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Population structure and pathogenicity tests of *Pyricularia oryzae* on wild and cultivated rice in Mali

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Abstract

Blast is a devastating disease of rice caused by the fungus *Pyricularia oryzae*. The role of infected straw and seed as sources of primary inoculum in blast disease epidemics is well known. The role of alternative hosts is yet to be confirmed. The current study sought to assess if wild rice is a major source of inoculum for cultivated rice by comparing the genetic structure of *P. oryzae* populations from both hosts. Two hundred and eighty-two strains were genotyped with 12 simple-sequence repeat (SSR) markers. Cross infectivity of 20 *P. oryzae* isolates was also assessed using pathogenicity tests. Samples were collected from cultivated and wild rice organs with blast symptoms in irrigated and lowland areas of Mali in Koulikoro, Sikasso and Ségou regions. Under controlled conditions, *P. oryzae* isolates from *Oryza longistaminata* wild rice were pathogenic to cultivated rice and had a similar range of cultivar compatibility to isolates from cultivated rice. Results of pathogenicity tests suggest that *P. oryzae* isolates from wild rice (*O. longistaminata* and *Oryza barthii*) have the potential to attack cultivated rice (*Oryza sativa*) in the field. However, populations of *P. oryzae* on cultivated and wild rice were genetically differentiated. A very large fraction (94.4%) of the multilocus genotypes identified were specific to one host species or the other. Thus, although physically close, each rice species hosts a different population of the pathogen, and our results support the hypothesis that wild rice is not a major source of blast inoculum for cultivated rice.

KEYWORDS
blast disease, inoculum source, Mali, population structure, *Pyricularia oryzae*, rice

1 | INTRODUCTION

Blast is recognized as one of the most devastating diseases of rice in the world (Savary et al., 2000). The disease is caused by the fungus *Pyricularia oryzae* (synonym *Magnaporthe oryzae*). This model pathosystem for research on host–pathogen interactions (Dean et al., 2012) is also a major challenge for food security (Pennisi, 2010). *P. oryzae* is an ascomycete fungus that reproduces mainly by asexual multiplication. This widely described asexual cycle is the only mode of reproduction observed in nature. The cycle starts with the adhesion of a spore to the leaf, followed very quickly (in less than 2 h) by the formation of a germ tube. The appressorium formed at the tip of the germ tube allows penetration into the plant cell. One to 2 weeks after infection, *P. oryzae* is able to sporulate and start a new cycle

(Ribot et al., 2008). Rice blast is distributed on all continents and in every area where rice is grown (Ou, 1985). Globally, despite the use of control methods, losses are estimated at 4% of the annual world rice harvest (Savary et al., 2019) and represent a lack of staple food for millions of people.

The genetic structure of *P. oryzae* populations infecting rice has been extensively studied on a global scale to understand the evolution of the pathogen and to adapt control techniques, in particular, the deployment of varietal resistance management strategies. Characterization of *P. oryzae* populations has been carried out at different geographic scales with different molecular markers and genome sequences (Adreit et al., 2007; Gladieux, Condon, et al., 2018; Tharreau et al., 2009; Thierry et al., 2022). Studies encompassing samples from different continents are consistent with clustering in four main genetic groups, each with a wide large geographical distribution, and with Asian isolates represented in all four groups (Gladieux, Condon, et al., 2018; Thierry et al., 2022). Although traces of ancestral recombination were identified (Gladieux, Condon, et al., 2018; Thierry et al., 2022), populations of the blast fungus on rice are clonal, with the potential exception of populations that could reproduce sexually in very limited areas of Asia (Saleh et al., 2012; Thierry et al., 2022). These studies also showed that Asia is the centre of diversity and the origin of the pathogen populations (Thierry et al., 2022). However, little data exist on the diversity and population structure of the pathogen in Africa. Mutiga et al. (2017) studied the genetic diversity of *P. oryzae* rice populations and evaluated the virulence spectrum of this pathogen in West Africa and East Africa. These authors identified seven genetic groups and showed differentiation between West and East African populations. Kassankogno et al. (2017) showed that populations from two neighbouring countries, Burkina Faso and Togo, are differentiated but that some multilocus genotypes are shared, suggesting some migration between these countries. A recent study (Odjo et al., 2021) confirmed this hypothesis and showed the presence in Africa and Madagascar of the four genetic groups previously described in Asia (Gladieux, Condon, et al., 2018; Thierry et al., 2022). Populations of *P. oryzae* in West Africa, East Africa and Madagascar were highly differentiated (Odjo et al., 2021).

Like any disease, knowing inoculum sources is necessary to develop sustainable control methods. Evidence that wild species are sources of inoculum for cultivated plants is scarce and often indirect for aerial fungal pathogens. The disease severity of ergot on brome grasses and in neighbouring fields of barley suggests that brome grasses could be a source of inoculum for ergot epidemics on barley (Wyka & Broders, 2023). Linde et al. (2016) assessed and compared the diversity of *Rhynchosporium commune* on weedy and cultivated barley using microsatellite (simple-sequence repeat, SSR) markers. The study showed that weedy barley grass is an important auxiliary host of *R. commune*, harbouring highly virulent pathogen types capable of transmission to barley. In contrast, several studies show the divergence of fungal pathogen populations on different hosts (Brunner et al., 2007; Linde & Smith, 2019; Stukenbrock et al., 2011).

