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RESEARCH

Magnaporthe oryzae Populations Adapted to Finger Millet and Rice Exhibit Distinctive Patterns of Genetic Diversity, Sexuality and Host Interaction

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Abstract In this study, host-specific forms of the blast pathogen *Magnaporthe oryzae* in sub-Saharan Africa (SSA) were characterised from distinct cropping locations using a combination of molecular and biological assays. Finger millet blast populations in East Africa revealed a continuous genetic variation pattern and lack of clonal lineages, with a wide range of haplotypes. *M. oryzae* populations lacked the *grasshopper* (*grh*) element (96%) and appeared distinct to those in Asia. An overall near equal distribution (47–53%) of the mating types *MAT1-1* and *MAT1-2*, high fertility status (84–89%) and the dominance of hermaphrodites (64%) suggest a strong sexual reproductive potential. Differences in pathogen aggressiveness and lack of cultivar incompatibility suggest the importance of quantitative resistance. Rice blast populations in West Africa showed a typical lineage-based structure. Among the nine lineages identified, three comprised ~90% of the isolates. Skewed distribution of the mating types *MAT1-1* (29%) and *MAT1-2* (71%) was accompanied by low fertility. Clear differences in cultivar compatibility within and between lineages suggest R gene-mediated interactions. Distinctive patterns of genetic diversity, sexual reproductive potential and pathogenicity suggest adaptive divergence of host-specific forms of *M. oryzae* populations linked to crop domestication and agricultural intensification.

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Introduction

Finger millet (*Eleusine coracana*) and rice (*Oryza sativa*) are important staples for millions of poor people in sub-Saharan Africa (SSA) and as such are key food security crops. Finger millet is widely grown in the semi-arid areas of Africa and plays a key role in the livelihood of small-holder farmers. Finger millet is being increasingly recognised as highly nutritious for the weak and immuno-compromised. Blast disease is very prominent among the constraints that affect the yield, utilization and trade of finger millet within East Africa [1]. The disease affects the crop at all growth stages and panicle blast is the most destructive form of the disease [2]. It is especially serious in western Kenya and north-eastern Uganda. Many of the widely grown landraces and varieties are susceptible to blast disease with losses of 10–50% being common [2, 3].

The demand for rice in SSA is increasing at double the rate of population growth in various West African countries, accounting for 20–50% of total calorific consumption. NERICA varieties of rice are also being introduced into East Africa on a massive scale [4]. With the intensification of rice cultivation, blast disease has become the most serious and destructive fungal disease of rice in SSA and different types of blast disease predominate in different production systems. Varying levels of yield losses (up to 77%) due to blast under different agro-ecological conditions have been reported in West Africa [5]. Globally, rice blast disease is mainly controlled using resistant cultivars. However, where blast is prevalent, resistance breakdown is well documented. Consequently, research efforts have focused on understanding the blast pathogen population structure by combining modern molecular-biotechnological approaches with traditional pathological assays [6–9]. These studies have led to novel rice blast resistance breeding and deployment strategies [10] and similar approaches are being tested with other systems [11, 12].

Blast disease of both finger millet and rice is caused by the ascomycete fungus *Magnaporthe oryzae* (anamorph: *Pyricularia oryzae* [13]), which is known to infect more than 50 monocot species. Despite the wide host range of the pathogen, *M. oryzae* populations mainly tend to exist as host-specific (adapted) forms, capable of infecting a single host. Lack of knowledge of the blast pathogen population biology on finger millet and rice in SSA has hindered efforts towards the identification and the development of resistant cultivars adapted to local agroecological conditions. In this study, more than 600 *M. oryzae* isolates from these crop systems in SSA have been characterised applying a combination of molecular tools and biological assays. *M. oryzae* populations on these key food security crops in Africa reveal distinctive patterns of genetic diversity, sexuality and pathogenicity, suggesting adaptive divergence of host-specific forms of the pathogen linked to domestication and agricultural intensification of finger millet and rice.

Materials and Methods

Fungal Isolates, Storage and Growth Conditions

Finger millet blast samples were collected from 23 locations mainly covering farmers' fields in north-eastern Uganda and western Kenya as well as screening sites at Serere, Uganda and Alupe, Kenya. 280 isolates were collected from cultivated (*E. coracana*) and wild millet (*Eleusine* sp./*E. indica*) as well as other weed hosts (*Cynodon dactylon*, *Dactylotenium aegyptium*, *Digitaria horizontalis*, *Digitaria scalarum*, *Isachne kiyalaensis* and *Pennisetum purpureum*) and leaf, neck and panicle blast samples following a macrogeographic survey of various districts in Kenya and Uganda. 48 isolates were collected from leaf, neck and panicle blast samples from nine varieties planted in a microgeographic survey experiment at the National Semi-arid Resources Research Institute (NaSARRI), Soroti district, Uganda. Further details of the 328 isolates are provided in Table S1. Rice blast samples were collected from 24 locations in Ghana, Nigeria, Burkina Faso and Côte d'Ivoire mainly covering screening sites and surrounding farmers' fields. Blast samples were collected from cultivated rice (*O. sativa*), wild rice (*O. barthii* and *O. longistaminata*), and various weed hosts (*Andropogon gayanus*, *Brachiaria mutica*, *Brachiaria* sp., *Eleusine* sp., *Paspalum scrobiculatum*, *Rottboellia exaltata*, *Rottboellia* sp. and *Setaria* sp.). A total of 633 mono-conidial cultures of *M. oryzae* were established by plating surface-sterilised blast samples on oatmeal agar medium and preserved on filter paper discs (Table 1; Tables S1, S3). Cultures were grown in 2× yeast extract glucose medium [14], and the mycelium was freeze-dried and stored at −20°C.

Molecular Analyses

For AFLP analysis (finger millet blast isolates), genomic DNA was extracted from 40 mg of freeze-dried mycelium using GenElute^{TM} Plant Genomic DNA Miniprep Kit (Sigma, UK). AFLP analysis was performed using a kit containing 96 *Eco*RI/*Mse*I primer combinations including two and three selective bases (Invitrogen, UK). For

Table 1 Blast pathogen *M. oryzae* and *M. grisea* isolates used in this study

Country	No. of locations[a]	Host	No. of isolates[b]
Uganda, East Africa	16	Finger millet	175
		Wild millet and weed hosts	24
Kenya, East Africa	7	Finger millet	98
		Wild millet and weed hosts	31
Ghana, West Africa	7	Rice	67
		Wild rice and weed hosts	4
Nigeria, West Africa	4	Rice	23
Cote d' Ivoire, West Africa	7	Rice	131
		Wild rice and weed hosts	8
Burkina Faso, West Africa	6	Rice	55
		Wild rice and weed hosts	17
Total number of isolates			633

Other isolates[c]

MG-R, MG 70-6, Guy11, TH3, JP15	Rice
G22	Finger millet
BR62	Wild millet
I-R-22	Laboratory strain
4136-4-3	Laboratory strain

Isolate stocks are held at the Division of Science, University of Bedfordshire

[a] Includes 48 isolates collected from nine varieties planted for a microgeographic survey experiment at NaSARRI, Soroti district, Uganda

[b] Further details of the isolates are provided in Tables S1 and S3

[c] Isolate R (4375.R.26) was provided by J. E. Hamer and M. Levy; Isolate MG 70-6 was provided by A. Ellingboe; Isolates Guy11, TH3, JP15, G22, BR62, I-R-22 and 4136-4-3 were from the collection held by N.J. Talbot at Exeter

MGR586 fingerprinting (rice blast isolates), DNA was extracted from 300 mg mycelial powder following the CTAB method [14] to obtain sufficient quantities. *Eco*RI DNA digestion and MGR586 hybridisation were carried out following standard protocols [6] along with an international reference strain R (isolate code 4375.R.26, Dr. John Hamer) used in three lanes across a gel as internal comparison. DNA profile data were employed to score the presence and absence of each fragment and generate a binary matrix. With the finger millet blast isolates, a composite binary matrix was generated by pooling the AFLP data from five primer combinations. The binary matrix was used in cluster analysis with boot strapping to identify genetic groups (lineages) utilising the GENSTAT and WINBOOT packages [7]. In the AFLP analysis, 15 randomly chosen finger millet blast isolates were used in initial screening of 96 primer combinations. In the subsequent detailed population analysis, these 15 isolates were repeated with each of the five selected primer combinations and served as internal controls to ensure the reproducibility and robustness of the AFLP profiles and haplotypes. With the MGR586 profiling, use of reference strain R in every single gel and repeat fingerprinting of 29 isolates (Table 1) obtained from wild rice and weed hosts along with lineage representatives served as quality control. In addition, consistency in DNA extraction, electrophoresis and PCR conditions were maintained, where appropriate, to ensure the reproducibility of the DNA profiles.

