Tracing the Origin and Evolutionary History of *Pyricularia oryzae* Infecting Maize and Barnyard Grass

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ABSTRACT

Blast disease is a notorious fungal disease leading to dramatic yield losses on major food crops such as rice and wheat. The causal agent, *Pyricularia oryzae*, encompasses different lineages, each having a different host range. Host shifts are suspected to have occurred in this species from *Setaria* spp. to rice and from *Lolium* spp. to wheat. The emergence of blast disease on maize in Iran was observed for the first time in the north of the country in 2012. We later identified blast disease in two additional regions of Iran: Gilan in 2013 and Golestan in 2016. Epidemics on the weed barnyard grass (*Echinochloa* spp.) were also observed in the same maize fields. Here, we showed that *P. oryzae* is the causal agent of this disease on both hosts. Pathogenicity assays in the greenhouse revealed that strains from maize can infect barnyard grass and conversely. However, genotyping with simple sequence repeat markers and comparative genomics showed that strains causing field epidemics on maize and on barnyard grass are

different, although they belong to the same previously undescribed clade of *P. oryzae*. Phylogenetic analyses including these strains and a maize strain collected in Gabon in 1985 revealed two independent host-range expansion events from barnyard grass to maize. Comparative genomics between maize and barnyard grass strains revealed the presence or absence of five candidate genes associated with host specificity on maize, with the deletion of a small genomic region possibly responsible for adaptation to maize. This recent emergence of *P. oryzae* on maize provides a case study to understand host range expansion. Epidemics on maize raise concerns about potential yield losses on this crop in Iran and potential geographic expansion of the disease.

Keywords: blast, emergence, evolution, genomics, host range, *Magnaporthe oryzae*, maize, new disease, pathogen detection

As for human diseases, the emergence of new plant diseases is favored by global changes and is an increasing matter of concern. In particular, epidemics caused by fungal pathogens have increased in frequency and are a recurrent threat to global food security (Fisher et al. 2012). The emergence of new plant diseases can be caused by human-driven or aerial dispersal of pathogens, increase in virulence, or infection of a novel host (Giraud et al. 2010). Emblematic examples are the introductions of *Cryphonectria parasitica* and *Phytophthora cinnamomi* into the United States from Japanese chestnut trees (*Castanea crenata*), which were imported and sold throughout the United States, resulting in the devastation of the American chestnut (*C. dentata*) (Milgroom et al. 1996). The causal agent of ergot disease was also introduced on sorghum to

American and Australian countries from Asian and African countries (Bandyopadhyay and Frederiksen 1999). The introduction of *P. infestans* from South America into Europe resulted in epidemics of late blight of potato, which caused a famine in Ireland in the nineteenth century (Bandyopadhyay and Frederiksen 1999). The blast disease was endemic to South America but recently emerged in Bangladesh (Islam et al. 2016) and it is now spreading to India (Chaudhuri 2017) and Africa (Tembo et al. 2020).

The fungal species *Pyricularia oryzae* causes blast diseases of staple crops such as rice and wheat, or other cereals of local importance (e.g., millet). Epidemics caused by *P. oryzae* annually destroy enough food supply to sustain millions of people (Pennisi 2010). The blast also has an economic impact through the cost of control methods to prevent or limit epidemics in recreation areas such as golf courses or stadiums (Uddin et al. 1999). Blast epidemics are highly destructive, leading to 100% yield loss on rice in some extreme cases, 40 to 100% on wheat in Brazil (Skamnioti and Gurr 2009), and over 90% destruction on turfgrass in several golf courses (Uddin et al. 1999). The blast pathosystem not only is a model for studying plant–pathogen interactions (Dean et al. 2012; Talbot 2003; Valent 1990) but also is emerging for investigating the host shifts, host-range expansions, and disease spillovers that occur among grass crops (Chiapello et al. 2015; Gladieux et al. 2018a). The rice blast fungus likely emerged on rice following a host shift from *Setaria* spp. (Couch et al. 2005). A host shift from ryegrass (*Lolium* spp.) to wheat was recently hypothesized following genomic studies of field strains (Farman et al. 2017; Gladieux et al. 2018a; Inoue et al. 2017). Epidemics of blast on maize were also previously observed in

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Gabon (Nottéghem 1990) and Japan (Yamanaka 1982) but seem to have been limited in time and space. During the epidemic in Gabon in 1985, three strains were isolated. One of these strains (GA1, also named GN0001 hereafter) was characterized and assigned to *P. oryzae* by phylogenetic analysis of multiple gene genealogies (Klaubauf et al. 2014; Lebrun 1991). GN0001 was inoculated by syringe injection on Asian rice (*Oryza sativa*), finger millet (*Eleusine coracana*), wheat (*Triticum aestivum*), pearl millet (*Pennisetum glaucum*), crabgrass (*Digitaria horizontalis*), and maize (*Zea mays*) and was pathogenic only on maize (Biju-Duval 1994).

