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Full Length Research Paper

Genetic diversity of the blast fungus, *Magnaporthe grisea* (Hebert) Barr, in Burkina Faso

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Trapping nurseries trialed at two screening sites in Burkina Faso appeared to be an effective tool to characterize the virulence spectrum of blast populations using limited equipment and reduced labor. It made it possible to identify the best site to be used for screening for durable resistance. The effectiveness of some resistance genes indicated that they could be pyramided to provide durable resistance to blast fungus in Burkina Faso. The study also revealed the possible existence of new pathotypes in Burkina Faso. Fifty-five isolates of the blast fungus, *Magnaporthe grisea,* collected from the nurseries and rice fields were analyzed using random amplified polymorphic DNA (RAPD) PCR. Five major groups (*Mg-1, Mg-2, Mg-3 Mg-4 and Mg-5*) were defined. *Mg-1, Mg-2* and *Mg-3* were the largest groups representing, 30.9, 25.5 and 30.9% of the 55 isolates analyzed. Only 9.1 and 3.6% belong to *Mg-4* and *Mg-5*, respectively. Our results confirmed that RAPD PCR offers an inexpensive and speedy means of generating markers for analyzing the population structure of the blast fungus.

Key words: *Magnaporthe grisea*, *Oryza* spp., Rice, graminaceous, pathogenicity, virulence spectrum, genetic fingerprinting, RAPD PCR, polymorphism.

INTRODUCTION

Blast disease is caused by *Magnaporthe grisea* (Hebert) Barr., anamorphe *Pyricularia grisea* Sacc. (Rossman et al., 1990) is an important fungal disease of rice known to occur in most rice producing areas of the world (Ou, 1985). The disease results in yield loss as high as 70 - 80% (Ou, 1985) when predisposition factors (high mean temperature values, degree of relative humidity higher than 85 - 89%, presence of dew, drought stress and excessive nitrogen fertilization) favor epidemic development (Piotti et al., 2005). Losses of nearly 80% have been reported in certain years in West Africa (Delassus, 1973). Particularly dangerous in upland rice, it also causes serious damage in rainfed lowland and irrigated systems in Burkina Faso, mainly when farmers seek to intensify production by the use of improved varieties and fertilizers (Séré, 1999). Therefore, blast constitutes one of the main constraints to intensification for increasing rice production.

The use of resistant varieties is the most economical and effective way of controlling rice blast mainly in resource-poor farmers' fields. Unfortunately, the causal fungus is able to overcome this resistance within two to three years after these plants are cultivated widely (Babujee and Gnanamanickham, 2000). The breakdown in resistance has been attributed to the high variability of the pathogen and there are numerous reports that this diversity may be due to continuous generation of novel pathogenic variation. Knowledge of the genetic variation within and among populations is an important component of understanding the population biology of pathogenic fungi and infers the impact of driving force influencing the evolution of pathogen populations (McDonald et al., 1997). Therefore, information on population diversity may be used for developing strategies to increase the durability of resistance (Xia et al., 2000).

There has been a tremendous accumulation of knowledge on pathogen population diversity in the past decade especially with the development of molecular techniques. In particular, DNA fingerprinting was used

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Table 1. Identity of varieties used in trapping nurseries at Farako-Ba and Banfora.

S/N	Varieties with known resistance genes	
	Varieties name	**Resistance genes**
1	Aichi Asahi	Pia +Pi19(t) [2]
2	Usen	Pia + Pi? [1]
3	Ishikari Shiroke	Pii +Piks [2]
4	Kanto 51	Pik [1]
5	K3	Pikh [3]
6	Tsuyuake	Pikm [2]
7	K60	Pikp + Pish [2]
8	Caloro	Piks [1]
9	Sha Tiao Tsao	Piks [1]
10	Shin 2	Piks + Pish [1]
11	Yashiro Moshi	Pita [2]
12	Pi N°4	Pita2+Pish [2]
13	Fukunishiki	Piz + Pish [2]
14	Bl 1	Pib + Pish [1]
15	K59	Pit + Piks [2]
16	Toride 1	Pizt+Pish [2]
17	Dular	Pika + Pi? [1]
18	Tetep	Pikh [3] + Pi-1 [4] +Pita[4] + Pi? [5]
19	K1	Pita [1]
Released varieties		
20	FKR 16 (4418)	Unknown
21	Dourado Précoce	Unknown
22	FKR 19	Unknown
23	FKR 28	Unknown
Susceptible checks		
24	Pekin	Unknown
25	Delta	Unknown

[1] Rathour et al. (2004)
[2] Hayashi et al. (1998)
[3] Kiyosawa and Ling (2001)
[4] Mackill and Bonman (1992)
[5] Inukai et al. (1994)

extensively to reveal the genetic diversity of plant pathogens needed for effective deployment of resistance and to identify shifts in races or population structures that might occur (Javan-Nikkhah et al., 2004).