Magnaporthe oryzae isolates were screened for the presence of the grasshopper (*grh*) DNA repeat element by PCR using two sets (PES and PKE) of primers spanning the *grh* repeat element sequence. PES primer set (PESF: 5′-GC GTTCGAAGCGTTGAAACAC-3′ and PESR: 5′-AGCTA TATAAGCCCTAAGGTATTGC-3′) and PKE primer set (PKEF: 5′-CGGAATTCTTCAGTCACGGGAACAAGC-3′ and PKER: 5′-TCCGAGGTGCACATGTGTGAAACGC-3′) were designed from known sequences (M77661 and M77662). *MAT1-1* (MAT1-1 F and R) and *MAT1-2* (MAT1-2 F and R) specific primers designed based on known sequences [15] were used to identify the mating type. Sequences of the primers used in PCR are: MAT1-1 F: 5′-TGCGAATGCC TACATCCTGTACCGC-3′; MAT1-1 R: 5′-CGCTTCTGA GGAACGCAGACGACC-3′; MAT1-2 F: 5′-TCTGCTTG AAGCTGCAATACAACGG-3′ and MAT1-2 R: 5′-CAT GCGAGGGTGCCATGATAGGC-3′. PCRs were carried out with 1 ng of genomic DNA at 60°C annealing temperature using REDTaq™ Ready Mix™ (Sigma, UK) according to standard procedures [16].

Mating Assays

Isolates were crossed with testers of opposite mating type according to standard procedures [17, 18]. The testers used included isolates 4136-4-3, TH3 and I-R-22 for *MAT1-1* and Guy11, JP15 and BR62 for *MAT1-2* [19] and K23/123 established in this study. Perithecia were observed under a microscope approximately 4 weeks after crossing. Crushed perithecia were observed to determine the presence of asci and ascospores, and the viability of the ascospores was tested on water agar plates by observing their germination.

Pathogenicity Assays

Seeds of finger millet varieties E11 (susceptible), P665, INDAF 5, SEREMI 1, SEREMI 2, SEREMI 3, PESE1 and GULU E (moderately resistant) chosen based on previous

field rating, were provided by NaSARRI. Thirty-one finger millet blast isolates selected mainly representing the sampling locations and the host range were tested on these varieties with three replicates for each variety and isolate combination. Previously characterised isolates K5/23 and D15/S6 were included in every single batch of the pathogenicity assays to monitor reproducibility and to set up repeat assays, if necessary. Spore suspensions (40 ml of 1×10^5 spores/ml) were prepared from 15-day-old OMA cultures using 0.5% gelatin solution. Finger millet seedlings were grown in trays for 2 weeks at 25–27°C and 12 h light. Each tray was isolated from its surroundings by placing it in a large polythene biohazard bag and sprayed with 10 ml of the inoculum. After spraying, the tops of the bags were sealed and the plants were incubated for 7 days. The total number of blast lesions on the fourth leaf and the approximate percentage area covered by lesions were recorded. The raw data were averaged between replicates and analysed by Genstat. Seeds of the international rice differentials Raminad Str. 3, Zenith, NP-125, Usen, Dular, Kanto 51, Sha-tiao-tsao and Caloro used in pathotyping of rice blast isolates [6] were provided by Africa Rice Center. Growth of rice seedlings, preparation of spore suspension and inoculation with three replicates were as described above. In pathotyping assays, 156 *M. oryzae* isolates representing various genetic lineages were tested. A generally compatible cultivar CO39 and a *M. oryzae* isolate of known pathotype (IB54) were employed to monitor reproducibility and to set up repeat assays, if necessary. Host responses were scored [14] and pathotype designations were assigned [20]. Uninoculated controls were maintained in the same greenhouse. All pathogenicity assays were conducted utilising the greenhouse facilities at the University of Warwick, UK.

Results

Characterisation of the Finger Millet Blast Pathogen Populations in East Africa

Genetic Diversity

A total of 328 *M. oryzae* isolates including 280 from 22 macro-geographic survey sites across Kenya and Uganda, and 48 from a micro-geographic survey at Serere, Uganda were characterised by AFLP analysis. Five primer combinations were chosen based on profile patterns, according to an initial screen of 96 primers using 15 *M. oryzae* isolates. Of the five primer sets, E-TC/M-CA revealed the highest genetic variation and E-GT/M-CT the least and up to 15 bands ranging between 50 and 800 bp were amplified from each isolate. No variation was observed in AFLP profiles in repeat assays, based on the 15 isolates used as internal controls. Comparison of the sequence from a set of bands with the *M. oryzae* genome (strain 70–15) revealed that these fragments represented loci on different chromosomes/linkage groups (data not shown). Each isolate with a unique profile was designated as a single haplotype (SH) and isolates with identical profiles as shared haplotypes (ShH). In UPGMA cluster analysis, 195 haplotypes identified among the 280 *M. oryzae* isolates from various parts of Uganda and Kenya showed less than 40% overall variation, with the exception of isolates from *Digitaria* spp. The *M. oryzae* haplotypes from different types of blast as well as those from different hosts were randomly distributed among the overall population represented on the dendrogram. A lack of distinct genetic groups or lineages and <50% bootstrap support for any of the isolate clusters observed with the finger millet blast system clearly supported a continuous genetic variation pattern of the pathogen population (Fig. 1). However, isolates from *Digitaria* spp. formed a distinct cluster with a high bootstrap value of 87% (Fig. 1).

A consensus tree based on the ITS region (approx. 550 bases) revealed two distinct groups designated A and B with 100% bootstrap support (Fig. S1). Group A included blast pathogen isolates from finger millet and wild millet and B comprised isolates from *Digitaria* species. The subgroups A1 and A2 did not differentiate the isolates with and without the *grh* element. As evidenced by 3.5–3.7% nucleotide sequence differences between them, *M. oryzae* isolates from *Eleusine* spp. (0–0.7%) were phylogenetically distinct to those from *Digitaria* spp. (0–0.4%).

The macro- and micro-geographic populations (328 isolates) were also screened by PCR to investigate the presence or absence of the *grh* repeat element (Fig. 2). Vast majority of the pathogen population (96%) did not contain the *grh*. Of the 13 isolates that yielded positive amplicons of 836 and 1,347 bp from two target regions (e.g. Fig. 2a), 12 were from four different districts in Kenya recovered from finger millet as well as wild millet. These 13 isolates represented different haplotypes with varied mating type and fertility status (Fig. 2b).

Haplotype Diversity and Distribution

Among the 195 *M. oryzae* haplotypes identified in macrogeographic surveys, 160 were SHs and 35 were ShHs representing 120 isolates, with each ShH comprising 2–12 isolates. Overall, 148 haplotypes from finger millet, 26 from wild millet with eight shared between finger millet and wild millet and 13 haplotypes from other weed hosts were identified. Haplotype diversity index ranged from 0.6 to 1.0 across various districts in the two countries. Between Kenya and Uganda, the number of *M. oryzae* haplotypes identified was comparable at 102 and 106, respectively.

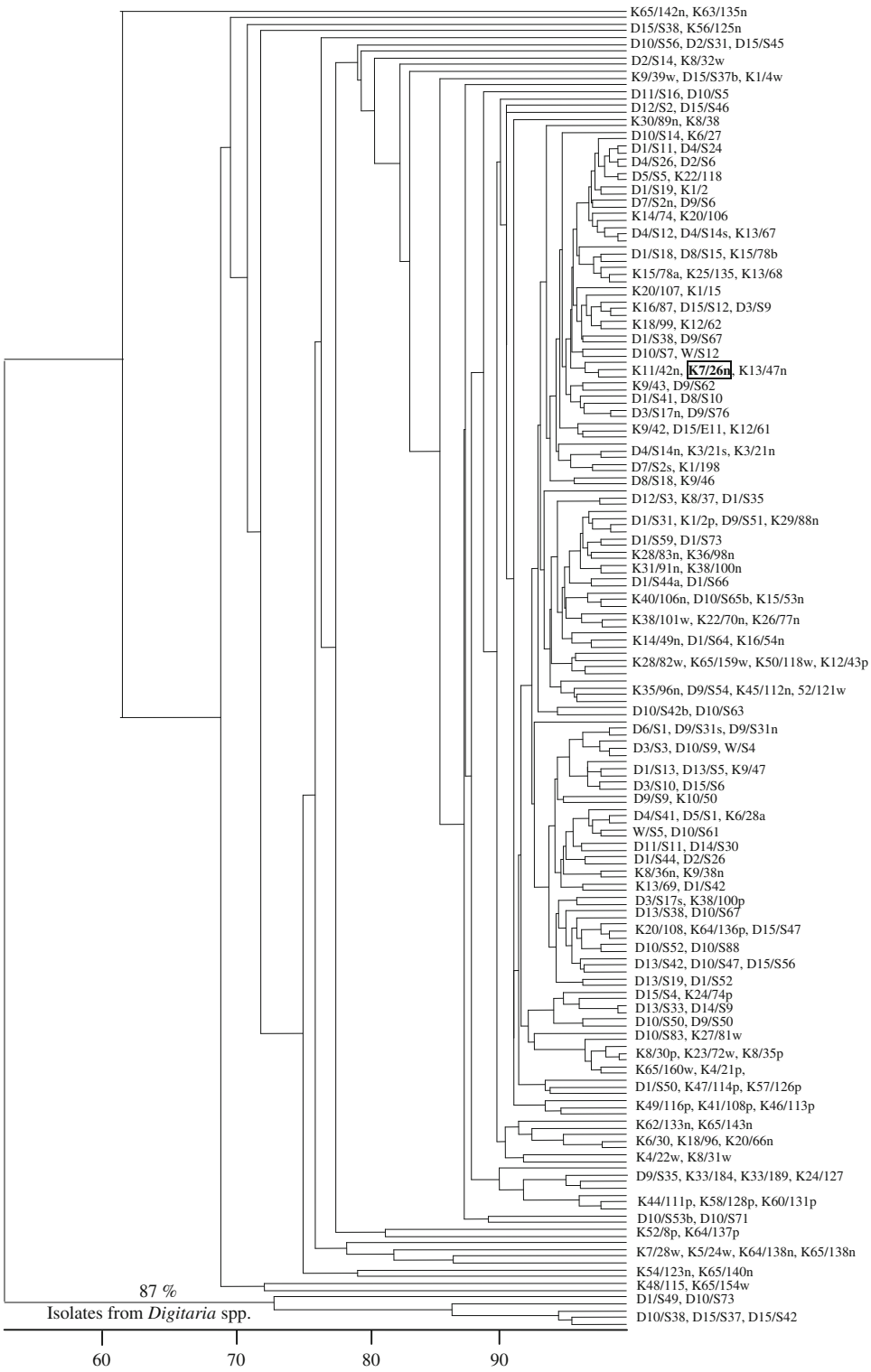
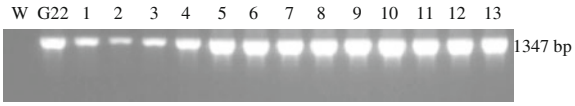