For blast disease, some primary inoculum sources are well known, such as infected seed and straw (Guerber & TeBeest, 2006; Hubert et al., 2015; Long et al., 2001; Raveloson et al., 2018). In contrast, the role of weeds as inoculum sources is controversial. Based on pathogenicity tests in controlled conditions, some weeds have been presented as sources of inoculum for blast epidemics on rice, including *Digitaria sanguinalis* (Choi et al., 2013), *Festuca arundinacea*, *Lolium multiflorum*, *Anthoxanthum odoratum*, *Phalaris arundinacea* (Kato et al., 2000), *Rottboellia exaltata*, *Echinochloa colona* and *Leersia hexandra* (Mackill & Bonman, 1986). But, to date, this hypothesis has not been confirmed by observations in the field. In contrast, genetic (Borromeo et al., 1993) and phylogenetic studies (Gladieux, Ravel, et al., 2018) have shown that strains causing epidemics on rice belong to a single clade, which is not sampled on other hosts (with the exception of a barley sample from Thailand). Similarly to weeds, certain species of wild rice were suspected of serving as alternative hosts. In Africa, the role of wild rice as an inoculum source was demonstrated for two important diseases on Asian cultivated rice: bacterial leaf streak (Wonni et al., 2014) and rice yellow mottle virus (Traoré et al., 2009). Pathogenicity tests of *P. oryzae* isolates from wild rice (*Oryza meridionalis*) in northern Australia showed that many local rice varieties of *Oryza sativa* are susceptible (Khemmuk et al., 2016). However, although on a limited number of samples (10 isolates), *P. oryzae* populations from wild rice (*O. rufipogon*) in Cambodia showed a different pathogenicity spectrum than populations from cultivated rice (Fukuta et al., 2014). This latter study suggests that both pathogen populations may be differentiated. Therefore, to date, there is no evidence that wild species of rice are inoculum sources for blast epidemics on cultivated rice.

Isolating *P. oryzae* from wild rice and showing that these isolates are pathogenic on cultivated rice is not sufficient to demonstrate a role as inoculum source. Both hosts could act as separated compartments and epidemics could be independent, that is, without demographic and genetic mutual impact on blast populations. Such an example of 'parallel' epidemics was observed for blast populations on indica and japonica varieties in the Yuanyang terraces of Yunnan province, China (Liao et al., 2016). *P. oryzae* isolates from indica varieties were genetically different from isolates from japonica varieties, although both types of varieties were cultivated in close proximity, and isolates from indica varieties had the potential to attack the cultivated japonica varieties. Mali offers an opportunity to address this question because *O. sativa*, the Asian rice (cultivated worldwide) cohabits with the African rice *O. glaberrima* (cultivated in some parts of West Africa), as well as with other wild rice species such as *O. longistaminata* and *O. barthii*. Wild rice is usually found in drains, dikes and dams that are close to cultivated rice fields. In this context, wild species of rice could be sources of inoculum for cultivated Asian rice. We took advantage of this specific context to test if the *P. oryzae* populations on both hosts were genetically similar. Our working hypothesis was that if wild rice is a major source of inoculum for cultivated rice, then there should be important gene flows between *P. oryzae* populations on both hosts and these populations should be genetically similar (i.e., poorly differentiated).

We also performed pathogenicity tests in controlled conditions to evaluate the infectivity on *O. sativa* of *P. oryzae* isolates collected on *O. longistaminata*.

2 | MATERIALS AND METHODS

2.1 | Sampling and conservation of samples

Sampling was carried out on leaves, panicle nodes (neck) and stems of cultivated and wild rice showing blast symptoms. They were collected from irrigated and lowland areas between 2017 and 2019, at different stages of rice development, in three regions (Koulikoro, Sikasso and Ségou) in Mali. Each sample was bagged in a paper envelope and labelled with date, variety, species, locality, organ, stage and GPS data, dried at room temperature then stored in the oven (BINDER) at 37°C until use. In total, nine sites were surveyed (Sélingué, Manikoura, Niéna, Baguineda, M'pegnesso, Niono Nango Sahel, Niono N3-Bis, Niono N7, Loulouni Faraka Banakoro; Figure 1; Table S1). Diseased organs (leaves, panicle nodes and stems) were collected on different plants distributed in the plots, while avoiding taking several plants from a disease focus. The number of diseased organs collected per plot depended on the incidence in the field. Disease incidence in wild rice was very difficult to assess because it was found in water-filled drains and was rarely seen on dikes. A sample was defined as a set of diseased organs collected from the same plot and on a single variety at a single date. On average, the estimated size of a plot was 0.25 ha. We isolated several strains per sample but mainly one per plant (a maximum of three diseased organs were collected per plant).

2.2 | Isolation and storage of isolates of *P. oryzae*

Diseased organs were placed on a moistened filter paper disc in sterile Petri dishes and incubated at 25°C for 24 h. The fragments were then observed under a binocular microscope (SZ-PT; Olympus) to assess the presence or absence of *Pyricularia* conidia. The conidia were collected with a glass needle and deposited on the surface of Bacto-agar medium (45 g/L Bacto-agar). The Petri dish was sealed with masking tape (Tesa) and incubated at 25°C for 24 h for germination. A germinated conidium was placed on rice flour medium (15 g agar, 20 g rice flour, 2.5 g yeast extract in 1 L of water and 500,000 units of penicillin G was added after autoclaving) and incubated at 25°C. A single strain was isolated per plant sampled. For storage, an actively growing mycelium plug was deposited on a sterilized filter paper (Whatman no. 5) and placed on rice flour agar medium (Silué & Nottéghem, 1990). Five to 7 days later, the mycelium-covered filter