The emergence of blast disease on maize was recently observed in Iran. Blast symptoms on maize were first observed in 2012 in Mazandaran Province (Pordel et al. 2016) and spread to two additional provinces in the following years. The fungus genus *Pyricularia* was systematically isolated from lesions. Our observations also revealed that barnyard grass (*Echinochloa* spp.), present as a weed in maize fields or nearby, was infected by *Pyricularia oryzae* and had severe blast symptoms as well. Here, we report on a new epidemic caused by *P. oryzae* on maize and present evidence that the emergence of blast disease on maize in Gabon and Iran was caused by independent events of host range expansion from the common weed barnyard grass to maize.

MATERIALS AND METHODS

Fungal isolation. Leaves of maize (*Z. mays*) with blast symptoms were collected in the Mazandaran, Gilan, and Golestan Provinces of Iran in August 2012 and 2013 and September 2016. All *Pyricularia* isolates were obtained by isolating single hyphal tips emerging from germinating conidia (Pordel et al. 2016). Newly sequenced genomes from seven isolates from Iran and one isolate from Gabon were analyzed together with 56 published genomes and 19 additional unpublished genomes from strains isolated from rice, ryegrass, and *Setaria* spp. In total, genomic data for eighty-five strains from 14 different hosts were included in the study (Supplementary Table S1).

DNA extraction, and microsatellite amplification. For each strain, a paper stock of sporulating mycelium was deposited on rice flour medium maintained in a climatic chamber for 4 to 5 days to obtain actively growing mycelium. Freshly grown mycelia were inoculated in 6 ml of 2 YEG liquid medium (2 g of yeast extract, 10 g of glucose, 3 g of KNO₃, 2 g of KH₂PO₄, 500,000 units of penicillin, and 1 liter of water). Cultures were maintained for 4 days in the dark at 25°C, before being collected for genomic DNA extraction following a previously published protocol (Adreit et al. 2007). Strains were genotyped using 10 simple sequence repeat (SSR) markers (Supplementary Table S2), previously developed for population genetics studies of *P. oryzae* (Adreit et al. 2007; Kaye et al. 2003). SSR markers were amplified by PCR (Qiagen Multiplex PCR kit) in a total volume of 5 µl, including 2.5 µl of Master Mix, 0.5 µl of 10× Mix primers, 0.5 µl of 5xQ solution, and 1.5 µl of genomic DNA (10 ng/µl). The PCR program was as follows: (i) pre-denaturation at 95°C for 15 min, (ii) denaturation at 94°C for the 30s, (iii) hybridization at 57 to 63°C for 90s, (iv) extension at 72°C for 60s, (v) repeat steps i through iv for 40 cycles, and (vi) final extension at 95°C for 30 min. Amplicons were separated and analyzed on a 16-capillary ABI Prism 3130XL machine (Applied Biosystems, Foster City, CA, U.S.A.) and the size of the amplicons was evaluated by fluorescence measurement. For this analysis, 1.5 µl of amplified products (1/70 dilution) was mixed with 15 µl of formamide HiDi and GeneScan-500LIZ size marker (Applied Biosystems). The raw data collected were then analyzed and converted to allele size with GENEMAPPER 4 software (Applied Biosystems). A network of the different multilocus genotypes was constructed with the goeBURST algorithm implemented in Phyloviz 2.0 v (Francisco et al. 2012).

Whole-genome sequencing and assembly. Sequencing was performed on Illumina HiSeq 3000 sequencers and produced 150

nucleotide paired-end reads with >50× depth. Low-quality reads were removed using the software Cutadapt (Martin 2011). De novo assembly was performed with ABySS software (Simpson et al. 2009). We evaluated the N₅₀ value of the assembled sequences following different K-mers values and chose the assembled sequences having the highest N₅₀ value for further analyses.

Protein-coding gene identification. Genes of each assembled sequence of *P. oryzae* isolates were predicted using BRAKER1 and AUGUSTUS v 3.0.3 software (Hoff et al. 2015). Gene prediction with BRAKER1 used RNA-sequencing data from different sources (Supplementary Table S3). Homology relationships among genes predicted in the 85 genomes were identified using OrthoFinder v2.4.0 (Emms and Kelly 2019). Population genomic analysis was based on a set of 7,466 single-copy orthologs. Sequences for each single-copy ortholog were aligned and cleaned with TranslatorX (Abascal et al. 2010) using default parameters.