Fig. 1 Dendrogram depicting the continuous variation pattern of the *Magnaporthe* species haplotypes associated with finger millet blast in East Africa based on AFLP (amplified fragment length polymorphism) profiles. AFLP data generated with five primer-pair combinations were pooled together to prepare a binary matrix which was analysed by the UPGMA (unweighted pair group method with arithmetic averages) method; bootstrap analysis of 100 replications of the data set revealed less than 50% support for any of the isolate clusters within *M. oryzae* indicating that the haplotypes do not form distinct genetic groups or lineages; isolate K7/26n representing the widely distributed ShH24 is shown in a *box*. *M. grisea* isolates from *Digitaria* species formed a distinct cluster from rest of the haplotypes with 87% bootstrap support, as shown in the dendrogram. Scale bar represents percentage similarity. For ShH with identical AFLP profiles, only representative isolates are shown in the dendrogram and the various isolates included by each of the ShH are shown in Table S2

Among these, 89 haplotypes were restricted to Kenya and 93 were restricted to Uganda, whilst 13 haplotypes occurred in both countries. Thus, *M. oryzae* isolates collected from each location represented genetically variable populations dominated by SHs. And the diversity index of SHs ranged from 0.5 to 0.8 across the various districts surveyed in the two countries. The diversity index of ShHs ranged from 0.2 to 0.5 among the survey locations in Kenya and Uganda. The ShHs revealed varying patterns of distribution with some that were restricted. Others such as ShH 24 represented by K7/26n (Fig. 2) was one of the widely distributed haplotypes recorded from both finger millet and wild millet, in two

A



B

Isolate	Country	District	Host	Haplotype[a]	Mating type[b]	Fertility[c]
K13/67	Kenya	Teso	*E. coracana*	SH	1.1	H
K15/53n[d]	Kenya	Teso	*E. coracana*	ShH-20	1.1	H
K1/15	Kenya	Teso	*Eleusine sp. (wild millet)*	SH	1.2	H
K33/184	Kenya	Kericho	*E. coracana*	SH	1.2	H
K33/189	Kenya	Kericho	*E. coracana*	SH	1.2	H
K24/127	Kenya	Kisii	*E. coracana*	SH	1.1	H
K44/111p[d]	Kenya	Kisii	*E. coracana*	SH	1.1	I
K48/115n	Kenya	Kisii	*E. coracana*	SH	1.2	M
K56/125n	Kenya	Gucha	*E. coracana*	SH	1.1	I
K58/128p	Kenya	Gucha	*E. coracana*	SH	1.1	H
K60/131p	Kenya	Gucha	*E. coracana*	ShH-08	1.1	I
K55/124p	Kenya	Gucha	*E. coracana*	ShH-05	1.2	M
D1/S11	Uganda	Pallisa	*E. coracana*	SH	1.2	I

Fig. 2 *Magnaporthe oryzae* isolates containing the *grh* element among the populations associated with finger millet blast in Kenya and Uganda. **a** PCR assay to amplify a *grh* fragment using primers PESF and PESR; *W* negative control without DNA, *G22* previously characterised isolate containing *grh*; Isolates *1–13* are listed in the table in the corresponding order. **b** Genotypic and phenotypic characteristics of the *M. oryzae* isolates (4%) containing the *grh* element. [a] Isolates were characterised using AFLP markers; *SH* Single haplotypes; *ShH-05, 08 and 20* Shared haplotypes. [b] Mating type was determined using a PCR assay. [c] Fertility status was determined as *H* hermaphrodites; *M* males and *I* infertile (in crosses, perithecia are produced by the test isolate and the standard tester; only by the standard tester, and no perithecia are produced, respectively); [d] K15/53n, high aggressiveness (+++) and K44/111p, low aggressiveness (+) as determined in pathogenicity tests on ten finger millet varieties. Isolate details are provided in Table S3

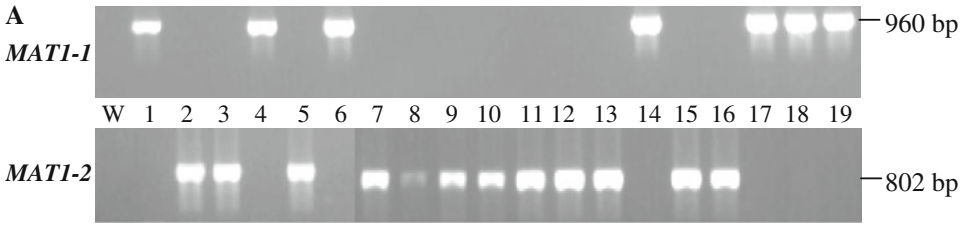
districts of Kenya and eight districts of Uganda and from leaf, neck and panicle infections during 2000 and 2002 (Table S2). Overall, 17 *M. oryzae* ShHs represented isolates collected from more than one form of blast, e.g. ShH 21 including leaf, neck and panicle blast isolates represented by K13/47n (Fig. 2; Table S2). Further, based on the varietal use pattern in Kenya, majority of the pathogen haplotypes identified were from varietal mixtures (57.3%). Among the 55 *M. oryzae* isolates from wild millet and other weed hosts, 47 were SHs (Table S2; Fig. 2). AFLP analysis of 48 *M. oryzae* isolates collected from nine finger millet varieties in a microgeographic survey at the screening site in Serere, Uganda, also showed the dominance of SHs. UPGMA analysis revealed their continuous variation pattern with no significant bootstrap support of any groups, although high similarity of some isolates was observed linked to the variety or plant part from which they originated (data not shown).

Distribution of Mating Types and Fertility Status

The PCR assay developed was effective in rapid identification of the mating type of 323 *M. oryzae* isolates (98.2%), along with six reference isolates (e.g. Fig. 3a). The five isolates from *Digitaria* spp. did not yield any PCR product, despite repeated attempts and positive amplification with other primers (data not shown), reflecting their distinctness within *Magnaporthe* species populations. *MAT1-1* and *MAT1-2* type *M. oryzae* isolates were found in near equal proportion in Uganda and Kenya, as shown in Fig. 3b. Site specific differences were observed in some districts, for example, in the Pallisa district in Uganda, *MAT1-1* and *MAT1-2* were 31 and 66%, respectively. Despite these differences, both mating types were widely prevalent. For example, among *M. oryzae* isolates collected in 2000, 47% were *MAT1-1* and 53% *MAT1-2* with a similar range for 2002. Of the 225 isolates from finger millet, *MAT1-1* and *MAT1-2* types were 48 and 52%, respectively, with a similar range among isolates from wild millet. In the microgeographic survey at Serere, *M. oryzae* isolates of both mating types were also found (Table S1).