However, the studies of genetic diversity of *M. grisea* in Burkina Faso have not previously been critically conducted, as very little research information is available. Therefore, the aim of this current study is to assess the pathological diversity of the blast pathogen at two screening sites and conduct genetic fingerprinting of *M. grisea* isolates for the first time in Burkina Faso using random amplified polymorphic DNA (RAPD) markers. It is anticipated that this study will lead to a better understanding of the diversity and distribution of blast pathogens both from rice and non-rice hosts, and to its potential application in rice breeding programs aiming at development of durable blast-resistant rice cultivars.

MATERIALS AND METHODS

Assessment of the pathological diversity

Trapping nurseries were conducted at two screening sites in Burkina Faso (Farako-Ba research station in upland condition, and Banfora Lowland rice experimental site) using 19 cultivars bearing known resistance genes, 4 released varieties and 2 varieties as susceptible checks (Table 1). Symptoms were recorded on each variety by considering plants without symptoms or with only hypersensitive lesions as resistant to the blast population on both sites (Thinlay et al., 2000).

Blast fungus collection and culture

Lesions were collected at the epidemic initiation and early in the morning when high spore production was observed on the lesions (Pinnschmidt et al., 1993). Additional samples were also collected from local and improved varieties in farmers' fields and from wild rice (*Oryza longistaminata*) and graminaceous weeds in the main rice-growing localities of the country (Table 2). With a sterile needle, a fragment of mycelium and conidiophores with spores was taken from sporulating lesions and moved gently on the surface of a water agar medium in Petri dishes (10 g of agar, 50 mg of penicillin and 50 mg of dihydrostreptomycin in an adequate quantity of distilled water for 1 liter of medium). The spores were then dispersed on the surface of the medium, and single ones were surrounded under a stereomicroscope. Six to ten hours later, the germinating single marked conidia were transferred to starch medium (10 g of soluble starch, 1 g of yeast extract, 10 g of agar, 50 mg of penicillin and 50 mg of dihydrostreptomycin in an adequate quantity of distilled water for 1 liter of medium). Such monoconidial *in vitro* cultures were grown at room temperature for 6 to 7 days before transfer for long term conservation according to method adapted from Valent et al. (1986). The conservation method used includes growing the fungus on filter papers overlaying the starch medium. After colonization, the paper discs are transferred aseptically under lamina flow in sterile paper filter bags, dried in glass boxes containing silica gel for 5 to 6 days, and then transferred into sealed plastic under vacuum. They are stored at 4°C.

Genomic DNA extraction

Mycelia and spores from 10 days old monoconidial culture are transferred into 75 ml of potato dextrose broth (pH 7.4) in a 250 ml conical flask and kept under constant shaking at 28°C for 6 days. The resulting mycelia ball was freeze-dried, and DNA was extracted using the standard CTAB (cetyl trimethyl ammonium bromide) method (Hamer and Givan, 1990).

RAPD-PCR analysis

RAPD-PCR analysis (Guthrie et al., 1992) was carried out on all the isolates collected both from rice and non-rice host. DNA primers tested were purchased from Operon Technologies (Alameda, California, USA) and each is 10 nucleotides long. Two concentrations of each DNA (24 ng and 96 ng per reaction) were used to test reproducibility and eliminate sporadic amplification products from the analysis. Eighty primers (OPA, OPB, OPC and OPZ series), including PAP2 and PAP3, were screened with three isolates (MGR 272, MGR 069 and MGR 097) for their ability to amplify the *M. grisea* DNA. The primers that showed polymorphism were used in amplifying the DNA from all *M. grisea* isolates. Amplifications were performed in a 25 µl reaction mixture consisting of genomic DNA, 1X reaction buffer (Promega), 100 µM each of dATP, dCTP, dGTP,

Table 2. Identity of *M. grisea* isolates used.