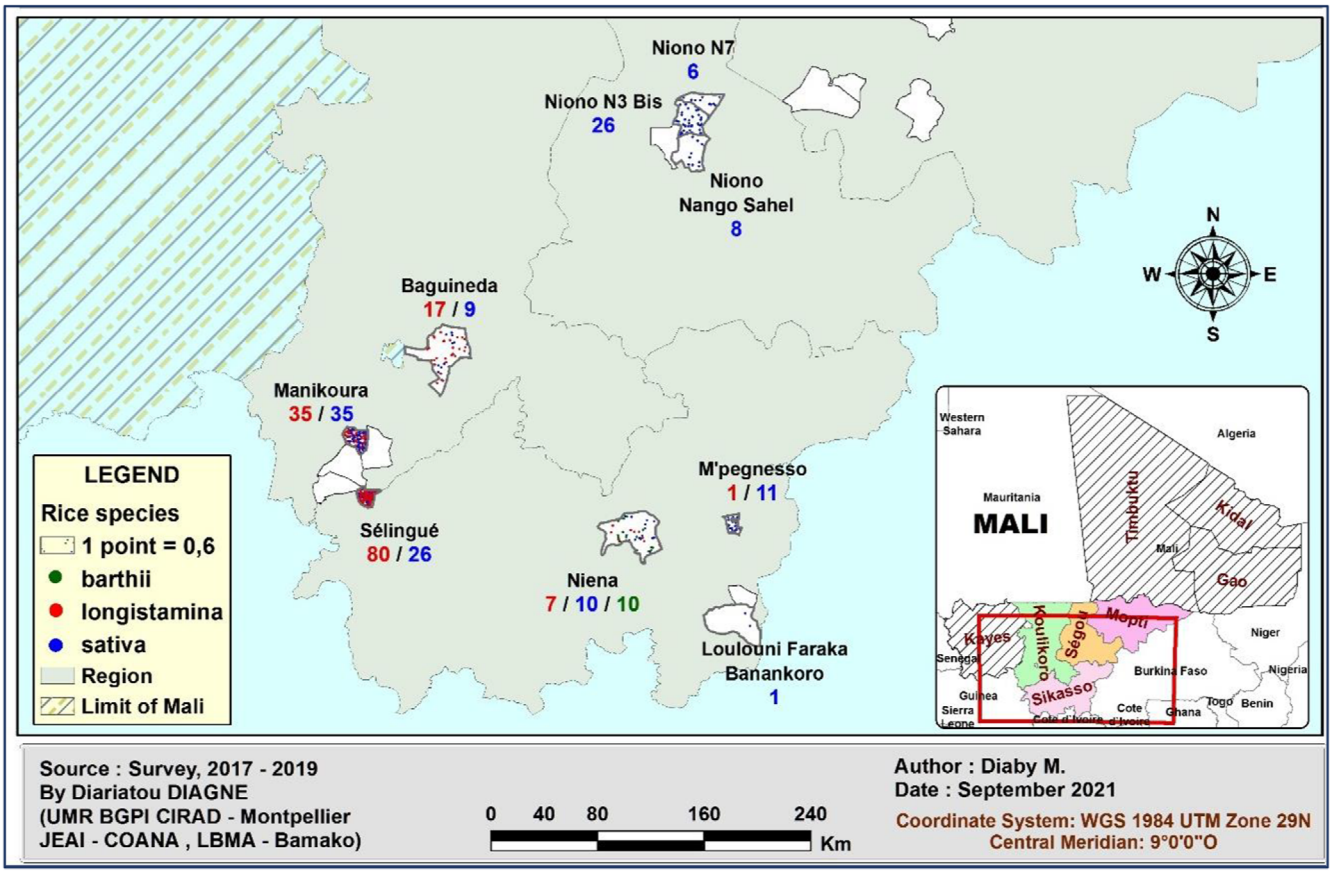

FIGURE 1 Mapping of *Pyricularia oryzae* strains isolated from *Oryza barthii* (green), *O. longistaminata* (red) and *O. sativa* (blue) by site and host species from 2017 to 2019.

Location	Number of MLGs	Number of isolates per host species			Total
		Oryza sativa	*Oryza longistaminata*	*Oryza barthii*	
Sélingué	58	25	79	–	104
Manikoura	27	32	35	–	67
Niéna	17	10	7	10	27
Baguineda	13	7	16	–	23
M'pegnesso	8	11	1	–	12
Niono Nango Sahel	5	8	–	–	8
Niono N3-Bis	8	25	–	–	25
Niono N7	6	6	–	–	6
Loulouni Faraka Banankoro	1	1	–	–	1
Not determined	5	9	0	0	9
Total		134	138	10	282

TABLE 1 Distribution of *Pyricularia oryzae* isolates by site and host species and number of multilocus genotypes (MLGs) per site.

paper was removed and placed in a sterile empty Petri dish with a lid and incubated at 35°C for 4 days for drying. It was then cut into small fragments and placed in sterile paper bags, placed in a plastic bag and sealed under vacuum for storage at −20°C (Valent et al., 1986).

A total of 282 strains from different varieties were isolated between 2017 and 2019 from nine locations (Table 1; Table S2). In addition, 11 Malian strains collected between 1986 and 2011 from *O. sativa* were included in the study as genotyping controls. These strains were previously characterized by Odjo et al. (2021).

2.3 | DNA extraction and SSR marker amplification

After 4 days of culture in rice flour liquid medium (20 g rice flour and 2 g yeast extract in 1 L of water), the mycelium was recovered in Eppendorf tubes, and genomic DNA was extracted according to the protocol described by Gladieux, Condon, et al. (2018).

A total of 282 isolates (271 from the 2017 to 2019 sampling and the 11 genotyping control strains) were genotyped with 12 SSR markers (Tables S3 and S5) previously used for *P. oryzae* genotyping (Adreit et al., 2007; Odjo et al., 2021; Saleh et al., 2012). These markers were amplified by PCR (QIAGEN Multiplex PCR kit) as previously described (Saleh et al., 2012) with a total volume of 5 µL, including 2.5 µL of Master Mix, 0.5 µL of 10× Mix primers, 0.5 µL of 5× Q solution and 1.5 µL of genomic DNA (10 ng/µL). The PCR programme was as follows: 95°C for 15 min; 40 cycles of 94°C for 30 s, 57–63°C for 90 s, 72°C for 60 s; and 72°C for 30 min. The resulting products were separated and analysed on a 16-capillary ABI Prism 3130XL machine (Applied Biosystems), and the amplicons were evaluated for size by fluorescence measurement. For this analysis, 1.5 µL of amplified products (1/70 dilution) were mixed with 15 µL formamide HiDi and GeneScan-500LIZ size marker (Applied Biosystems). Amplifications were repeated twice, and control strains were included in all amplification plates to verify calibration. When inconsistent results were obtained between two technical replicates, amplification was performed a third time. Isolates where data was missing more than twice were excluded from the analysis.