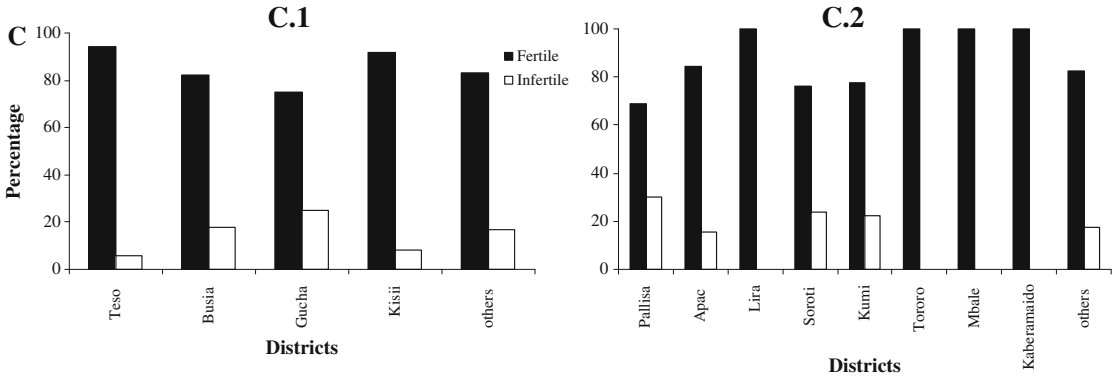
In crosses with testers, including a *MAT 1-2* isolate K23/123 identified in this study, perithecia were formed in 2–3 weeks. More than 80% of the *M. oryzae* populations were fertile ranging from 75–94.4% in Kenya to 69–100% in Uganda, including the Serere site, with 100% fertility recorded in at least four districts (Fig. 3c.1, c.2). Further, 88% of the isolates from finger millet, 86% from wild millet and also isolates from other weeds were fertile. However, the five isolates from *Digitaria* species were either very poorly fertile or infertile in crosses with the six standard testers as well as six local hermaphroditic isolates of both mating types. Crosses with 237 fertile *M. oryzae* isolates revealed asci and viable ascospores with >90% germination on 4% water agar (Fig. 3d). In crosses among *M. oryzae* isolates from the same locations, more than 60% led to the production of mature perithecia (data not shown). Within the fertile populations, the degree of fertility ranged from high (>20 perithecia per 5 mm^2 area in agar culture) in 64.4% of the isolates, to intermediate (10–19 perithecia per 5 mm^2) in 24%, and to low (<10 perithecia per 5 mm^2) in 11.6% (Fig. 3c.3). This varied across locations in both the countries, e.g. in Mbale, Uganda all the fertile *M. oryzae* isolates showed high degree of fertility, with an average of 72.7% across all locations (Fig. 3c.3). *M. oryzae* isolates from the *Eleusine* spp. showed high fertility, whereas isolates from other weed hosts showed intermediate to low degrees of fertility.

Among the fertile *M. oryzae* isolates identified in Kenya and Uganda, 63.6% behaved as hermaphrodites, 30.7% males and 5.7% females (Fig. 3c.3). However, their distribution pattern across different locations in each country varied. For instance, in Uganda, in Mbale district, all the fertile isolates were hermaphrodites, whereas in Tororo, Kaberamaido and Kumi districts only hermaphrodites (42–78%) and males (22–58%) were observed. Lira district



B

Country/Region	No. of isolates[a]	*MAT1-1*[b]	*MAT1-2*
Kenya	129	59	70
Uganda	151	70	76
East Africa	**280**	**129**	**146**



C.3

Country/ Region	Sexuality			Fertility status		
	Percentage of isolates					
	He	M	F	Hi	I	L
Kenya	62.6	30.6	6.8	56.2	32.2	11.6
Uganda	64.6	30.8	4.6	72.7	15.7	11.6
East Africa	63.6	30.7	5.7	64.4	24.0	11.6

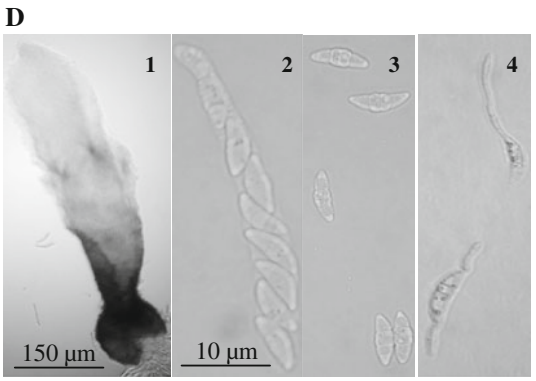


Fig. 3 Mating type distribution and fertility status among *Magnaporthe* species populations associated with finger millet blast production systems in East Africa. **a** PCR-based identification of the mating type alleles *MAT1-1* and *MAT1-2*. *1–6* are previously characterised isolates of known mating type and *7–19* are examples of isolates of unknown mating type characterised in this study; *W* is PCR negative control without DNA; *1, 4 and 6* are *MAT1-1* isolates 4136-4-3, I-R-22 and TH3, respectively; *2, 3 and 5* are *MAT1-2* isolates BR62, Guy11 and JP15, respectively; *14, 17, 18 and 19* are isolates D3/31s, D9/S59, D9/S78, D10/S54, respectively, identified as *MAT1-1*; *7–13, 15 and 16* are isolates D1/S38, D1/S44, D1/S53b, D1/S72, D2/S24, D2/S26, D2/S28, D4/S38 and D9/S32, respectively, identified as *MAT1-2*. **b** Distribution of mating types across key cropping locations in Kenya and Uganda. [a] Among the total number of isolates obtained from Kenya and Uganda, mating type of the five isolates from *Digitaria* species could not be determined by the PCR assay; three of these isolates could be typed in mating crosses and two isolates could not be typed either by PCR or by crossing; Table S3 provides details of the isolates. **c** Mating behaviour and fertility status of the *Magnaporthe* species isolates. Others in *c.1* are the districts of Homabay, Kericho and Suba in Kenya; Others in *c.2* are the districts of Katakwi, Masindi, Busia, Iganga, Kamuli, Nakasongola and Bugiri in Uganda; *c.3*: *He* hermaphrodites, *M* males and *F* females (in crosses, perithecia are produced by the study isolate and the standard tester; only by the standard tester and only by the study isolate, respectively); *Hi* high, *I* intermediate and *L* low indicate the level of fertility (no. of perithecia per 5 mm^2 in crosses on agar plates). **d** *1–4* are: mature perithecium releasing asci, mature ascus containing ascospores, individual ascospores and germinating ascospores, respectively, from dual culture crosses on agar plates

had the lowest percentage of *M. oryzae* isolates behaving as hermaphrodites (34%) and a higher proportion of males (45%). Similarly, at the microgeographic survey site in Serere, the proportion of males was high (75.7%), followed by hermaphrodites (21.6%) and females (2.7%).

Pathogenicity of M. oryzae Isolates

Thirty-one isolates representing diverse sampling locations and the host range were tested. These were compatible to the eight finger millet varieties used and only showed differences in aggressiveness. In regression analysis, with an average line for two controls K5/23 and D15/S6, isolates D8/S15, D14/S30 and K21/68n from finger millet were among the highly aggressive. Isolates K5/24w and K7/28w from wild millet also belonged to this category, whilst isolate K48-115n from finger millet was the least aggressive (Fig. 4a). From the single residual maximum likelihood (REML) analysis, it was evident that in terms of the aggressiveness of the *M. oryzae* isolates, country, host and plant part from which they originated were not significant factors. The eight finger millet varieties showed varied levels of blast infection which was the lowest on PESE I and SEREMI I and the highest on Gulu E and Indaf 5 (Fig. 4b). Typical spindle-shaped blast symptoms were observed on all the varieties tested (e.g. Fig. 4c) with 30 isolates from *Eleusine* spp. Isolate D15/S37 from *Digitaria* sp. produced oval spots; no blast infection was observed on healthy controls. Based on the ANOVA analysis, the overall differences between isolates and varieties were