S/N	Isolate Code*	Host Plant	Locality
1	MGR 284	Caloro	Farako-Ba
2	MGR 305	Aichi Asahi	Farako-Ba
3	MGR 009	Yashiro Moshi	Farako-Ba
4	MGR 008	Yashiro Moshi	Farako-Ba
5	MGR 003	Yashiro Moshi	Farako-Ba
6	MGR 011	Tsuyuake	Farako-Ba
7	MGR 011b	Tsuyuake	Farako-Ba
8	MGR 012	Tsuyuake	Farako-Ba
9	MGR 099	K3	Farako-Ba
10	MGR 013	Shin 2	Farako-Ba
11	MGR 253	Dular	Farako-Ba
12	MGR 253b	Dular	Farako-Ba
13	MGR 010	Usen	Farako-Ba
14	MGR 283	Caloro	Farako-Ba
15	MGW 018	*Rottbollia sp*	Farako-Ba
16	MGR 417	4418	Farako-Ba
17	MGR 259	Delta	Farako-Ba
18	MGW 294	*Brachiaria sp*	Farako-Ba
19	MGR 026	Pekin	Farako-Ba
20	MGW 034	*Paspallum scrobiculatum*	Farako-Ba
21	MGW 033	*Paspallum scrobiculatum*	Farako-Ba
22	MGW 031	*Paspallum scrobiculatum*	Farako-Ba
23	MGR 025	Pekin	Farako-Ba
24	MGW 030	*Paspallum scrobiculatum*	Farako-Ba
25	MGR 023	Pekin	Farako-Ba
26	MGR 234	FKR 28	Farako-Ba
27	MGR 274	Caloro	Farako-Ba
28	MGR 258	Dourado	Farako-Ba
29	MGR 010	Usen	Farako-Ba
30	MGR 097	K60	Farako-Ba
31	MGR 096	Kanto 51	Farako-Ba
32	MGR 020	Pi N°4	Farako-Ba
33	MGR 302	Aichi Asahi	Farako-Ba
34	MGW 057	*Oryza longistaminata*	Vallée du Kou
35	MGW 052	*Oryza longistaminata*	Vallée du Kou
36	MGW 046	*Oryza longistaminata*	Vallee du Kou
37	MGR 021	Pekin	Farako-Ba
38	MGR 126	Ishikari Shiroke	Farako-Ba
39	MGR 079	Local variety	Labola
40	MGR 075	Local variety	Labola
41	MGW 018	*Setaria pallidae-fusca*	Sideradougou
42	MGR 247	FKR 19	Sideradougou
43	MGR 069	Local variety	Sideradougou
44	MGW 039	*Oryza longistaminata*	Sideradougou
45	MGR 243	FKR 19	Sideradougou
46	MGW 038	*Oryza longistaminata*	Labola
47	MGW 037	*Oryza longistaminata*	Sideradougou
48	MGR 231	Fukunishiki	Banfora
49	MGR 114	Ishikari Shiroke	Banfora
50	MGR 016	Shat Tiao Tsao	Banfora
51	MGR 272	Caloro	Banfora
52	MGW 036	*Oryza longistaminata*	Banfora
53	MGR 067	LOCAL variety	Koumadougou
54	MGR 062	LOCAL variety	Koumadougou
55	MGR 238	FKR 19	Banfora

*MGR = isolates from rice plants.
MGW = isolates from weeds.

and dTTP, 0.2 µM Operon random primer, 2.5 µM $MgCl_2$ and 1U of Taq polymerase (Boehringer, Germany). A single primer was used in each reaction. The reaction mixture was overlaid with 50 µl of mineral oil to prevent evaporation. Amplification was performed in a thermowell microtiter plate (Costa Corporation) using a Perkin Elmer programmable Thermal Controller model 9600. The cycling program was (i) 1 cycle of 94^{o}C for 3 min; (ii) 45 cycles of 94^{o}C for 1 min for denaturation, 40^{o}C for 1 min for annealing of primer and 72^{o}C for 2 min for extension; and (iii) a final extension at 72^{o}C for 7 min. The amplification products were resolved by electrophoresis in a 1.4% agarose gel using TAE buffer (45 mM Tris-acetate, 1 mM EDTA, pH 8.0) at 100 V for 2 h. A 1 kb ladder (Life Technologies, Gaithersburg, MD, USA) was included as a molecular size marker. Gels were visualized by staining with ethidium bromide solution (0.5 µg/ml) and banding patterns were photographed over UV light using a red filter.