2.4 | Analysis of genotyping data

The raw data collected were analysed and transcribed into allele sizes using the GeneMapper v. 4.1 (Applied Biosystems) tool. We defined a multilocus genotype (MLG) as a unique combination of alleles across all loci genotyped. Strains were grouped in MLGs using a script developed by Sébastien Ravel (Odjo et al., 2021; Ravel, 2020). The first strain of the genotyping dataset was assigned to MLG1. The second strain was assigned to MLG1 if its genotype was identical to the genotype of the first strain at all loci; the second strain was assigned to MLG2 if its genotype was different for at least one allele at one locus. The procedure was repeated until the genotype of each strain was compared to the MLGs previously identified. Genotypes with missing data may be 100% similar to more than one MLG. In this case, we chose not to assign that genotype to any MLG. Thus, not all isolates could be assigned to an MLG. A discriminant analysis of principal components (DAPC) was performed using the 'adegenet' package of the R software v. 3.2.2 (Jombart, 2008; Jombart & Ahmed, 2011; R Core Team, 2017). DAPC was performed to cluster, without a priori, the MLGs into genetic groups. The mean number of alleles per locus (N_a) and the unbiased genetic diversity (H_{nb}) (Nei, 1987) were calculated using Genetix v. 4.05 (Belkhir et al., 2004). The genetic diversity of *P. oryzae* populations on wild and cultivated rice in Mali was analysed. The mean number of private alleles (N_p) was estimated as the number of alleles present in a single genetic group, combining all markers. Genetic differentiation between populations was estimated with the F_{ST} index (Weir & Cockerham, 1984) calculated with Genepop v. 4.2 (Raymond & Rousset, 1995; Rousset, 2008). We analysed the genetic differentiation between *P. oryzae* populations on wild (*O. longistaminata*) and cultivated (*O. sativa*) rice within each of the four genetic groups.

2.5 | Pathogenicity testing of wild rice isolates on cultivated rice

To assess the potential of strains isolated from wild rice to be infectious and cause epidemics on cultivated rice, a subset of strains was randomly selected. Thus, the pathogenicity of 20 Malian isolates of *P. oryzae* collected from 2017 to 2019 was assessed on *O. sativa* rice varieties. Among them, 12 isolates were collected from wild rice *O. longistaminata*, four isolates from wild rice *O. barthii* and four isolates from cultivated rice *O. sativa* (used as controls). Isolates were selected to represent the genetic diversity observed (Table 2).

Isolates were inoculated on 11 certified varieties of *O. sativa* cultivated in Mali (08Fan2, ADNY 11, ARICA 10, ARICA 9, Bw 348–1, Kogoni, Nenekala, Shwetasoke, Swarna 2, Wapmo [Was 197] and Was 49), and *O. sativa* susceptible check variety Maratelli. Fifteen seeds per accession were sown in peat soil (Jiffy compost, reference 284795). The 12 accessions were grown in a 33 × 45 × 5 cm plastic tray and cultivated in the greenhouse (26°C day, 21°C night; relative humidity 50%–70%). Inoculations were carried out 28 days after sowing (4- to 5-leaf stage) by spraying 30 mL per tray of a spore suspension (Gallet et al., 2016). Each accession × strain combination was tested three times in independent assays (i.e. plants sown and inoculated at different dates). *P. oryzae* isolates were cultured for 1 week on rice flour agar medium following the conditions described above for isolate storage. To prepare the spore suspension, 5 mL of sterile distilled water was applied to the isolate in culture, and the mycelium was scraped off with a plastic spreader. The suspension was then collected in a tube after filtering through a funnel fitted with gauze. The concentration of spores was quantified using a Malassez cell. The spore suspension was adjusted to 25,000 spores/mL and 0.5% gelatin. The inoculated plants were incubated at 25°C and 100% relative humidity for 1 day and then transferred for 7 days to a growth chamber (12 h photoperiod; 26°C day, 21°C night). Disease symptoms were assessed 7 days after inoculation. The scoring was done according to the 1–6 scale described by Gallet et al. (2016). Scores 1–3 were considered as incompatible reactions (resistant variety), whereas scores 4–6 were considered as compatible reactions (susceptible variety). When scores of the three replicates were consistently assigned to the same type of reaction, the corresponding strain × accession interaction was considered either compatible or incompatible. When data from the three replicates were inconsistent, the corresponding strain × accession interaction was considered as missing data (Table 2). The compatible reaction ratio per strain (rows) and per original host (columns) were calculated as follows: number of compatible reactions divided by the total number of determined interactions.

3 | RESULTS

Oryza longistaminata was commonly found in the localities of Sélingué (Sikasso region), Manikoura and Baguineda (Koulikoro region), whereas *O. barthii* was found only in Niéna (Sikasso region). Blast was observed and sampled in all these sites and on all rice species present. There was no blast disease on *O. longistaminata* and *O. barthii* in Niono (Figure 1). To compare *P. oryzae* populations on wild and cultivated rice in Mali, we carried out analyses at two geographic scales (whole country/localities) and at two genetic levels of the pathogen population (genotype/genetic clusters). Because *P. oryzae* populations on rice are clonal in most of the rice-growing areas, including Africa, most population genetic analyses we conducted were based on MLGs rather than on allelic frequencies.

3.1 | Population diversity and structure at the whole country scale

The genetic diversity was similar for *P. oryzae* populations sampled on wild rice (*O. longistaminata*) and on cultivated rice (*O. sativa*) at the whole country scale. The unbiased genetic diversity (H_{nb}) was 0.62 and 0.58, and the mean number of alleles per locus (N_a) was 9 and 8, respectively (Table 3). The two populations were moderately differentiated, as indicated by a significant ($p = 1.8e^{-42}$) but relatively low F_{ST} value (0.16) for *P. oryzae* compared with the study by Saleh et al. (2014), which showed that pairwise F_{ST} values between genetic clusters ranged from 0.27 to 0.63. The average number of private alleles (N_p) was 3.1 and 2.2, respectively (Table 3). These relatively high N_p also supported differentiation between the two populations. The sample size ($n = 10$) of the population sampled on *O. barthii* was considered too small to calculate parameters of genetic diversity and differentiation.

Two hundred and fifty-four out of 282 isolates genotyped were unambiguously assigned to 125 MLGs. Eighty-three MLGs were represented by a single isolate (hereafter named single MLGs), and 42 MLGs were represented by two or more isolates (hereafter named multi MLGs; Table 4). Among these latter, 35 were sampled only on a single host: 18, 14 and 3 on Asian cultivated rice (*O. sativa*) and on wild rice species *O. longistaminata* and *O. barthii*, respectively. There was a limited number of shared MLGs between hosts, with only six sampled on both *O. sativa* and *O. longistaminata* and one on both *O. longistaminata* and *O. barthii* (Table 4).