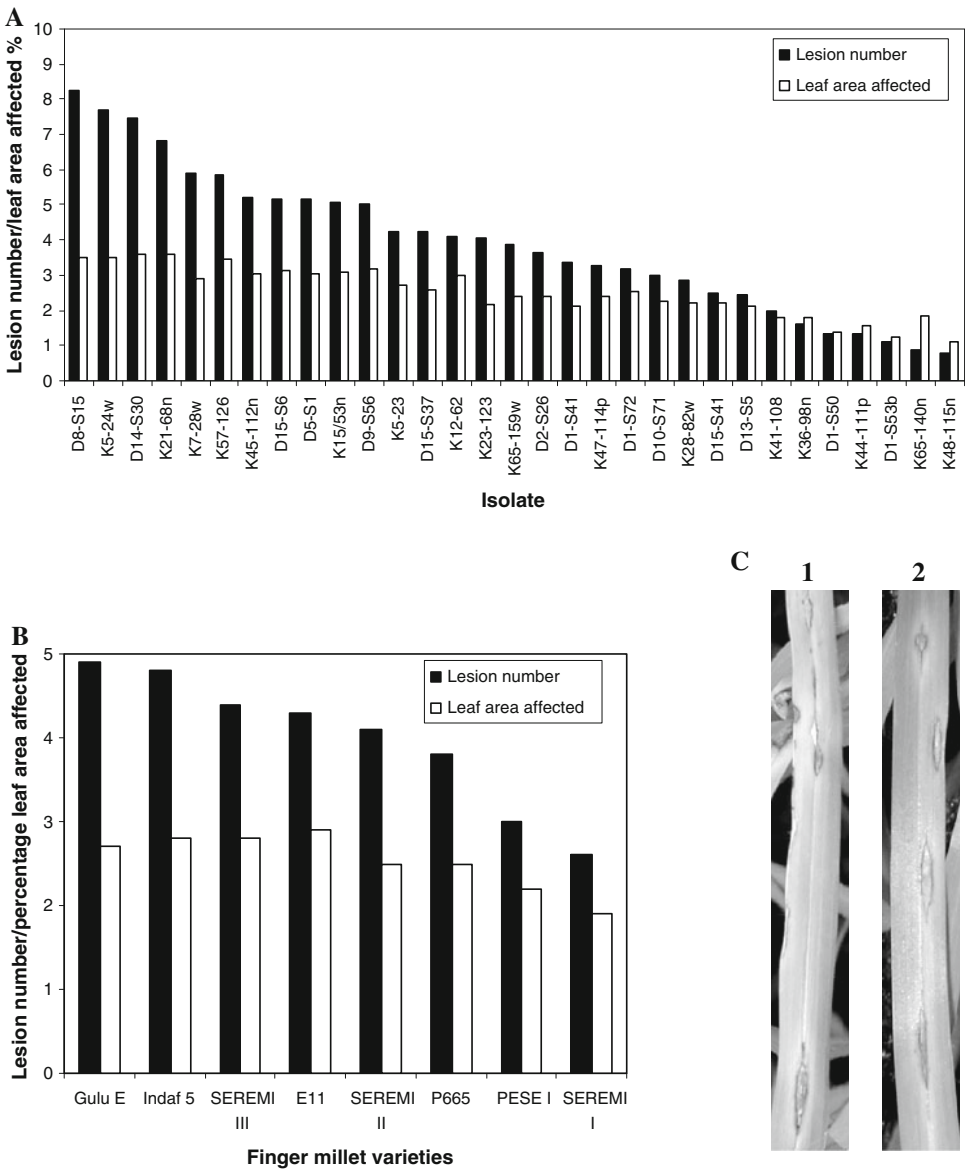


Fig. 4 *Magnaporthe oryzae* populations associated with finger millet blast in East Africa revealed only differences in aggressiveness and not cultivar incompatibility in pathogenicity tests under controlled conditions. **a** Aggressiveness of 31 isolates based on data pooled from infection levels on eight finger millet varieties; **b** reaction of eight finger millet varieties based on data pooled from the infections caused by 31 isolates; **c** typical blast symptoms on the leaves of finger millet seedlings caused by isolates from finger millet (*1* isolate K57/126) and wild millet (*2* isolate K28/82W); details of the isolates used are presented in Table S1

highly significant for the lesion numbers and leaf area affected.

Characterisation of the Rice Blast Pathogen Populations in West Africa

Genetic Diversity

MGR586 fingerprint analysis of 305 *M. oryzae* isolates from rice and associated weed hosts at key sites in Ghana, Nigeria, Burkina Faso and Côte d'Ivoire (Table 1) revealed typical lineage-based pattern in the West African region. In each of the countries, pathogen populations were partitioned into two to five distinct lineages with high bootstrap support up to 100% (e.g. Fig. 5a). Among these, one to two lineages were dominant, including 88–95% of the isolates. Other lineages were minor and of restricted occurrence. For example, among lineages GH-1 to GH-4 identified in Ghana, GH-1 was dominant comprising 56% of the isolates and present on at least 24 rice cultivars across the country except in the eastern region. Lineage GH-2 comprised 32% of the isolates but was mainly restricted to the Volta region,

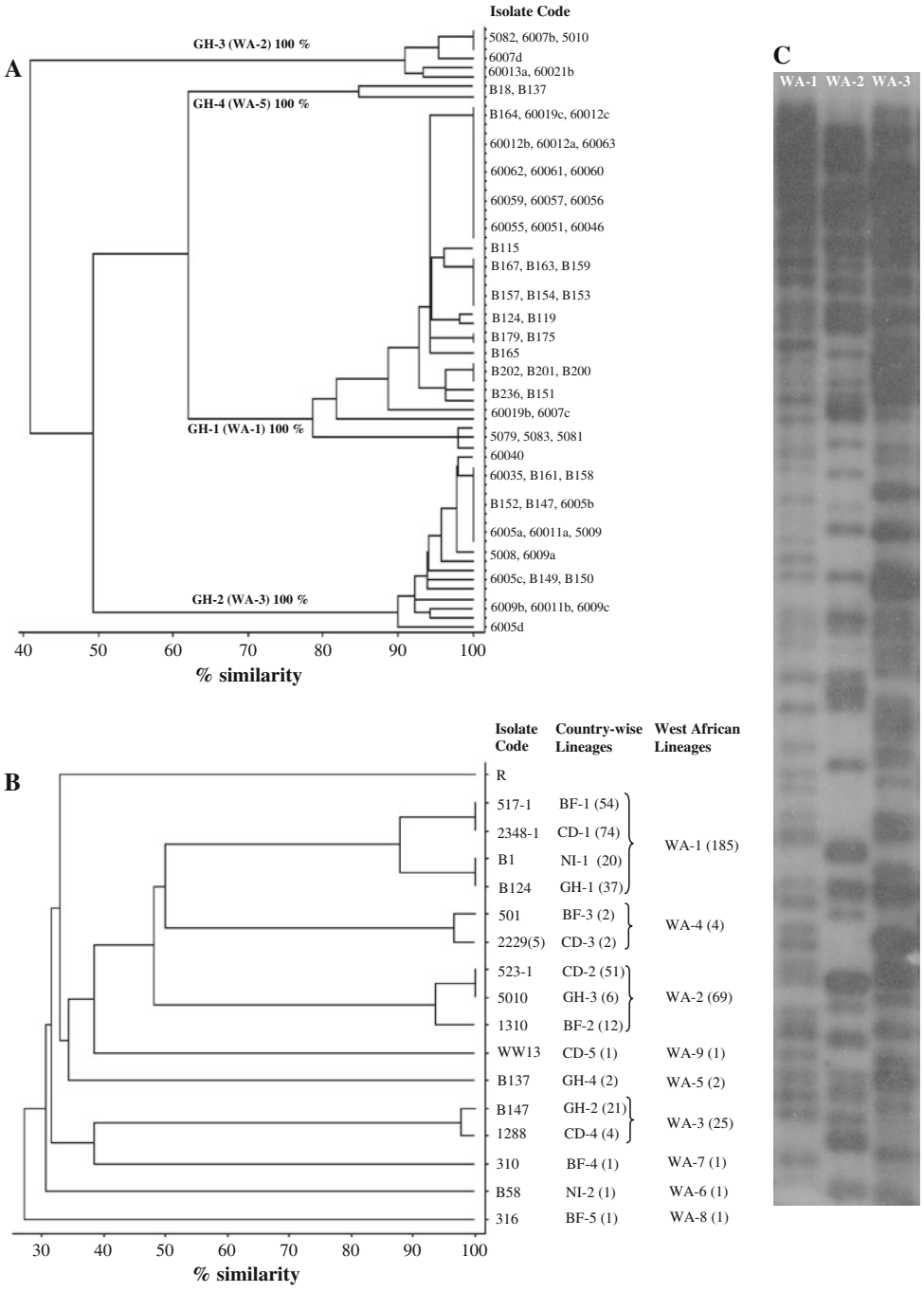


Fig. 5 Dendrograms depicting the typical lineage-based pattern of the *M. oryzae* populations associated with rice blast in West Africa based on MGR586 fingerprints. **a** An example of the limited number of lineages (GH-1 to GH-4 in Ghana) comprising the pathogen populations in a country in the West African region. **b** Nine distinct lineages identified in West Africa based on a comparison of various lineages common to the four countries with a representative isolate shown for each of the country-wise lineages; WA-1 to WA-4 are the major lineages, whilst WA-5 to WA-9 are of restricted occurrence. MGR586 fingerprint data was converted to a binary matrix which was analysed by the UPGMA (unweighted pair group method with arithmetic averages) method; bootstrap values of 100 replications of the data set are shown in percentages for each of the lineages; 305 isolates were analysed in all, and their details are provided in Table S3; *BF* Burkina Faso, *CD* Côte d'Ivoire, *GH* Ghana, *NI* Nigeria and *WA* West Africa; *R* refers to the international reference isolate used in MGR586 fingerprint analysis. **c** MGR586 fingerprints of *M. oryzae* isolates representing the three major blast pathogen lineages in West Africa and Ghana (WA-1 = GH-1, WA-2 = GH-3 and WA-3 = GH-2)

