADN - *Pyricularia grisea* - Arroz

*I*ntroduction

Rice blast is a widespread and damaging disease of cultivated rice caused by the fungus *Pyricularia grisea* Sacc. (Teleomorph: *Magnaporthe grisea* Barr, [1]).

Pyricularia grisea is noted for expressing a large number of virulent forms or pathotypes. In the field, *Pyricularia grisea* is reproduced asexually [2]. Although the sexual stage (teleomorph) can be demonstrated in the laboratory [3] the vast majority of field isolates are infertile.

Single genes (*PWL1 and PWL2*) that determine specificity toward the weeping lovegrass and rice hosts have been identified in independent crosses [3, 4]. The *PWL* gene family that determines specificities toward other grass species has also been documented [5]. Although the rice blast pathosystem is believed to follow a gene-to-gene relationship [6-8], examples of both monogenic and polygenic inheritance of host species specificity have been demonstrated [4, 9-11].

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Virulence analysis has led to conflicting opinions on the degree of variability of *Pyricularia grisea* [2, 12]. The sole tool characterizing this virulence diversity has been a pathogenicity assay. The assay sorts isolates into pathotypes by the symptoms they cause on each one of a set of differentially resistant rice cultivars. Assay results can be strongly influenced by host age and condition, inoculum quality and environmental variations during the assay [13]. Although one international set of eight differential cultivars has been established to standardize the assay [14], it has become clear that this set does not expose all the pathotype diversity occurring in many rice production regions [15, 16].

The use of resistant cultivars has been the preferred method for controlling this disease and considerable effort has been directed toward the understanding of genetic resistance mechanisms. However, blast resistance is rarely effective for more than 2-3 years [15]. It is not known whether the ability of the pathogen to overcome resistant cultivars reflects shifts in the frequency of formerly rare pathotypes, the frequent occurrence of genetic changes to new forms of virulence or a combination of both phenomena [17]. Consequently, detailed genetic information on population structure is essential for understanding the virulence dynamics of the pathogen and devising more effective strategies to reduce the impact of rice blast disease.

Hamer *et al*. [18] identified a family of dispersed repetitive DNA sequences, called MGR that was diagnostically conserved in *Pyricularia grisea* rice pathogen genomes. DNA gel blot analysis, using an MGR sequence (pCB586) as a hybridization probe, yielded mitotically stable and isolate-specific EcoRI RFLP profiles, containing 50 or more resolvable fragments of 0.7 Kb to 20 Kb in length [19].

DNA fingerprinting has been widely used to study the population structure of *Pyricularia grisea* in several countries [17, 19-26]. In these studies, the populations of *Pyricularia grisea* consisted of genetic groups of closely related strains, which were inferred to represent clonal lineages, except for some populations from the Indian Himalayas whose genetic structure may be affected to some extent by sexual recombination [25].

Each lineage has a specific virulence spectrum, characterized by uniform incompatibility with one or a combination of host resistance variants [27-29]. Zeigler *et al.*, [30] have hypothesized that lineage exclusion could be a means to achieve durable resistance in rice cultivars. Based on virulence data of lineage, this approach will combine resistance genes in a cultivar that will then confer resistance to the entire pathogen population. These authors have postulated that the durability of resistance developed by combining the resistance effective against all members of all lineage groups will be substantially longer than those combined on the basis of field performance by donors or incompatibility with selected varieties.

Additionally, the knowledge on the genetic structure of the rice blast pathogen population across the world suggests that the complexity and degree of genetic diversity in the pathogen population is very closely related to the diversity of cultivated varieties and to the period of time that rice has been grown in that region. For instance, in terms of lineage number, pathogen populations are more complex in older rice system of Asia [21-23, 26] than in America and European countries [17, 19, 20, 24] where *M. grisea* populations originated from a few introductions that occurred only a few centuries ago. The larger lineage number has been found in locations of the Indian Himalayas [25], which is considered the center of rice origin [31].

According to these results and considering that rice was introduced in Cuba relatively recently; a low lineage number can be expected in the Cuban pathogen population. The present study was aimed at determining the pathogen population structure in the two main rice production regions as a first step to improve breeding strategies for the selection of a more durable resistance to blast in Cuban rice cultivars.

Materials and Methods

Field sampling and monoconidial cultures

In the present study, 149 and 107 monoconidial isolates were obtained during 1995-1997 from lesions resulting on 35 varieties (Table 1) growing on blast nurseries at Los Palacios (LP) and Sur del Jíbaro (SJ), two of the main rice production regions

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Table 1. Rice varieties, mutant lines and their progenitors used in the field sampling strategy.