DAPC was carried out to cluster MLGs in genetic groups without a priori, that is, using only the genotypic information of the strains. To determine the number of clusters (*K*), variation of the Bayesian information criterion (BIC) value was calculated for varying values of *K*. BIC value regularly decreased with increasing *K* values (Figure S1). Because the curve did not show a marked inflection, we could not determine an optimal *K* value based on this approach. Instead, we applied the recommendation of the DAPC tutorial described by Jombart and Collins (2015) and tried to find the value of *K* that 'better summarizes the data than others'. The graphic display of DAPC (Figure 2) results showed that the genotypes were clearly separated into a minimum number of three groups (Figure 2). Additional analysis of DAPC results (Figure 2; Figures S2 and S3) showed that clustering in more than five groups seemed to create an unnecessary (overlapping groups) and unreliable (assignation of genotypes

TABLE 2 Pathogenicity testing of *Pyricularia oryzae* on cultivated rice varieties.

				Oryza sativa variety												
Original host	Isolate	MLGs[a]	Genetic cluster	Kogoni	Nenekala	ADNY 11	Swarna 2	ARICA 10	ARICA 9	Wapmo (was 197)	Was 49	08Fan2	Bw 348–1	Shwetasoke	Maratelli	Ratio of compatible interactions per strain
Oryza barthii	ML0312	94	2	−	ND[b]	−	−	−	+	+	+	+	+	+	+	0.64[c]
	ML0321	97	4	−	+[d]	+	−	−	+	+	+	+	+	+	+	0.75
	ML0320	98	4	−	−	−	+	−	+	+	+	+	+	+	+	0.66
	ML0322	99	4	−	−	−	−	+	+	+	+	+	+	+	+	0.66
Oryza longistaminata	ML0097	9	1	−	−	−	−	−	−	−	−	−	−	−	−	0.00
	ML0134	26	2	−	−	−	−	−	−	−	−	−	−	+	−	1.00
	ML0160	40	2	−	−	−	−	−	−	−	−	+	+	+	−	0.25
	ML0176	48	2	−	−	−	−	−	−	−	−	+	+	+	−	0.25
	ML0296	92	2	−	−	−	−	−	−	−	ND	+	+	+	−	0.27
	ML0093	8	3	−	−	−	−	−	−	−	−	+	+	−	−	0.17
	ML0156	18	3	−	−	−	−	−	−	−	−	+	+	+	−	0.25
	ML0138	28	3	−	−	−	−	−	−	−	−	−	−	−	+	0.08
	ML0145	31	3	−	−	−	−	−	−	−	−	+	+	+	−	0.25
	ML0089	111	3	−	−	−	−	−	−	−	−	+	+	+	−	0.25
	ML0103	13	4	−	−	−	−	−	−	−	−	−	−	+	+	0.17
	ML0328	101	4	−	−	−	+	+	+	+	+	+	+	+	+	0.75
Oryza sativa	ML0223	121	1	+	−	−	−	−	−	−	−	+	ND	+	+	0.36
	ML0276	124	2	−	−	−	−	−	−	−	−	+	+	+	−	0.25
	ML0339	103	3	−	−	−	+	+	+	+	+	+	+	+	+	0.75
	ML0130	116	4	−	−	−	−	−	−	−	−	−	−	−	−	0.00
Ratio of compatible interactions per original host species[e]	*O. barthii* isolates			0.00	0.33	0.25	0.25	0.25	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	O. longistaminata isolates			0.00	0.00	0.00	0.08	0.08	0.08	0.08	0.09	0.66	0.66	0.75	0.25	
	O. sativa isolates			0.25	0.00	0.00	0.25	0.25	0.25	0.25	0.25	0.75	0.66	0.75	0.50	

[a]MLGs, multilocus genotypes.

[b]ND: missing data.

[c]The number of determined interactions was 12 for all strains except ML0223, ML0296 and ML0312 (11 determined interactions).

[d]+, compatible interaction; −, incompatible interaction.

[e]The number of determined interactions were 4, 12 and 4 for *O. barthii*, *O. longistaminata* and *O. sativa* isolates, respectively, except on varieties Nenekala (three determined interactions for *O. barthii*), was 49 (11 determined interactions for *O. longistaminata*) and BW348-1 (three determined interactions for *O. sativa*).

changing from one run to the other) level of clustering. Hence four genetic clusters (G1, G2, G3 and G4) were defined.

As expected, and because genotypes were clustered based on genetic similarity, F_{ST} values were high between the different genetic groups (Table 5). We then analysed the genetic differentiation between *P. oryzae* populations on wild *O. longistaminata* and cultivated *O. sativa* rice within each of the four genetic clusters. We observed that the populations isolated from *O. longistaminata* and *O. sativa* were significantly differentiated. F_{ST} indices between the two populations ranged between relatively low to average values (0.41, 0.16 and 0.31 for G1, G2 and G3, respectively; Table 5). In contrast, for the G4 cluster, populations from *O. longistaminata* and *O. sativa* were not differentiated ($F_{ST}=0.02$, $p=0.096$; Table 5). A bias due to the small sample size ($n=3$) of the population collected on *O. sativa* could explain this exception.

3.2 | Population diversity and structure at the local scale

To compare populations at the local scale, we focused on Sélingué, Manikoura, Niéna and Baguineda localities, where the sample size was sufficient for comparisons between populations isolated from wild rice and cultivated rice (Figure 1; Table 1).

In Sélingué, 10 single MLGs and two multi MLGs (representing a total of nine isolates) were specific to cultivated rice *O. sativa* (Figure 3a). Similarly, we identified 30 single MLGs and 11 multi MLGs (30 isolates) specific to wild rice *O. longistaminata* (Figure 3a; Table S4). Four MLGs were shared between both species. All four genetic clusters (GCs) were identified on *O. sativa* and *O. longistaminata*. Cluster G1 was dominant on *O. sativa* (12/25 isolates), whereas G2 (37/79 isolates) and G3 (27/79 isolates) were dominant on *O. longistaminata* (Table 6).

In Manikoura, 14 single and three multi MLGs (13 isolates) were specific to *O. sativa*, and seven single and two multi MLGs (24 isolates) were specific to *O. longistaminata* (Figure 3b; Table S4). There was no genotype shared between wild and cultivated rice in Manikoura. Genetic clusters G1 and G3 were sampled on *O. sativa*, G3 being more represented (30/32 isolates), whereas the four genetic clusters were identified on *O. longistaminata* with G2 as the dominant one (26/35 isolates; Table 6).