whilst GH-3 and GH-4 were minor lineages (Fig. 5a; Table S3). Similarly, among the five lineages in Burkina Faso, BF-1 was dominant including 77% of the isolates and was widely prevalent on 18 rice cultivars, wild rice (*Oryza longistaminata*) as well as weed hosts (*Bracharia* sp., *P. scrobiculatum* and *Setaria* sp.). In Nigeria, NI-1 was the major lineage (87% isolates) present at the three main sites on 20 cultivars. Of the five lineages in Côte d'Ivoire, CD-1 was dominant (56% isolates) and was wide spread on at least 25 different rice cultivars (Fig. 5b; Table S3). Sixteen isolates (approx. 5%) originating from rice, wild rice and weeds produced only up to nine MGR586 hybridising bands (Table S3). Nine distinct West African blast pathogen lineages WA1–WA9 were identified including lineages common among the four countries by combined analysis of the MGR586 fingerprints of isolates representing country-wise lineages. Lineage WA-1 (64% isolates) represented the dominant lineage in each of the four countries. WA-2 (24%) was recorded in Ghana, Côte d'Ivoire and Burkina Faso but not in Nigeria. WA-3 (8.6%) was present in Ghana and Côte d'Ivoire, whilst WA-4 to WA-9 were minor lineages of restricted occurrence (Fig. 5b; Table S3). As some of the isolates from wild rice and weed hosts showed typical rice-pathogen like MGR586 fingerprints, 29 isolates obtained from these hosts along with the lineage representatives and the reference strain R were repeat fingerprinted and no variation was observed.

Distribution of Mating Types and Fertility Status

PCR screening of more than 150 *M. oryzae* isolates revealed skewed distribution pattern of the two mating types with 29% of the isolates possessing *MAT1-1* and 71% *MAT1-2* overall. Among the four countries, the frequency of *MAT1-1* varied from 12 to 41% and *MAT1-2* from 59 to 88%. In crosses with standard testers, these isolates revealed very low fertility. For example, one isolate was male fertile and in two other isolates, perithecia formed by the tester were barren and none of the isolates were either hermaphroditic or female fertile (data not shown).

Pathotype Diversity and Distribution

Virulence spectrum analysis of 156 *M. oryzae* isolates representing various genetic lineages on a set of differentials revealed clear differences in compatibility to different rice cultivars (e.g. Fig. 6a, b). This led to the identification of 16–25 pathotypes in each of the four West African countries and their distribution patterns (Tables S3, S4). Some pathotype groups were dominant indicating the compatibility of the isolates to the commonly grown cultivars and also the narrow genetic base of the rice germplasm, e.g. IB (59% isolates) in Ghana, IC (42%) in Burkina Faso and IB (41%) in Nigeria. In Côte d'Ivoire, however, pathotype groups IA, IB, IC and ID ranged between 16 and 29% suggesting their adoption to a wider range of rice cultivars. Similarly, a diverse range of pathotypes was present at some sites, e.g. 13 pathotypes belonging to groups IA, IB, IC and ID at the Man site in Côte d'Ivoire. A number of isolates showed distinct virulence spectrum defined by a particular pathotype, but there were also examples where different isolates belonged to the same pathotype. For example, IB-1 included 27% of isolates tested in Ghana and was recorded in most of the locations surveyed (Table S3).

In total, 52 distinct pathotypes were recorded from the four West African countries, and among these, 35% were represented by two or more isolates and the rest by single isolates. Some of the pathotypes were recovered from different cultivars at the same site, e.g. IB-13 in Ghana and IG-1 in Burkina Faso. Some pathotypes were widely prevalent and were recovered from a range of rice cultivars/lines in more than one country, for instance, IB-1 from 21 different cultivars in Burkina Faso, Côte d'Ivoire, Ghana and Nigeria and IA-80 from nine different cultivars in Burkina Faso, Côte d'Ivoire and Nigeria. Some of the isolates originating from wild rice as well as weed hosts (e.g. *Paspalum* and *Setaria*) were pathogenic on the rice differentials and belonged to pathotype groups IB and IC (Tables S3, S4). A small proportion of isolates with limited MGR586 copy numbers were non-pathogenic on rice differentials as well as susceptible checks B40 and CO39.

Lineage–Pathotype Relationships

Pathotype diversity was clearly higher when compared with the genetic lineages identified in the West African region. Varied patterns of compatibility and incompatibility were observed among the blast pathogen populations partitioned into various lineages (Fig. 6b; Table S4). Some lineages represented a narrow virulence spectrum, e.g. GH-1 and GH-2 in Ghana mainly included isolates belonging to the pathotype group IB (up to 68%). Some of these pathotypes were closely related (e.g. IB-1, IB-5, IB-7 and IB-9) differing by a few compatibility reactions on the differentials. Other lineages exhibited a more complex virulence spectrum, e.g. lineage BF-1 represented 21 pathotypes belonging to diverse groups IA, IB, IC, IF, IG and IH. Similarly, in Côte d'Ivoire CD-1 represented pathotypes belonging to groups IA, IB, IC, ID, IF and IG. Overall, IB and IC, represented by all major lineages, were the dominant pathotype groups. And all isolates belonging to IB shared virulence on Zenith (Pi-z, Pi-I and Pi-a genes) and avirulence on Raminad str 3 [unknown R gene(s)]. Similarly, all isolates of IC shared virulence on NP-125 and avirulence on Raminad str 3 and Zenith (Table S4).

A

Compatibility (C) and incompatibility (IC) of *M.oryzae* isolates on rice differentials (%)																
	A		B		C		D		E		F		G		H	
Lineage	C	IC	C	IC	C	IC	C	IC	C	IC	C	IC	C	IC	C	IC
GH-1 (WA-1)	9	91	74	26	85	15	94	6	68	32	71	29	94	6	94	6
GH-2 (WA-3)	-	100	68	32	53	47	42	58	21	79	26	74	100	-	100	-
GH-3 (WA-2)	17	83	33	67	50	50	67	33	0	100	83	17	83	17	83	17
NI-1 (WA-1)	21	79	58	42	74	26	84	16	58	42	52	48	74	26	52	48
CD-1 (WA-1)	32	68	47	53	47	53	63	37	16	84	16	84	89	11	89	11
CD-2 (WA-2)	22	78	29	71	43	57	78	22	14	86	57	43	71	29	71	29
BF-1 (WA-1)	12	88	20	80	68	32	48	52	32	68	68	32	84	16	72	28
BF-2 (WA-2)	20	80	20	80	20	80	40	60	20	80	40	60	100	-	100	-

B

Fig. 6 *Magnaporthe oryzae* populations associated with rice blast in West Africa revealed clear differences in compatibility and incompatibility to rice differentials carrying various R genes in pathogenicity tests under controlled conditions. **a** Compatibility and incompatibility (%) of *M. oryzae* isolates representing the major lineages in each of the four West African countries. **b** An example of the compatible and incompatible interactions shown by these isolates on rice differentials *A–H* representing Raminad Str. 3, Zenith, NP-125, Usen, Dular, Kanto 51, Sha-tiao-tsao and Caloro, respectively. 156 isolates representing the major lineages in each of the four countries were pathotyped on the rice differentials. Details of the isolates and their reactions are available in Tables S4 and S3

Discussion

Magnaporthe oryzae populations adapted to finger millet and rice in SSA revealed distinctive patterns of genetic diversity, mating type distribution and fertility status, and host compatibility reflecting the very different histories and patterns of cultivation of these crops in this region. Finger millet was domesticated in East Africa where landraces and traditional varietal mixtures have been cultivated for thousands of years, and the crop was introduced into Asia more recently [21]. The finger millet blast pathogen populations in East Africa revealed high haplotype diversity and a lack of clonal lineages both at the macro- and micro-geographic levels. The continuous genetic variation pattern of the finger millet blast pathogen populations in this region, linked to high sexual reproduction and recombination potential is congruent with the model of ancient and recombining populations [18]. The presence of some haplotypes in various locations in both Kenya and Uganda reflects pathogen movement linked to anthropogenic activities [1, 22, 23]. This is the first report of the presence of *M. oryzae* isolates containing the *grh* element in East Africa, possibly introduced through recent germplasm exchange. Finger millet blast pathogen isolates from different parts of Asia commonly contained the *grh* element [11, 24, 25]. However, in a small number of isolates tested from East Africa in an early study, this element was not found based on Southern hybridisation analysis [24]. The current study has shown that finger millet blast is caused by at least two distinct forms of *M. oryzae* as 96% of the pathogen populations in East Africa lacked the *grh* repeat. The fact that two distinct target regions resulting in 836- and 1,347-bp products were consistently amplified in 4% of the isolates whereas neither were amplified in the others suggests that these are not false positives due to mutational changes in the sequence. These two forms could have

evolved independently, although horizontal transfer of the *grh* element into a sub-population cannot be ruled out at this stage [25].