Phylogenetic analysis

Positions of unequivocally scorable RAPD bands were transformed into a binary character matrix (“1” for the presence and “0” for the absence of a band at a particular position). Pairwise distance matrices were compiled by the NTSYS-pc 2.0 software (Rohlf, 1993) using the Jaccard coefficient of similarity. Phylogenetic tree was created by the unweighted pair-group method arithmetic (UPGMA) average cluster analysis (Sneath and Sokal, 1973; Swofford and Olsen, 1990).

RESULTS AND DISCUSSION

Virulence of the blast population at two screening sites

The term virulence is used to differentiate the ability of a blast fungus strain to overcome the resistance gene of a particular rice variety. The virulence spectrum of an isolate or a population refers to the number of varieties with different resistance genes to be attacked. The trapping nurseries developed at two screening sites indicated that 12 of the varieties bearing known resistance genes were attacked at Farako-Ba; meanwhile, only four developed susceptible lesions at Banfora (Table 3). The virulence spectrum of the blast fungus population was therefore broader at Farako-Ba than at Banfora. The higher diversity of the blast population at Farako-Ba might be due to the diversity of rice germplasm cultivated over the years at this experimental station as host genotype is known to influence the composition of the pathogen population (Chen et al., 1995; Park et al., 2003). Such diversity indicates that Farako-Ba is a better screening site for durable resistance than Banfora, as resistance breakdown had been most often attributed to a poor pre-release challenge by an adequate pathogen population (Mekwatanakarn et al., 2000).

The M. grisea population at both Farako-Ba and Banfora sites could not match the single gene Pita in K1 and the multiple genes Pib+Pish, Pit+Piks, Pizt+Pish, *Pikh+Pi1+Pita+Pi in BL1, K59*, Toride 1 and Tetep, respectively. However, Yachiro Mochi bearing the same resistance gene as K1 (*Pita*) is susceptible at Farako-Ba. Therefore, the resistance of K1 is not only due to Pita but to an additional undetermined resistance gene. Furthermore, the resistance of Sha Tia Tsao at Farako-Ba might be due to an additional resistance gene since Caloro, bearing the same Piks gene, is attacked. On the other hand, all the strains that could attack Ishikari Shirokee should have been virulent on Sha Tiao Tsao. Therefore, the lack of susceptible lesions on Sha Tiao Tsao at Farako-Ba while Ishikari Shiroke was attacked means that Sha Tiao Tsao had an undermined efficient resistant gene against the Farako-Ba blast population. Such a situation is not surprising in identifying and characterizing resistant genes using Japanese differential cultivars. Some resistant genes detected using Japanese fungus strains, could not be detected with Philippines isolates. Similarly, Japanese and Kiyosawa differential varieties include some resistance genes which could be recognized by Japanese differential fungus strains. Therefore, new resistance genes are found using isolates from different origins. For instance, Fujisaka 5 was first selected as a differential variety with a single resistance gene (*Pii*). Later, an additional gene, *Piks*, was found in Fujisaka 5 (Kiyosawa and Ling, 2001). *Piks* was found in Japanese cultivars only with a Filipino fungus strain. In Tetep, Kiyosawa (1976) first estimated the presence of Pikh. Thereafter, Pi-1, Pita and an unknown gene were found within Tetep (Kiyosawa and Ling, 2001). In the same way, Pish was identified later in Shin 2, Kusabue, Fukunishiki, Pino4 and Bl1 previously known, respectively, to carry Piks, Pik, Piz, Pita2 and Bl1 (Inukai et al., 1994). Moreover, some of the differential lines could carry several major and environmentally-sensitive minor resistance genes causing intermediate and poorly repeatable reactions even in well-controlled conditions (Mekwatanakarn et al., 2000).

Molecular characterization of blast fungus isolates

Ten primers out of 82 primers tested showed polymorphism among individual isolates. The amplification reactions with the 10 primers generated 153 bands, 108 of them being polymorphic (Table 4) with sizes ranging between 150 and 1,000 base pairs (Figure 1). The amplified DNA distinct band pattern obtained allowing the identification of each individual. For instance, isolate MGW036 presents unique bands when its DNA is amplified with most of the primers tested (Figure 1).These bands could be used to characterize and identify it.