In Niéna, five single and one multi MLGs (four isolates) were specific to *O. sativa*, one single and three multi MLGs (nine isolates) were specific to the wild rice *O. barthii*, and six single MLGs were specific to *O. longistaminata* (Figure 3c; Table S4). There was no genotype shared between wild and cultivated rice. A dominance of cluster G4 on the two wild species in Niéna was observed. G2 (1/10 isolates) and G4 (9/10 isolates) were found on *O. barthii*, while G3 (1/7 isolates) and G4 (6/7 isolates) were found on *O. longistaminata* (Table 6). G1 (2/10 isolates) and G3 (8/10 isolates) were sampled on *O. sativa*.

In Baguineda, we identified four single and one multi MLGs (two isolates) specific to *O. sativa* and four single and three multi MLGs (10 isolates) specific to *O. longistaminata* (Figure 3d; Table S4). There was no genotype shared between wild and cultivated rice in Baguineda. G1 (seven isolates) was the only genetic cluster sampled on *O. sativa*, whereas G2 (3/16 isolates) and G3 (13/16 isolates) were identified on *O. longistaminata* (Table 6).

TABLE 3 Genetic diversity of *Pyricularia oryzae* populations on wild (*Oryza longistaminata*) and cultivated (*O. sativa*) rice in Mali.

		Diversity parameter		
Host	N[a]	H_{nb}[b]	N_a[c]	N_p[d]
O. longistaminata	138	0.62	9	3.1
O. sativa	134	0.58	8	2.2
Total	272			

Note: The 12 simple-sequence repeat markers listed in Table S3 were used for the genotyping.

[a]N, total number of individuals.

[b]H_{nb}, unbiased gene diversity.

[c]N_a, mean number of alleles per locus.

[d]N_p, mean number of private alleles per locus.

3.3 | Pathogenicity of isolates from *O. longistaminata* on cultivated rice

Most of the varieties (including the susceptible check Maratelli) were incompatible with the majority of isolates, except three varieties, namely 08Fan2, Bw 348–1 and Shwetasoke (Table 2). The average ratio of compatible interactions between *P. oryzae* isolates and *O. sativa* varieties was similar for isolates from *O. longistaminata* and *O. sativa*: 0.34 (range: 0–0.75) and 0.31 (range: 0–0.75),

TABLE 4 Distribution of multilocus genotypes (MLGs) by host species.

	Species-specific			Shared		
MLGs[a]	*Oryza sativa*	*Oryza longistaminata*	*Oryza barthii*	*O. sativa*/*O. longistaminata*	*O. sativa*/*O. barthii*	*O. longistaminata*/*O. barthii*
Single MLGs	36	47	0	–	–	–
Multi MLGs	18	14	3	6	0	1

Note: The 12 simple-sequence repeat markers listed in Table S3 were used for the genotyping.

[a]Single MLGs are represented by one isolate. Multi MLGs are represented by two or more isolates.

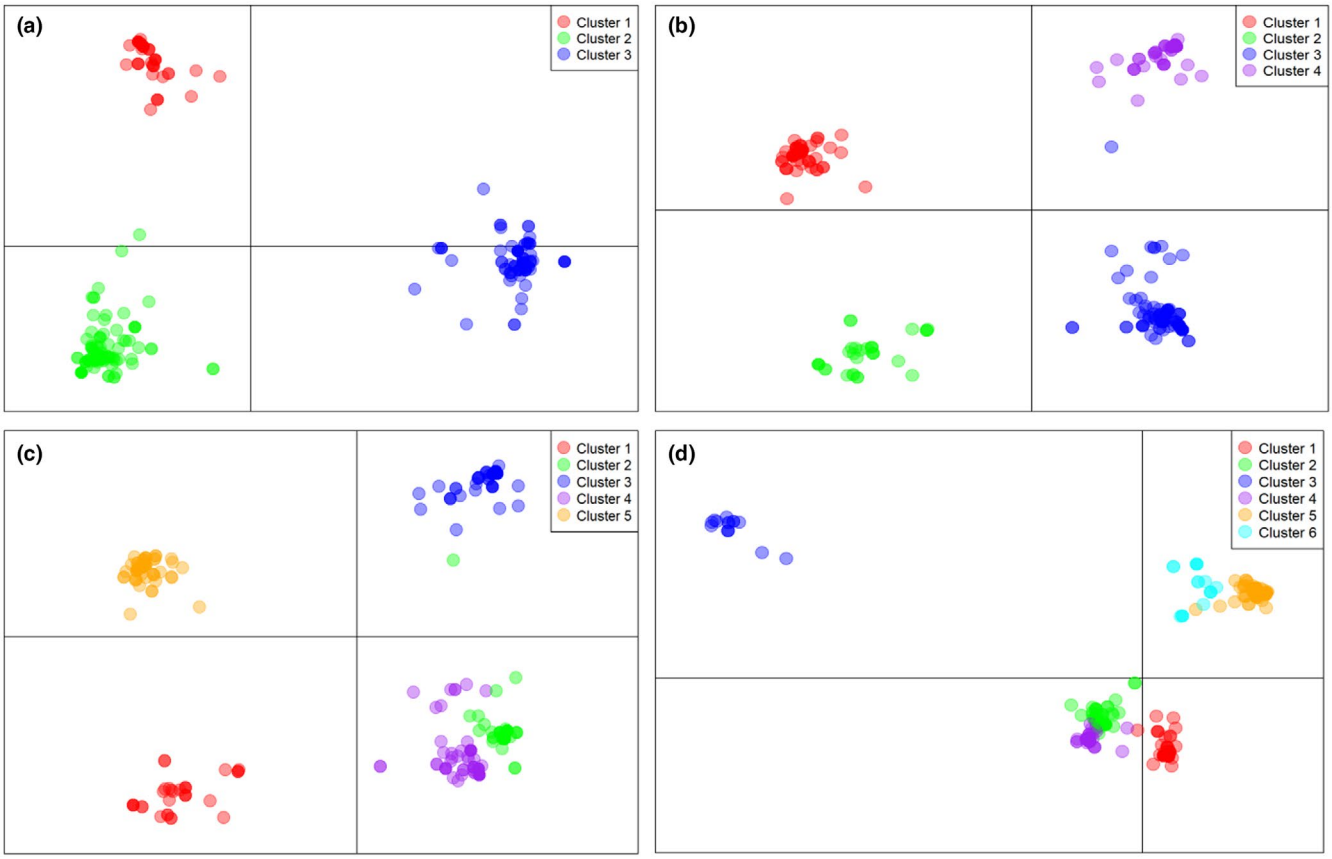

FIGURE 2 Clustering of genotypes by the discriminant analysis of principal components (DAPC) method with the number of clusters (*K*) varying between three (a), four (b), five (c) and six (d). Each colour represents a different cluster.