The rice blast pathogen populations in West Africa, characterised in this study, and globally reveal a lineage-based structure. Rice cultivation in West Africa represents a unique situation as Asian varieties belonging to *O. sativa* introduced about 450 years ago largely replaced the African rice *O. glaberrima* [21]. Contrasting differences in the number of blast pathogen lineages observed in different geographic locations [6–8, 18, 26, 27] have been attributed to the history of rice cultivation and the varietal use patterns. Dominance of a limited number of pathogen lineages in each of the four West African countries over a 5-year period is similar to the situations reported in Japan and Korea, linked to extensive cultivation of a limited number of host genotypes [28, 29].

Blast pathogen populations specific to finger millet and rice in SSA showed clear differences in mating type distribution pattern and fertility status. In East Africa, an overall near equal distribution of *MAT1-1* and *MAT1-2* was established with the finger millet blast system, consistent with a strong sexual reproduction and recombination potential. Occurrence of isolates of both mating types on some finger millet varieties underlines the potential for sexual reproduction in the field. Although location-specific differences were observed in their frequency, both mating types were found in most areas surveyed. The relationship between the distribution of the mating types and the fertility status of the isolates was complex with some correlation to high lands and cool conditions. In parts of Asia, limited data indicates ~30% fertility and variable distribution of mating types in finger millet blast [30]. Sexual reproduction in the finger millet blast system appears to be conditioned by complex interactions based on the pathogen populations, the host genotype and the agroecological conditions.

In the rice blast system in West Africa, skewed distribution of the two mating types with the dominance of *MAT1-2* and very low fertility were observed suggesting that sexual recombination is unlikely to play a role in pathogen diversity as reported in various geographic locations [18, 19, 31, 32]. Similarly, on other hosts such as wheat and turf grasses only one mating type was found in each location or where both mating types were present, their ratios were highly skewed [33, 34].

The fertility status of the finger millet blast pathogen populations in East Africa, where sexually fertile and hermaphroditic isolates were frequently recovered, is in contrast to the general situation with blast on other hosts and geographic locations. This clearly fits the model that sexual fertility may be encountered in regions in which a pathogen originally evolved or regions where conditions reflect the organism's evolutionary history [35]. Finger millet blast pathogen appears similar to a few fungi in which strong potential for mating has only recently been observed [36]. This suggests a role for sexual reproduction and recombination in the continuous genetic variation pattern observed among the finger millet blast pathogen populations in East Africa. The PCR assay developed in this study enabled the identification of the mating type of 473 *M. oryzae* isolates fully in agreement with results from mating compatibility assays. This assay proved to be both rapid and robust compared with the biological and molecular assays used previously [32–34]. *Magnaporthe* sp. isolates from *Digitaria* spp., which formed a distinct genetic cluster based on AFLPs and ITS sequences, did not yield positive amplicons potentially due to sequence differences in the idiomorphs. This is in conformity with the recent distinction of blast pathogen isolates from *Digitaria* spp. as *M. grisea* and those from a number of other hosts including rice and finger millet as *M. oryzae* [13].

This study has shown clear differences in the host interaction patterns between the blast pathogen populations adapted to finger millet and rice. In the finger millet blast system, compatibility between the various isolates and the varieties tested with only quantitative differences in disease levels, suggests that polygenic quantitative resistance is more common than qualitative resistance conditioned by major R genes [37, 38]. ShH representing *M. oryzae* isolates from different types of blast revealed that genetically similar isolates are capable of causing different forms of the disease [37]. Finger millet varieties in general show a consistent reaction to different types of blast, with limited exceptions [2, 38, 39]. Furthermore, pathogenicity and molecular data from the present study have shown that weed management is a key component in effective blast disease control in the low input systems in East Africa [37]. At least eight haplotypes were common to the cultivated and wild millet suggesting the importance of the wild hosts in pathogen epidemiology [37]. However, isolates from *Digitaria* spp. were not readily encountered on the cultivated millet, potentially related to their atypical nature reflected by the oval spots produced by isolate D15/S37. The presence of ShH in two countries suggests pathogen transmission through seed exchanges and cross border trade [1, 23]. Varied levels (5–66%) of finger millet seed infection by *M. oryzae* and the potential to initiate blast disease highlight the need to use pathogen-free clean seed [37, 38, 40].

The rice blast pathogen populations in West Africa showed clear differences in cultivar compatibility. The level of pathotype diversity recorded in this region is similar to areas such as Americas and Europe [6, 8], in contrast to traditional rice growing areas such as Thailand with a huge range of pathotypes [26]. Differences in the

distribution patterns of the pathotype groups and dominant pathotypes across the four West African countries suggest adaptation to the local environment and/or commonly grown cultivars, driven by the selection pressure of host resistance genes [7]. High mutation rates at loci modulating host-compatibility can lead to pathotype changes in asexual fungi such as *M. oryzae*. A high degree of variation in *M. oryzae* isolates in the telomere regions containing a number of critical pathogenicity genes, and altered gene expression patterns due to ectopic recombination known in various pathogens, could lead to pathogenic adaptation [12]. Furthermore, in contrast to some other locations [41], several *M. oryzae* isolates from wild rice and weed hosts in West Africa showed typical MGR586 profiles and were pathogenic on rice differentials. Their epidemiological significance needs to be carefully considered, as a lineage initially identified from *Panicum repens* became dominant in South India [42]. *M. oryzae* is capable of generating considerable genetic variation even in the absence of sexual reproduction with the potential for adaptive divergence linked to agricultural intensification [43, 44].

With the finger millet blast system, resistance breeding has proven difficult and the methodologies are still emerging [45, 46]. Effective rice blast management based on the pathogen population structure and epidemic history has been demonstrated [10, 42, 47, 48]. Present study has provided insight into the biology of the blast pathogen on finger millet and rice, forming a framework for future resistance breeding and disease management programmes of these major diseases in SSA.

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References

1. Lenne, J. M., Takan, J. P., Mgonja, M. A., Manyasa, E. O., Kaloki, P., Wanyera, N., et al. (2007). Finger millet blast management: A key entry point for fighting malnutrition and poverty in East Africa. *Outlook on Agriculture, 36*, 101–108.
2. Takan, J. P., Akello, B., Esele, P., Manyasa, E. O., Obilana, A. B., Audi, P. O., et al. (2004). Finger millet blast pathogen diversity and management in East Africa: A summary of project activities and outputs. *International Sorghum and Millets Newsletter, 45*, 66–69.
3. Mitaru, B. N., Kargia, J. T., & Munene, C. (1993). Finger millet production and utilization in Kenya. In K. W. Riley, S. C. Gupta, A. Seetharam, & J. N. Mushonga (Eds.), *Advances in small millets* (pp. 247–254). New Delhi: Oxford and IBH.
4. Africa Rice Center. (2007). *Africa rice trends*. http://www.warda.org/publications.
5. Singh, B. N., Jones, M. P., Fomba, S. N., Sere, Y., Sy, A. A., Akator, K., et al. (2000). Breeding for blast resistance in lowland rice in West Africa. In D. Tharreau, M.-H. Lebrun, N. J. Talbot, & J.-L. Notteghem (Eds.), *Advances in rice blast research* (pp. 112–128). Netherlands: Kluwer Academic.
6. Levy, M., Romao, J., Marchetti, M. A., & Hamer, J. E. (1991). DNA fingerprinting with a dispersed repeated sequence resolves pathotype diversity in the rice blast fungus. *Plant Cell, 3*, 95–102.
7. Chen, D., Zeigler, R. S., Leung, H., & Nelson, R. J. (1995). Population structure of *Pyricularia grisea* at two screening sites in the Philippines. *Phytopathology, 85*, 1011–1020.
8. Roumen, E., Levy, M., & Notteghem, J. L. (1997). Characterisation of the European pathogen population of *Magnaporthe grisea* by DNA finger printing and pathotype analysis. *European Journal of Plant Pathology, 103*, 363–371.
9. Correa-Victoria, F. J., Escobar, F., Prado, G., & Aricapa, G. (2000). Population dynamics of the rice blast pathogen in a screening site in Colombia and characterization of resistance. In D. Tharreau, M.-H. Lebrun, N. J. Talbot, & J.-L. Notteghem (Eds.), *Advances in rice blast research* (pp. 214–220). Netherlands: Kluwer Academic.
10. Zeigler, R. S., & Correa-Victoria, F. J. (2000). *Applying Magnaporthe grisea population analyses for durable rice blast resistance. APSnet feature: Pathogen population genetics and breeding for disease resistance*. http://www.apsnet.org/online/feature/.
11. Viji, G., Gnanamanickam, S. S., & Levy, M. (2000). DNA polymorphisms of isolates of *Magnaporthe grisea* from India that are pathogenic to finger millet and rice. *Mycological Research, 104*, 161–167.
12. Farman, M. L., & Kim, Y.-S. (2005). Telomere hypervariability in *Magnaporthe oryzae*. *Molecular Plant Pathology, 6*, 287–298.
13. Couch, B. C., & Kohn, L. M. (2002). A multilocus gene genealogy concordant with host preferences indicates segregation of a new species, *Magnaporthe oryzae*, from *Magnaporthe grisea*. *Mycologia, 94*, 683–693.
14. Valent, B., Farall, L., & Chumley, F. G. (1991). *Magnaporthe grisea* genes for pathogenicity and virulence identified through a series of backcrosses. *Genetics, 127*, 87–101.
15. Kang, S., Chumley, G. F., & Valent, B. (1994). Isolation of mating-type genes of the phytopathogenic fungus *Magnaporthe grisea* using genomic subtraction. *Genetics, 138*, 289–296.
16. Talhinhas, P., Sreenivasaprasad, S., Neves-Martins, J., & Oliveira, M. H. (2002). Genetic and morphological characterisation of *Colletotrichum acutatum* causing anthracnose of *Lupinus* species. *Phytopathology, 92*, 986–996.
17. Itoi, S., Mishima, T., Arase, S., & Nozu, M. (1983). Mating behaviour of Japanese isolates of *Pyricularia oryzae*. *Phytopathology, 73*, 155–158.
18. Kumar, J., Nelson, R. J., & Zeigler, R. S. (1999). Population structure and dynamics of *Magnaporthe grisea* in the Indian Himalayas. *Genetics, 152*, 971–984.
19. Notteghem, J. L., & Silué, D. (1992). Distribution of the mating type alleles in *Magnaporthe grisea* populations pathogenic on rice. *Phytopathology, 82*, 421–424.
20. Ling, K. C., & Ou, S. H. (1969). Standardization of the international race numbers of *Pyricularia oryzae*. *Phytopathology, 59*, 339–342.
21. National Research Council. (1996). Finger millet. In *Lost crops in Africa, Vol. 1: Grains* (pp. 39–58). National Research