Using 108 RAPD markers to construct phylogenetic relationships among 55 *M. grisea* isolates led to classification into five major groups (*Mg-1, Mg-2, Mg-3 Mg-4 and Mg-5*) at a 65% similarity coefficient while all the isolates were distinct at 100% similarity coefficient (Figure 2). *Mg-1, Mg-2* and *Mg-3* were the largest groups representing respectively 30.9, 25.5 and 30.9% of the 55 isolates analyzed. Only 9.1 and 3.6 % belong to *Mg-4*

Table 3. Reaction of 24 varieties in trapping nurseries at Farako-Ba research station and Banfora rice experimental site in Burkina Faso.

Varieties names	Resistance genes	Reaction to natural blast epidemic	
		Farako-Ba	Banfora
Aichi Asahi	Pia +Pi19(t)	+	-
Usen	Pia + Pi?	+	+
Ishikari Shiroke	Pii +Piks	+	+
Kanto 51	Pik	+	-
K3	Pikh	+	-
Tsuyuake	Pikm	+	-
K60	Pikp + Pish	+	-
Caloro	Piks	+	+
Sha Tiao Tsao	Piks	-	+
Shin 2	Piks + Pish	+	-
Yashiro Moshi	Pita	+	-
Pi N°4	Pita2+Pish	+	-
Fukunishiki	Piz + Pish	-	+
Bl 1	Pib + Pish	-	-
K59	Pit + Piks	-	-
Toride 1	Pizt+Pish	-	-
Dular	Pika + Pi?	+	-
Tetep	Pikh + Pi-1 + Pita + Pi?	-	-
K1	Pita	-	-
FKR 16 (4418)	Unknown	+	-
Dourado Précoce	Unknown	+	-
FKR 19	Unknown	-	+
FKR 28	Unknown	+	-
Pekin	Unknown	+	-
Delta	Unknown	+	-

+ Compatible reaction (presence of susceptible lesions).
- Incompatible reaction (absence of susceptible lesions).

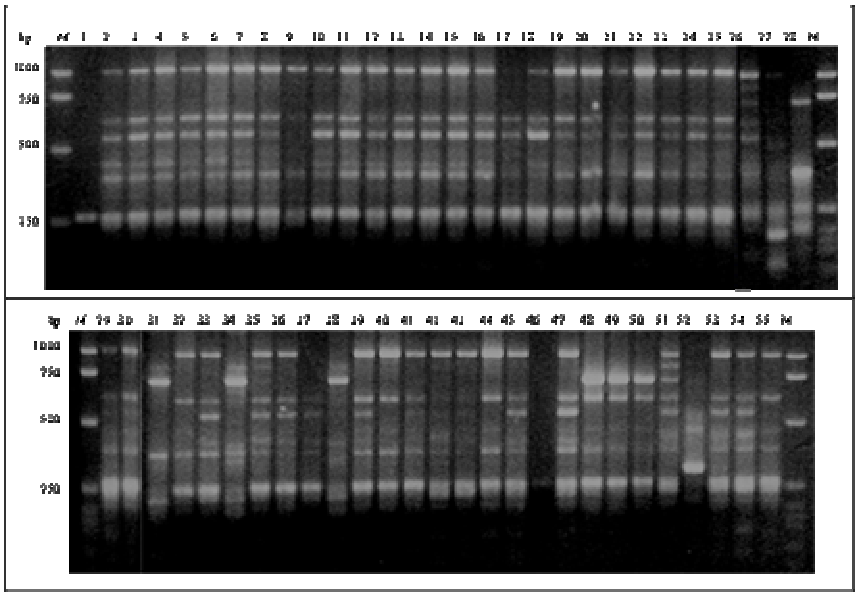

Figure 1. DNA fingerprinting patterns of 55 *M. grisea* isolates using OPB4 RAPD primer. M: 1kb molecular size marker.

Table 4. Oligonucleotide primers that showed genetic discrimination among the *M. grisea* isolates using RAPD-PCR analysis.