TABLE 5 F_{ST} statistics between genetic clusters of *Pyricularia oryzae* populations on wild (*Oryza longistaminata*) and cultivated (*Oryza sativa*) rice in Mali.

		Host							
		O. longistaminata				*O. sativa*			
Host	Genetic cluster	1	2	3	4	1	2	3	4
O. longistaminata	G1	–	*	*	*	*	*	*	*
	G2	0.65	–	*	*	*	*	*	*
	G3	0.64	0.66	–	*	*	*	*	*
	G4	0.46	0.59	0.51	–	*	*	*	0.10
O. sativa	G1	0.41	0.72	0.73	0.66	–	*	*	*
	G2	0.64	0.16	0.63	0.47	0.76	–	*	*
	GG3	0.65	0.65	0.31	0.50	0.73	0.62	–	*
	G4	0.44	0.54	0.49	0.02	0.71	0.43	0.49	–

Note: Values above diagonal are probability of Fisher's exact test; below diagonal are F_{ST} values. Values for populations from the same genetic cluster but from different hosts are underlined.
*$p \leq 0.001$.

respectively (Table 2). Isolates from *O. barthii* appeared to have higher frequencies of compatible interactions than isolates from *O. sativa*: 0.68 (range: 0.64–0.75). This broad virulence spectrum may be explained by a bias due to the small number of isolates from *O. barthii* tested and probably a narrower genetic diversity because they came from the same place and have closely related genotypes.

4 | DISCUSSION

The role of wild rice as a potential inoculum source for blast disease on cultivated rice is poorly documented. To our knowledge, two studies addressed this question through pathogenicity tests in controlled conditions (Fukuta et al., 2014; Khemmuk et al., 2016), but no population studies were carried out to detect potential migrations of

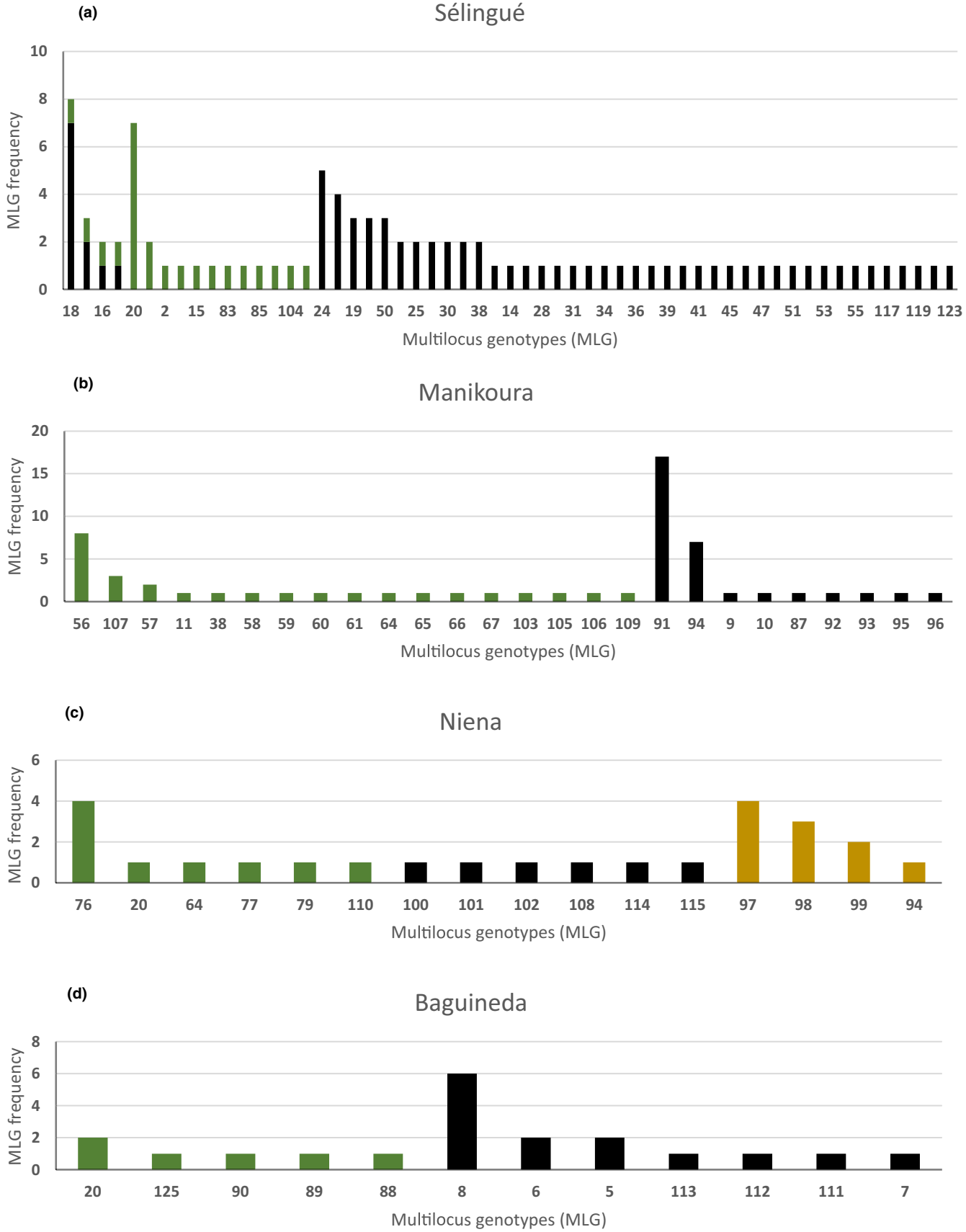

FIGURE 3 Distribution of multilocus genotypes (MLGs) by site and host species. Number of isolates per MLGs in four localities: (a) Sélingué, (b) Manikoura, (c) Niena, (d) Baguineda. Black: *Oryza longistaminata*; green: *O. sativa*; mustard: *O. barthii*.