Variety/lines	Progenitors.	Origin	Field evaluation[a]
IR-8	Peta / Dee Geo Woo Gen	Philippines	S
IR-759	IR-8 // Peta / Dawn	Philippines	S
IR-837	IR 262 / Niaw San Pahtawang	Philippines	S
IR880-C9	IR-8[3] / IR-749	Philippines	S
IR1529ECIA	IR-305 / IR-24	Philippines	S
BNK	Peta[3] / Taichung native 1 // PN16	Thailand	S
Naylamp	IR-8 / IR-12	Peru	S
Cica4	IR-8 / IR-12	Colombia	S
Cica8	Cica4 // IR665 / Tetep	Colombia	R
Línea2	P1223 / P1225	Colombia	R
Colombia1	Napal / Takao Iku 18	Colombia	R
Oryzica Llanos5	P5269 / P2060-F4-2-5-2	Colombia	R
Jucarito-104	IR-480-5-9-2 / IR-930-16-1	Cuba	S
Amistad-82	IR1529ECIA / VNIIR3223	Cuba	S
IR759-54-2-2	Line of IR759	Cuba	S
Caribe-1	Unknown	Cuba	S
Caribe-7	IR-8 / CEB29	Cuba	S
Perla de Cuba	Unknown	Cuba	S
IACuba-14	CP1-C8 / ECIA22-8-163	Cuba	S
IACuba-16	PNA46-110 / CP1-C8	Cuba	S
IACuba-17	Jucarito-104 / Century Patna	Cuba	S
IACuba-18	IRAT-13 / ECIA31-104-2-1-2 // Amistad-82	Cuba	S
IACuba-19	Jucarito-104 // Jucarito 104 / Cica-8	Cuba	S
IACuba-20	ICA-10 / ECIA-31-104-2-1-2	Cuba	S
IACuba-21	14 MeV fast neutron mutant line of Jucarito-104	Cuba	I
IACuba-23	14 MeV fast neutron mutant line of Jucarito-104	Cuba	S
IACuba-24	Jucarito-104 / IAC-10 // Jucarito-104 / Siguaraya	Cuba	S
IACuba-25	Somaclone of Amistad-82	Cuba	S
L-3001	14 MeV fast neutron mutant line of Jucarito-104	Cuba	S
L-3078	14 MeV fast neutron mutant line of Jucarito-104	Cuba	S
L-3292	14 MeV fast neutron mutant line of Jucarito-104	Cuba	S
L-3361	14 MeV fast neutron mutant line of Jucarito-104	Cuba	S
L-3499	14 MeV fast neutron mutant line of Jucarito-104	Cuba	S
L-3767	14 MeV fast neutron mutant line of Jucarito-104	Cuba	S
L-3782	14 MeV fast neutron mutant line of Jucarito-104	Cuba	S

a: Blast nurseries evaluations realized during 1997 at LP region. (1-3, (R) Resistant; 3-4, (I) Intermediate; > 4, (S) Susceptible, according to [32]).

located in the western and central parts of Cuba, respectively.

Isolate cultures were established by placing thoroughly washed leaf pieces bearing a single lesion on a water-agar plate and subsequently transferring the growing conidia to plates containing rice polish-agar as indicated by Levy *et al* [17]. In most cases, monoconidial isolates were subsequently obtained from each single-lesion culture; the monoconidial isolates are named hereafter as a numbered version of the original lesion culture. The identification of isolates as *Pyircularia grisea* was confirmed by light microscopy. Samples of each isolate were stored on dried filter paper at the Centro de Aplicaciones Técnológicas y Desarrollo Nuclear (Center for Technological Applications and Nuclear Development) monoconidial bank for further analysis. Isolates were reactivated by placing slivers of filter paper on rice polish-agar plates.

DNA isolation and MGR-DNA fingerprinting

Genomic DNA was extracted from lyophilized ground mycelia [33]. DNA concentrations were determined in a TKO 100 minifluorometer (Hoeffer, San Francisco, CA) with the DNA-specific fluorescent dye, Hoechst 33258 according to manufacturer's instructions.