S/N	Operon Code	Nucleotide Sequence 5' to 3'	No. of Fragments Amplified	No. of Polymorphic Bands
1	OPA-01	CAGGCCCTTC	15	11
2	OPA-03	AGTCAGCCAC	14	9
3	OPA-07	GGTGACGCAG	18	14
4	OPA-11	CAATCGCCGT	14	10
5	OPA-13	CAGCACCCAC	15	9
6	OPB-04	GGACTGGAGT	18	10
7	OPB-06	TGCTCTGCCC	10	8
8	OPB-10	CTGCTGGGAC	16	13
9	PAP2	TACAACGAGG	18	12
10	PAP3	TGGATTGGTC	15	12
		TOTAL	153	108

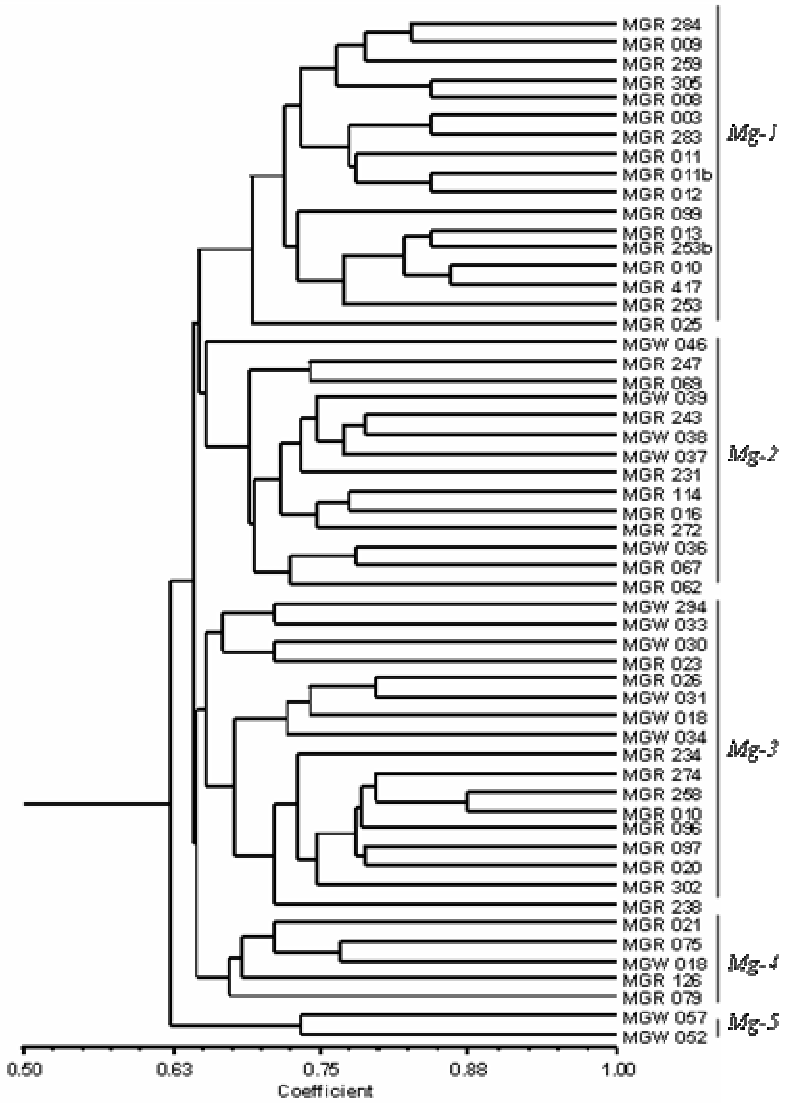

Figure 2. Phylogenetic diversity of 55 *M. grisea* isolates identified using 108 RAPD markers.

and *Mg-5* respectively (Table 5).

The study revealed that among the five groups, only *Mg-1* was found at Farako-Ba while *Mg-2, Mg-3* and *Mg-4* were distributed in two or three 3 localities. Isolates originating from the same host plants and from the same localities belong to different groups. Similar results were obtained by Xia et al. (1993) who found four lineages from a single cultivar in two fields in a single region in the USA. Therefore, sampling for assessing blast pathogen genetic diversity should take such results into consideration in order to ensure that the individuals sampling do reflect the genetic diversity of the population.