TABLE 6 Distribution of genetic clusters by species and site, number of *Pyricularia oryzae* isolates per site, host species and genetic cluster.

Host species	Genetic cluster	Koulikoro		Ségou			Sikasso					Total
		Baguineda	Manikoura	Niono N7	Niono N3-bis	Niono Nango Sahel	Loulouni Faraka Banakoro	M'pegnesso	Niena	Sélingué	Not determined	
Oryza barthii	G2	–	–	–	–	–	–	–	1	–	–	1
	G4	–	–	–	–	–	–	–	9	–	–	9
	G1	–	3	–	–	–	–	1	–	3	–	7
Oryza longistaminata	GG2	3	26	–	–	–	–	–	–	37	–	66
	G3	13	3	–	–	–	–	–	1	27	–	44
	G4	–	3	–	–	–	–	–	6	12	–	21
Oryza sativa	G1	7	2	6	25	8	–		2	12	3	65
	G2	–	–	–	–	–	–	2	–	6	–	8
	G3	–	30	–	–	–	–	9	8	5	6	58
	G4	–	–	–	–	–	1	–	–	2	–	3
Total		23	67	6	25	8	1	12	27	104	9	282

P. oryzae genotypes from one host to the other. Under the hypothesis that wild rice is a regular source of inoculum for blast epidemics on cultivated rice, populations of the pathogen on both hosts are expected to be the same. The present genetic study shows that *P. oryzae* populations on cultivated and wild rice in Mali are significantly but barely differentiated. This result suggests that, at the country scale, the gene pool shared between both populations is broad. However, comparisons of the clones and genetic clusters composing pathogen populations from each host show a clear differentiation, for both geographic scales examined (country and local). Each population is mainly composed of specific MLGs. At the country scale, few MLGs were sampled on both hosts (six out of 125). At the local scale, this number was even smaller because only four MLGs were sampled in the same area on wild and cultivated rice. Although the same four genetic groups were sampled on both hosts, their frequencies were different between hosts. At the country scale, the genetic clusters GC1 and GC4 were mainly composed of strains collected on wild rice species (91% and 89%, respectively), while GC1 was mainly composed of strains isolated from cultivated rice (90%). GC3 showed a more balanced ratio (43% of strains isolated from wild rice). These results show that wild rice (*O. longistaminata*) and cultivated rice (*O. sativa*) in Mali are hosting different populations of the blast fungus. These results do not support the hypothesis that wild rice could be the main source of inoculum for cultivated rice.

Maratelli is considered as a susceptible check because of its broad range of compatibility with *P. oryzae* isolates from rice. Results from different published studies with a very diverse collection of *P. oryzae* isolates from *O. sativa* showed that 94% of the 172 isolates tested were compatible (Gallet et al., 2016; Thierry et al., 2022). In this study, Maratelli was incompatible with an unexpectedly high ratio of isolates (50%). Most of these incompatible isolates (9/10) were isolated from *O. longistaminata*. Malian isolates from wild rice may carry avirulence factors that impede them from infecting Maratelli. Two avirulence genes to Maratelli have already been identified and mapped (Mandel et al., 1997). Alleles of these genes triggering an incompatible reaction in Maratelli originated from non-rice *P. oryzae* isolates.

The relatively small sample size of some populations studied here is likely to create bias and should lead to cautious interpretation of data. A first example is the low differentiation between populations from *O. sativa* and *O. longistaminata* within the G4 cluster. In this particular case, F_{ST} values were calculated based on a sample of three and 21 strains isolated from *O. sativa* and *O. longistaminata*, respectively. The sample size of strains isolated from cultivated rice is clearly not sufficient to have an accurate estimate of allelic frequencies and to draw conclusions from the differentiation parameters inferred from these frequencies. A second example is the results of pathogenicity tests of strains isolated from *O. barthii*. Plants of this species with blast symptoms were rarely encountered in the field in Mali and, consequently, it was difficult to isolate a sufficient number of strains for appropriate comparisons. However, because reports on *P. oryzae* isolated from *O. barthii* are rare, we believe that reporting preliminary

results is of interest. Nevertheless, the sample size remains small (10 strains) and potentially not representative of the diversity in Mali to draw conclusions on the compatibility range of this population on varieties of *O. sativa*. All 10 strains were collected from the same place at the same date and represent a narrow genetic base (only four MLGs). It is thus not surprising that these strains share similar pathogenicity spectra. We cannot exclude that strains isolated from *O. barthii* have a broader compatibility range on *O. sativa* than *O. sativa* strains but this hypothesis requires confirmation with a larger number of strains.

Pathogenicity tests on cultivated rice also revealed that isolates from wild rice are pathogenic to cultivated rice under controlled conditions. In addition, isolates from wild rice did not appear to be less pathogenic on *O. sativa* varieties cultivated in Mali than *O. sativa* isolates, that is, under controlled conditions, isolates of wild rice (*O. longistaminata*) are pathogenic to a small number of varieties. Therefore, the isolates from *O. longistaminata* have the potential to infect *O. sativa* varieties cultivated in Mali. Similar results were observed with *P. oryzae* isolates from wild rice (*O. meridionalis*) in Australia. These isolates were pathogenic to local rice varieties after artificial inoculation (Khemmuk et al., 2016). Despite this potential, and based on population genetic structure, *P. oryzae* isolates generating epidemics on *O. longistaminata* do not seem to cause epidemics on *O. sativa* in the field. Two non-exclusive causes can be provided to explain this observation. First, our evaluation of compatibility did not take into consideration other pathogenicity components that may be important in field epidemics (e.g., production of spores by lesion area unit). Secondly, isolates from *O. longistaminata* are less fit on *O. sativa* than isolates from *O. sativa* and are thus excluded when competing in the field. In *P. oryzae*, fitness differences were observed between populations from two rice subspecies and were hypothesized to contribute to the pathogen population structure (Liao et al., 2016). Additional experiments measuring quantitative differences in pathogenicity and fitness would be needed to test these hypotheses for *P. oryzae* populations of wild and cultivated rice.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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