Four micrograms of chromosomal DNA from each sample were digested with EcoRI and the electrophoresis is made on 0.8 % agarose gel. A reference standard, consisting of an EcoRI digest of DNA from a *Pyricularia grisea* laboratory strain, was cofractionated on all gels to facilitate direct comparisons between electrophoresis experiments [19]. This reference strain exhibited a MGR-DNA profile of 30 RFLPs of known length ranging from 0.9 to 38.2 Kb under the electrophoretic conditions. Capillary blots of the fractionated DNA were placed onto a nylon membrane (Hybond-N+, Amersham Corp.) with 0.4 M NaOH, 1M NaCl as transfer agent. The subclone pCB586 [18], peroxidase-direct labeled, was used as a probe and detected by a luminescence-based peroxidase assay following the manufacturer's instructions (ECL-direct labeled system and detection kit, Amersham corp.).

Analysis of lineage structure

Each MGR-DNA band position between 1 and 38 Kb was visually scored, (1) for the presence or (0) for the absence for each isolate. The resulting binary matrix was used to construct a matrix of similarities between all pairs of isolates according to Dice's coefficient [34]. Based on this similarity matrix, the unweighted pair group arithmetic mean analysis (UPGMA) in the SAHN program of the NTSYS-pc package [35] was used to produce a cluster phenogram of isolates. Statistical confidence intervals from the branching arrangements of the phenogram were determined by a bootstrap procedure [36] with 1000 repetitive samplings of the RFLP data and computing the 95 % range of the branch point values in the resultant UPGMA phenogram using the SAS software package [37]. Similarity values of 85 % were used to define lineage groups.

Measures of genetic variation and differentiation

Genetic diversity was analyzed considering three types of variants: individual bands, each distinct band pattern or haplotype and each genetic lineage previously determined. Gene diversity [38], based on individual band data was calculated using the following equation: $H_i = 1 - \sum X_i^2$ where H_i is the probability that two individuals randomly selected from the sample could be different at locus i (band i). Since two states (alleles) are possible at each locus: $\sum X_i^2 = X_1^2 + X_0^2$, being X_1 the proportion of individuals that presented band i and X_0 the proportion of those without it.

Then, mean average diversity [38] was calculated as:

$$H_{AV} = (n/n-1)\sum_{b=1}^{B} H_b / b$$

Where: n and b are the individual and band numbers, respectively.

The haplotype and lineage diversity were also calculated as follows:

$$H = 1 - \sum_{k=1}^{K} f_k^2$$

Where K is the number of haplotypes or lineages and f_k is the haplotype or lineages frequency.

The partitioning of genetic variation in the complete pathogen population was studied considering the two locations (LP and SJ) as subgroups. The coefficient of genetic differentiation (G_{ST}) was estimated as [39]: $G_{ST} = 1 - (H_S / H_T)$, where H_S is a weighted average of the estimated gene, haplotype or lineage diversities in the collection, and H_T is an estimate of the gene, haplotype or lineage diversity of the total pathogen population. The lineage and haplotype frequencies of these two subgroups were compared by the X^2 test ($p<0.05$).

Results

Lineage and haplotype determinations

Pathogen isolates were collected from 35 varieties both in LP (149 isolates) and SJ (107 isolates) locations. MGR-DNA fingerprinting revealed between 60 to approximately 80 resolvable bands (1 to 38 Kb) for each isolate. Based on at least two gel runs per isolate 134 haplotypes (distinct DNA banding patterns) were distinguished. Haplotypes contained different numbers of isolates (1 to 11).

A phenogram using all DNA fingerprinting data of all the pathogen collections (256 isolates) was constructed [40]. This analysis showed that the 134 haplotypes were partitioned into four distinct genetic groups (putative lineages defined at 85 % of MGR-similarity, Figure 1) as follows: 116 into lineage A, 12 into lineage B, 2 into lineage C and 4 into lineage D.

Within group average similarity estimates and the standard deviations were 0.984±0.003, 0.975±0.017, 0.975±0.015 and 0.990±0.012 for lineages A, B, C and D, respectively. The maximum consensus similarity between groups was 85 % (lineage B versus lineage C), the minimum was 75 % (lineages A, B and C versus lineage D), and the average among all lineages was 81.4 %.