Council: Board on Science and Technology for International Development.
22. Oryokot, J. O. E. (2001). Finger millet (*Eleusine coracana* (L.) Gaertn). In J. K. Mukiibi (Ed.), *Agriculture in Uganda. Crops, Vol. II* (pp. 29–41). Uganda: Fountain Publishers/CTA/NARO.
23. Nangoti, N., Kayobyo, G., & Rees, D. J. (2004). Seed demand and supply in eastern and northern Uganda—implications for government and non-government interventions. *Uganda Journal of Agricultural Sciences, 9*, 778–784.
24. Dobinson, K. F., Harris, R. E., & Hamer, J. E. (1993). *Grasshopper*, a long terminal repeat (LTR) retroelement in the phytopathogenic fungus *Magnaporthe grisea*. *Molecular Plant-Microbe Interactions, 6*, 114–126.
25. Tanaka, M., Nakayashiki, H., Tosa, Y., & Mayama, S. (2007). *The course of evolution of Magnaporthe oryzae Eleusine pathotype inferred from phylogenetic trees and structures of the flanking region of avirulence gene PWL1*. Asilomar-fungal genetics conference, California, USA, 20–25 March 2007.
26. Mekwatanakarn, P., Kositratana, W., Levy, M., & Zeigler, R. S. (2000). Pathotype and avirulence gene diversity of *Pyricularia grisea* in Thailand as determined by rice lines near-isogenic for major resistance genes. *Plant Disease, 84*, 60–70.
27. Correll, J. C., Harp, T. L., Guerber, J. C., Zeigler, R. S., Liu, B., Cartwright, R. D., et al. (2000). Characterisation of *Pyricularia grisea* in the United States using independent genetic and molecular markers. *Phytopathology, 90*, 1396–1404.
28. Don, L. D., Kusaba, M., Urashima, A. S., Tosa, Y., Nakayashiki, H., & Mayama, S. (1999). Population structure of the rice blast pathogen in Japan examined by DNA fingerprinting. *Annals of the Phytopathological Society of Japan, 65*, 15–24.
29. Park, S.-Y., Milgroom, M. G., Han, S.-S., Kang, S., & Lee, Y.-H. (2003). Diversity of pathotypes and DNA fingerprint haplotypes in populations of *Magnaporthe grisea* in Korea over two decades. *Phytopathology, 93*, 1378–1385.
30. Viji, G., & Gnanamanickam, S. S. (1998). Mating type distribution and fertility status of *Magnaporthe grisea* populations from various hosts in India. *Plant Disease, 82*, 36–40.
31. Mekwatanakarn, P., Kositratana, W., Phromraksa, T., & Zeigler, R. S. (1999). Sexually fertile *Magnaporthe grisea* rice pathogens in Thailand. *Plant Disease, 83*, 939–943.
32. Dayakar, B. V., Narayanan, N. N., & Gnanamanickam, S. S. (2000). Cross compatibility and distribution of mating type alleles of the rice blast fungus *Magnaporthe grisea* in India. *Plant Disease, 84*, 700–704.
33. Viji, G., & Uddin, W. (2002). Distribution of mating type alleles and fertility status of *Magnaporthe grisea* causing gray leaf spot of perennial ryegrass and St. Augustine grass turf. *Plant Disease, 84*, 877–884.
34. Tredway, L. P., Stevenson, K. L., & Burpee, L. L. (2005). Genetic structure of *Magnaporthe grisea* population associated with St. Augustine grass and tall fescue in Georgia. *Phytopathology, 95*, 463–471.
35. Leslie, J. F., & Klein, K. K. (1996). Female fertility and mating type effects on effective population size and evolution in filamentous fungi. *Genetics, 144*, 557–567.
36. Paoletti, M., Rydholm, C., Schwier, E. U., Anderson, M. J., Szakacs, G., Lutzoni, F., et al. (2005). Evidence for sexuality in the opportunistic fungal pathogen *Aspergillus fumigatus*. *Current Biology, 15*, 1242–1248.
37. Pande, S., Mukuru, S. Z., King, S. B., & Karunakar, R. I. (1995). Biology of, and resistance to finger millet blast in Kenya and Uganda. In S. Z. Mukuru & S. B. King (Eds.), *Proceedings of the eighth EARSAM regional workshop on sorghum and millets*, 30 Oct–5 Nov 1992, Sudan (pp. 83–92). Andhra Pradesh: ICRISAT.
38. Takan, J. P. (2007). *A study on the genetic diversity, sexuality and pathogenicity of the finger millet blast pathogen Magnaporthe grisea populations in two East African countries*. PhD thesis (pp. 315). UK: University of Exeter.
39. Somasekhara, Y. M., Viswanath, S., & Anilkumar, T. B. (1991). Evaluation of finger millet [*Eleusine coracana* (L) Gaertn] cultivars for their reactions to blast (*Pyricularia grisea* Sacc). *Tropical Agriculture, 68*, 231–234.
40. Shetty, S. H., Gopinath, A., & Rajashekar, K. (1985). Relationship of seed-borne inoculum of *Pyricularia grisea* to incidence of blast of finger millet in the field. *Indian Phytopathology, 38*, 154–156.
41. Borromeo, E. S., Nelson, R. J., Bonman, J. M., & Leung, H. (1993). Genetic differentiation among isolates of *Pyricularia* infecting rice and weed hosts. *Phytopathology, 83*, 393–399.
42. Gnanamanickam, S. S., Lavanya, B., Priyadarsini, V. B., Dayakar, B. V., Leenakumari, D., Sivaraj, R., et al. (2000). Lineage-exclusion resistance breeding: Pyramiding of blast resistance genes for management of rice blast in India. In D. Tharreau, M.-H. Lebrun, N. J. Talbot, & J.-L. Notteghem (Eds.), *Advances in rice blast research* (pp. 172–179). Netherlands: Kluwer Academic.
43. Dean, R. A., Talbot, N. J., Ebbole, D. J., Farman, M. L., Mitchell, T. K., Orbach, M. J., et al. (2005). The genome sequence of rice blast fungus *Magnaporthe grisea*. *Nature, 434*, 980–986.
44. Couch, B. C., Fudal, I., Lebrun, M.-H., Tharreau, D., Valent, B., Kim, P.-V., et al. (2005). Origins of host-specific populations of the blast pathogen *Magnaporthe oryzae* in crop domestication with subsequent expansion of pandemic clones on rice and weeds of rice. *Genetics, 170*, 613–630.
45. Parlevliet, J. E. (1988). Identification and evaluation of quantitative resistance. In K. L. Leonard & W. E. Fry (Eds.), *Plant disease epidemiology. Genetics, resistance, and management* (pp. 215–247). New York: McGraw Hill.
46. Oduori, C. O. A. (2005). *The importance and research status of finger millet in Africa*. McKnight Foundation Collaborative Crop Research Program workshop on Tef and finger millet: Comparative genomics of the Chloridoid cereal (pp. 12). Biosciences for East and Central Africa (BECA), ILRI, 28–30 June 2005, Nairobi, Kenya.
47. Talbot, N. J. (2002). Molecular variability studies of *Magnaporthe grisea* and application in disease control. In F. Kempken (Ed.), *The Mycota XI Agricultural applications* (pp. 153–169). Berlin: Springer-Verlag.
48. Zhu, Y., Chen, H., Fan, J., Wang, Y., Li, Y., Chen, J., et al. (2000). Genetic diversity and disease control in rice. *Nature, 406*, 718–722.