The high distinction pattern of each isolate obtained in this study suggests possible and frequent occurrence of mutants in *M. grisea* in different host cells (Bronson et al., 1990; Chumley and Valent, 1990; Levy et al., 1991; Klister and Miao, 1992). The limited number of morphological and cultural characters of *M. grisea*, and the lack of standardization of cultural conditions and virulence tests among the different researchers have led to confusion and uncertainty in the characterization of this pathogen (Babujee and Gnanamanickham, 2000). Distinct phenotypes usually consist of isolates that are genetically less related and such identification of isolates using cultural and morphological techniques often lacks consistency and precision (Babujee and Gnanamanickham, 2000). In the current study, we have found that identification of genetic diversity in *M. grisea* depends on different host cells and occurrence of mutants. For instance, 17 isolates genotyped as *Mg-1* were originated only from cultivated rice varieties from Farako-Ba experimental station, while 14 isolates from *O. longistaminata* and associated cultivated rice were genotyped as *Mg-2*. Furthermore, the *Mg-3* genotype consists of 22 isolates originated from other rice-related weeds and associated cultivated rice varieties while two isolates that do not interact with a cultivated rice variety but originated only from *O. longistaminata* were genotyped *Mg-4*.

The possible population structure, frequency and distribution of *M. grisea* genotypes in Burkina Faso have been revealed by this study. RAPD markers indicated possible relationship between host origin, mutation and genetic variation among *M. grisea* isolates, and this demonstrated the fingerprinting and diagnostic potential of RAPD. Obviously, for these DNA band patterns to have practical meaning in the areas of plant pathology, population biology and molecular epidemiology, specific DNA bands must be related to host origins, mutation and virulence genes (Welsh and McClelland, 1990). This could be accomplished by a systematic comparison of DNA band patterns among fungi, contrasting for the different host origins, mutation and the virulence genes present. A similar approach has been used to differenttiate aggressive from non-aggressive isolates of the oilseed rape pathogen *Phoma lingam* (Schafer and Wostmeyer, 1992).

The DNA fingerprint defined for each race of *M. grisea* should be useful for epidemiological surveys, disease diagnoses, and in the identification of new virulent strains, isolates and their origin. This information could be useful in rice breeding programs aiming at development of a lineage exclusion method (Zeigler et al., 1994) in breeding for durable blast-resistant rice cultivars to different rice ecologies and localities in Burkina Faso. However, our study didn't reveal any correspondence between virulence of *M. grisea* and the fingerprinting group as each group is composed of several virulence strains. A study conducted by Rathour et al. (2004) indicated that no correlation was observed between RAPD pattern and the virulence characteristic of the pathogen. The degree of correlation between lineages and pathotypes varies among populations, strong in the USA, Colombia and Europe, but moderate in the Philippines and Vietnam, while no clear correlation was observed in Japan (Park et al., 2003). Therefore, the relationship between virulence and lineage seems to be less complex in countries with a short history of rice cultivation and with a limited number of lineages than in those countries with a long history and high number of lineages (Piotti et al., 2005).

Conclusion

In order to analyze the pathogenic population structure of the blast fungus in Burkina Faso, trapping nurseries of varieties with known resistance genes were installed. Our study indicated that such nurseries are an effective tool to characterize the virulence spectrum of blast populations. They also avoid the difficulties of collecting representative samples and minimizing variability inherent to the experimental system. The nurseries made it possible to characterize the best site to be used for screening for resistance to blast disease. The results also revealed the effectiveness of some resistance genes: *Pita*, *Pib* associated with *Pish*, *Pit* and the three genes (*Piks*, *Pizt* and *Pish*) associated in Tetep. Pyramiding some of these resistance genes can provide durable resistance to the blast fungus in Burkina Faso as the resistance of cultivars that carry several resistance genes should be longer-lasting than those carrying single genes (Kiyosawa, 1982). However, the reaction of varieties with known resistance genes indicated that some of them may bear additional undermined resistance genes which mean that the blast fungus pathoype in Burkina Faso, as revealed by the reaction of the varieties carrying known resistant genes, is different from what was found so far in Asia. Therefore, it appeared necessary to reassess the behavior of the resistance genes identified in Asia against the Burkina Faso blast population because the precise delineation of pathogenic variability in the rice production area is a prerequisite for identifying rice genotypes with a broader resistance spectrum (Rathour et al., 2004). The

Table 5. DNA fingerprint groups and corresponding isolates with their host plant and locality of origin