The variation of within-group estimated similarities were surveyed by bootstrap analysis. All putative lineages were significantly different. The bootstrap analysis of the consensus MGR phenogram also indicated that, in terms of the degree of difference be-

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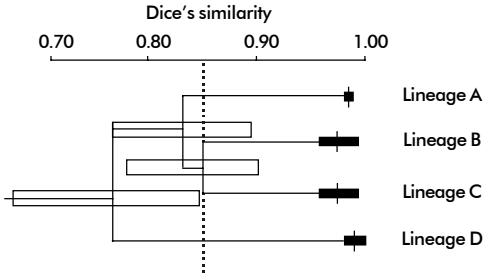

Figure 1. Phenogram of the MGR-DNA fingerprinting of genetic groups for *Pyricularia grisea*. Stippled error bars denote the 95 % confidence interval of branch point values from the bootstrap analysis of 256 MGR-DNA fingerprinting data. Filled bars denote ranges of variation of similarity values within lineage ($S_m \pm 1.96$ SD/$\sqrt{n}$) at a 95 % confidence level. S_m, SD and n are mean similarity, standard deviation and isolate numbers within each lineage, respectively. Vertical lines within filled bars denote mean similarity in each lineage.

tween lineages there were four significant ($p < 0.05$) branch points: one separating lineage D from the preceding genetic groups, another separating lineages B and C from lineage A, the third separating B and C lineages and a fourth allowed to separate lineage B isolates into two significantly different and internally cohesive clusters (data not shown).

Thus, only the four previously defined genetic groups were considered for further analyses. Although in the present study, we considered that lineages were defined at 85 % of MGR-similarity [17], inoculation experiments could be necessary to confirm the lineage number of this pathogen population.

The pathogen population at the LP region was characterized by the presence of all fungal genetic lineages. Lineage A was the only lineage found at the SJ region and the predominant one in the whole fungal population (Table 2). This genetic group characterized 84.7 % of the isolations studied that were obtained from 33 different rice genotypes. In contrast, lineages B, C and D were isolated from the pathogen population at a lower frequency (8.6, 2.7 and 3.9 % of the isolations studied, respectively) and these were recovered from one or a few very closely related rice genotypes. The difference in lineage number between the two locations was statistically demonstrated ($X^2 = 32.07$, $p < 0.001$).

A similar haplotype diversity of the pathogen population at the LP and SJ regions was apparent. Sixty nine haplotypes were distributed among the four genetic lineages (51 within lineage A, 12 within lineage B, 2 within lineage C and 4 within lineage D) on the LP region; while the fungus population at the SJ region showed 66 lineage A haplotypes. No significant difference with respect to haplotype number between the two locations was detected (X^2 test, $p < 0.001$). However, the haplotypes that characterized each location were different, with the unique exception of A_{17}.

The haplotype distribution among the 35 host varieties surveyed in blast nurseries at the LP and SJ regions are presented in Table 3. Note that 124 haplotypes (92.5 %) were recovered from one specific cultivar and only ten haplotypes were present in two or more rice genotypes.

Table 2. Summary of the origin and diversity of Cuban isolates of *Pyricularia grisea* within four MGR-DNA defined lineages.

Fungus Lineages	No. of isolates[a] (LP/SJ)	No. of MGR haplotypes[b] (LP/SJ)	No. of cultivars of origin[c] (LP/SJ)
A	110/107	51/66	14/28
B	22/0	12/0	7/0
C	7/0	2/0	1/0
D	10/0	4/0	3/0

a: The number of fungus isolates was 256

b: The number of fungus haplotypes was 134

c: The number of rice genotypes used in the sampling strategy was 35

Gene, haplotype and lineage diversity between isolate collections

The gene, haplotype and lineage diversity index estimated within and between the two pathogen collections, corroborated the results based on the pairwise MGR-DNA genetic distance estimated among isolates (Table 4).

A higher gene and lineage diversity was shown by the LP pathogen collection. Lineage diversity concept at the SJ region was not applicable because a unique lineage (Lineage A) characterized this region. Both

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Table 3. Haplotype organization of the rice blast fungus populations at the LP and SJ regions, in Cuba.

Variety or lines	Number of isolates per lineage A	B	C	D	Haplotypes[a]
IR-8	7				A_{15}, ***A_{20}***, A_{21}, ***A_{66}***, A_{74}
IR-759	8	1			***A_{14}***, A_{22}, A_{23}, ***A_{79}***, B_2
IR-837	10				A_{18}, A_{40}, A_{57}, A_{70}
IR880-C9	5				***A_{7}***, A_{9}, A_{28}, A_{77}, A_{86}
IR1529ECIA			7		C_1, C_2
BNK	7				A_{96}, A_{97}, A_{98}
Naylamp	4				A_{58}, ***A_{65}***
Cica 4	8	2			***A_{14}***, ***A_{17}***, A_{53}, A_{55}, A_{61}, ***A_{68}***, A_{82}, B_{12}
Cica 8	6				A_{84}, A_{85}, A_{92}, A_{93}
Línea 2	5				A_{54}, A_{56}, ***A_{78}***
Colombia 1	6				A_{35}, A_{62}, ***A_{78}***, A_{81}
Oryzica Llanos 5	4				A_{36}, ***A_{64}***, A_{71}
Jucarito 104	45	3			A_6, ***A_7***, A_8, A_{10}, A_{11}, A_{27}, A_{29}, A_{31}, A_{33}, A_{34}, A_{45}, A_{47}, A_{48}, A_{49}, A_{52}, A_{59}, A_{60}, A_{63}, A_{83}, A_{94}, A_{95}, A_{115}, A_{116}, B_{10}, B_{11}
Amistad-82	2				***A_{68}***
IR759-54-2-2	1				A_{30}
Caribe-1	10				***A_{17}***, ***A_{20}***, A_{51}, A_{87}
Caribe-7	6			2	***A_{14}***, A_{46}, D_2, D_4
Perla	11				***A_{14}***, A_{41}, A_{42}, A_{43}, A_{44}, A_{50}, ***A_{65}***
IACuba-14	3				***A_{64}***, ***A_{66}***, A_{76}
IACuba-16	8				A_1, A_2
IACuba-17	11				A_3, A_4, A_5, A_{37}, A_{38}
IACuba-18	4	3		6	A_{88}, A_{89}, B_1, D_1
IACuba-19	13				A_{12}, A_{13}, ***A_{68}***, ***A_{79}***, A_{80}, A_{90}, A_{91}
IACuba-20	8				A_{16}, A_{24}, A_{25}, A_{26}, A_{39}, A_{69}
IACuba-21	2			2	A_{72}, A_{73}, D_3
IACuba-23	1	5			A_{67}, B_3, B_4, B_5
IACuba-24		4			B_7, B_8, B_9
IACuba-25	3	4			***A_{68}***, A_{75}, B_6
L-3001	2				A_{113}, A_{114}
L-3078	3				A_{107}, A_{108}, A_{111}
L-3292	2				A_{19}, A_{101}
L-3361	4				A_{104}, A_{105}, A_{106}, A_{112}
L-3499	2				A_{32}, A_{103}
L-3767	5				***A_{17}***, A_{99}, A_{100}, A_{109}, A_{110}
L-3782	1				A_{102}
Totals	217	22	7	10	

a: haplotypes recovered from two or more rice genotype are given in italic and bold face.

populations presented a similar level of haplotype diversity. Gene and lineage diversity of the total fungal population were low (H_T = 0.107 and H_T = 0.272, respectively); in contrast, haplotype diversity was relatively high (H_T = 0.988). As expected, the level of diversity revealed by a highly polymorphic marker may notably increase when considering each distinct banding combination or pattern (haplotype) as variants instead of individual bands.

The main contribution to total diversity was the within-collection source in gene, haplotype and lineage diversity analyses. The estimates of the genetic differentiation coefficient were consistently low in the three analyses.

Table 4. Characterization of blast collections from Los Palacios and Sur de Jíbaro regions in Cuba. Genetic, haplotype and lineage diversity.

	Locations		Diversity statistics			
	LP	*SJ*	H_T	H_S	H_{ST}	G_{ST}
Gene diversity analysis	0.119[a]	0.066	0.107	0.098	0.009	0.084
Haplotype diversity analysis	0.976[b]	0.979	0.988	0.977	0.011	0.011
Lineage diversity analysis	0.426[c]	-	0.272	0.248	0.024	0.088

a: gene diversity within a collection or the probability that two individuals selected randomly from the collection could be different [39] considering each individual band as a polymorphic unit.
b,c: haplotype or lineage diversity within a collection or the probability that two individuals selected randomly belong to different haplotypes or lineages, respectively.
H_T: total diversity calculated as the weighted means of the gene, haplotype and lineage frequencies for the complete sample.
H_S: within-collection gene, haplotype and lineage diversity is the mean of the diversity for the considered collection, weighted by sample size.
H_{ST}: between collections gene, haplotype and lineage diversity.
G_{ST}: coefficient of genetic differentiation between collections.

Discussion

The present work identified 134 fungal haplotypes that were partitioned into four statistically different but highly related genetic groups (mean similarity values between groups from 75 to 85 %). This suggests that the lineages in the pathogen population arose from a common ancestor. Additionally, the presence of a reduced number of pathogen lineages can be considered as evidence of a primary state in their genetic microevolution.