Group	Isolate Code	Host Plant	R gene	Locality
Mg-1	MGR 417	4418	?	Farako-Ba
	MGR 305	Aichi Asahi	Pia +Pi19(t)	Farako-Ba
	MGR 284	Caloro	Piks	Farako-Ba
	MGR 283	Caloro	Piks	Farako-Ba
	MGR 259	Delta	?	Farako-Ba
	MGR 253	Dular	?	Farako-Ba
	MGR 253b	Dular	?	Farako-Ba
	MGR 099	K3	Pikh	Farako-Ba
	MGR 025	Pekin	?	Farako-Ba
	MGR 013	Shin 2	Piks+Pish	Farako-Ba
	MGR 011b	Tsuyuake	Pikm	Farako-Ba
	MGR 011	Tsuyuake	Pikm	Farako-Ba
	MGR 012	Tsuyuake	Pikm	Farako-Ba
	MGR 010	Usen	Pia+Pi?	Farako-Ba
	MGR 009	Yashiro Moshi	Pita	Farako-Ba
	MGR 008	Yashiro Moshi	Pita	Farako-Ba
	MGR 003	Yashiro Moshi	Pita	Farako-Ba
Mg-2	MGR 272	Caloro	Piks	Banfora
	MGR 247	FKR 19	?	Sideradougou
	MGR 243	FKR 19	?	Sideradougou
	MGR 231	Fukunishiki	Piz	Banfora
	MGR 114	Ishikari Shiroke	Pii+Piks	Banfora
	MGR 067	LOCAL variety	?	Koumadougou
	MGR 062	LOCAL variety	?	Koumadougou
	MGR 069	Local variety	?	Sideradougou
	MGW 036	*Oryza longistaminata*	?	Banfora
	MGW 038	*O. longistaminata*	?	Labola
	MGW 039	*O. longistaminata*	?	Sideradougou
	MGW 037	*O. longistaminata*	?	Sideradougou
	MGW 046	*O. longistaminata*	?	Vallee kou
	MGR 016	Shat Tiao Tsao	Piks	Banfora
Mg-3	MGR 302	Aichi Asahi	Pia +Pi19(t)	Farako-Ba
	MGW 294	*Brachiaria sp*		Farako-Ba
	MGR 274	Caloro	Piks	Farako-Ba
	MGR 258	Dourado	?	Farako-Ba
	MGR 238	FKR 19	?	Banfora
	MGR 234	FKR 28	?	Farako-Ba
	MGR 097	K60	Pikp+Pish	Farako-Ba
	MGR 096	Kanto 51	Pik	Farako-Ba
	MGW 034	*Paspallum scrobiculatum*	?	Farako-Ba
	MGW 033	*P. scrobiculatum*	?	Farako-Ba
	MGW 031	*P. scrobiculatum*	?	Farako-Ba
	MGW 030	*P. scrobiculatum*	?	Farako-Ba
	MGR 026	Pekin	?	Farako-Ba
	MGR 023	Pekin	?	Farako-Ba
	MGR 020	Pi N°4	Pita2+Pish	Farako-Ba
	MGW 018	*Rottbollia sp*	?	Farako-Ba

Table 5. Contd.

	MGR 010	Usen	Pia	Farako-Ba
Mg-4	MGR 126	Ishikari Shiroke	Pii+Piks	Farako-Ba
	MGR 021	Pekin	?	Farako-Ba
	MGR 079	Local variety	?	Labola
	MGR 075	Local variety	?	Labola
	MGW 018	*Setaria pallidae-fusca*	?	Sideradougou
Mg-5	MGW 057	*O. longistaminata*	?	Vallée du Kou
	MGW 052	*O. longistaminata*	?	Vallée du Kou

development of near-isogenic lines from IRRI (International Rice Research Institute) offers the possibility of evaluating single gene effects.

Molecular markers are used extensively to characterize plant pathogens and elucidate population genetic structure and the evolutionary relationship of plant pathogens (Traoré et al., 2005; Fargette et al., 2004). Most of the molecular studies carried out on the blast pathogen were done using restriction fragment length polymorphism (RFLP) techniques with the probe MGR-586 (Levy et al., 1991; Chen et al., 1995; Mekwatanakarn et al., 2000). However, such techniques are expensive and time-consuming, making them prohibitive for the analysis of a large number of samples (George et al., 1998) as well as for laboratories with modest facilities. Our results confirm that DNA (RAPD) offers an inexpensive and speedy means to generate molecular markers for analyzing the population structure of blast fungus. However, further investigation is needed on the relationship between DNA fingerprinting group and pathotypes in order to develop methods to establish population structures and define efficient resistance genes for use in breeding for durable blast resistance.

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