The high similarity estimates between lineages of this fungal population largely contrasts with the estimates reported in other countries. For instance, in Colombia the similarity value between lineages ranged from 37 to 85 % [17]; Chen *et al*. [22] reported similarity estimates from 49 to 85 % in Philippines; Roumen *et al*. [24], studying the European fungal population obtained similarity values from 55 to 65 %. The reduced number of rice cultivars planted extensively in Cuba and their low genetic diversity [41-43] could explain the high genetic similarity between blast pathogen lineages. As it has been suggested, there is a strong relationship between the genetic diversity of the material planted in one location and the diversity of the genetic lineage of the local fungus population [22].

Reports on rice blast haplotype numbers in pathogen populations across the world range from 15-18 in the Unites States [19, 20] up to 115-130 in Colombia [17] and the Philippines [22]. According to this, the total haplotype number (134) determined from 35 host cultivars in this study is one of the highest found for this fungus. This result can be explained by the fact that the MGR-DNA fingerprints of Cuban pathogen isolates were characterized by a high band number (nearly 80 bands), which enhancing the probability of identifying distinct band patterns (haplotypes). We visually confirmed that MGR-DNA fingerprinting from Cuban pathogen isolates are more complex than Colombian isolates (data not shown). Additionally, the use of different detection techniques (eg: radioactivily, colorimetric or enhance chemoluminescence) during DNA assays can contribute to differences in haplotype number between the pathogen populations mentioned above.

The results presented in Table 3 point to the presence of a predominant variety-haplotype association in both locations, and confirm that host selection plays a determinant role in modeling rice blast population structure [22]. Host genotype is an important factor that should be considered in the selection of an adequate field sampling strategy in the characterization of this pathogen population.

As mentioned above, the main contribution to total diversity was the within-collection source. Estimates of the genetic differentiation coefficient were consistently low both in gene, haplotype and lineage diversity analyses, indicating that very little diversity is determined by differences between pathogen populations at two locations. Xia *et al*. [20] obtained similar results comparing two rice blast populations in the United States.

The higher gene and lineage diversity found in the LP region could be explained by the high incidence of rice blast for more than twenty years; in contrast, the first epidemic outburst reported in SJ was in 1996. The predominance of lineage A in both regions may result from a probable introduction of rice blast from the LP to the SJ region. Similar migration events have been suggested in the Colombian pathogen population [27].

With one exception (A_{17}), haplotypes at LP were not found in the SJ region, suggesting frequent genetic changes between haplotypes. Xia and Correll [44] demonstrated a low hybridization potential between genetically distinct haplotypes. This suggests that the haplotype changes found in pathogen populations are produced more through mutations than through recombination.

Locations characterized by highly diverse pathogen populations have been recommended for obtaining varieties with more durable resistance to rice blast [27]. Therefore, differences in lineage number between locations suggest the possible use of the LP region for breeding.

Almost all-current Cuban cultivars are susceptible to rice blast. The occurrence of individuals from lineage A on a high number of Cuban commercial varieties shows that the diversity of resistance employed in the rice breeding program is low and must be broaden. Our recent studies confirmed this limited genetic base [41-43]. Interestingly, the predominant lineage A was not recovered in the varieties IACuba24 and IR1529ECIA, suggesting the presence of resistance genes in both varieties. However, pathogenicity studies using the Cuban commercial varieties will be needed to confirm this hypothesis.

This is the first report of plant pathogen population using molecular markers in Cuba. Our results provide an insight into the pathogen population structure.

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However, MGR-586 polymorphism only partially reveals the true genomic diversity of pathogen DNA. It is a highly dispersed sequence with previous successful applications in studies of rice blast biology. Thus, diversity estimates reported in the present study must be considered cautiously and confirmed with other marker systems. The use of microsatellite markers [45] and other transposable DNA sequences found in different regions of the fungal genome [46, 47] could broaden the molecular database of the Cuban pathogen population for further analyses as indicated by other authors [48, 49].

A strategy combining pathogenicity tests, DNA fingerprinting and molecular marker-assisted gene tagging is providing a way to define the genetic organization and distribution of rice blast pathogen diversity and to incorporate the appropriate resistance genes. Focusing on resistance genes to the lineage of the pathogen, rather than on genes that are effective against specific pathotypes, may allow the identification of suitable resistance sources for their inclusion in a breeding program for developing stable rice blast resistance.

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