Population genomic analysis. Maximum-likelihood trees were constructed using RAXML v8.2.12 (Stamatakis 2014) with the general time-reversible γ model, and bootstrap support was estimated based on 100 replicates. SplitsTree v4.13 (Huson and Bryant 2006) analysis was conducted on the concatenation of sequences at single-copy orthologs, excluding sites with missing data, sites with gaps, singletons, and monomorphic sites, to infer a neighbor-net phylogenetic network and test the null hypothesis of clonality using the PHI test. Population subdivision was analyzed using Structure v2.3.4 (Falush et al. 2003; Hubisz et al. 2009), based on multilocus haplotype profiles identified from ortholog alignments. Structure was run with 20,000 steps, following 10,000 burn-in, with 12 repeats. The presence of one single clustering solution (i.e., a single clustering mode) was checked visually across all 12 repeats for all K values.

Comparative genomics of effectors. To identify candidate genes potentially involved in host expansion, we used the table of homology relationships produced using OrthoFinder (Emms and Kelly 2019). We searched for groups of orthologs that are present in one lineage and absent or pseudogenized in another lineage. To reduce the number of orthogroups to be analyzed, we first removed groups of orthologs that were present in all lineages. Then, for the remaining groups of orthologs, we used Blastn to confirm that the absence of a given ortholog in the assembly of a given isolate was not caused by annotation error. All Blastn hits with an intron of less than 1,000 bp, coverage greater than 90% of the gene length, and 80% identity were counted as present. Once this correction was made, we looked for sequences that would have been pseudogenized by the insertion of a transposable element. We used RepeatMasker and the Repbase database to generate an annotation file of transposable elements and we used these annotation files to identify sequences with insertion of transposable elements. Finally, we used the orthology table corrected for annotation errors and pseudogenization by transposable elements to identify candidate genes as genes being present in the lineage and absent or inactivated by insertion of transposable elements in the other lineage.

Mating type determination and attempts to induce the formation of the teleomorph. The two primer sets designed by Tredway et al. (2005) for *MAT1-1* and *MAT1-2* amplification were used in a multiplex PCR to determine the mating type of maize and barnyard grass strains. The reaction mixture had a total volume of 20 µl and contained 3 µl of genomic DNA (5 to 20 ng µl⁻¹), 2/25× PCR buffer, 0.2 mM each dNTP, 5 pmol of each primer, 5 mM MgCl₂, and 2.5 U of Taq polymerase. The initial denaturation step was done at 95°C for 3 min; followed by 35 cycles of 94°C for 60 s, 60°C for 60 s, and 72°C for 60 s; a final elongation step at 72°C for 10 min was included. The products were separated on a 0.8% (wt/vol) agarose gel and visualized by ethidium bromide staining.

Male and female fertility were determined by in vitro crossing experiments. Sexual competence assays consisted of pairing actively growing mycelia of field strains and fertile tester strains (Br48 with Mat1-1 and Guy11 with Mat1-2) on rice flour-agar

medium. The plates were incubated at 25°C under cycles of 12 h of darkness and 12 h of light for 3 weeks. The mating type of field strains was designated as the opposite of the tester strain with which perithecia were produced and female fertility was declared when field strains formed perithecia (Nottéghem and Silué 1992). Male fertility was declared when field strains induced formation of perithecia (Nottéghem and Silué 1992).

Pathogenicity test. Eleven maize strains and four barnyard grass strains were tested in greenhouse assays for pathogenicity on wheat (*T. aestivum* variety Thésée), rice (*O. sativa* variety

Maratelli), wild foxtail millet (*Setaria viridis*, red-green foliage variety; Graines Voltz International, Colmar, France), barnyard grass (*Echinochloa crus-galli*), and maize (*Z. mays* varieties B73, M017, and one hybrid line). Seed of the different hosts were planted in 10-cm-diameter plastic pots filled with compost. Six seeds were planted per pot. Pots were kept in the greenhouse for 21 days and watered as needed to maintain the substrate wet. Plants were fertilized with NPK (15:10:15) liquid fertilizer once a week. Strains were cultured on rice flour-agar medium for 15 days at 25°C under a regime of 12 h of darkness and 12 h of fluorescent light. Conidia

Fig. 1. Schematic representation of sampling locations of *Pyricularia oryzae* on maize and barnyard grass in Iran.

TABLE 1. Pathogenicity results of *Pyricularia oryzae* strains on maize, barnyard grass, rice, wheat, and wild foxtail millet^a

Fungal strain	Host of origin	Locality (province, city)	Year	<i>Zea mays</i>	<i>Echinochloa crus-galli</i>	<i>Oryza sativa</i>	<i>Triticum aestivum</i>	<i>Setaria viridis</i>
IR0010	<i>Z. mays</i>	Mazandaran, Gharakhil	2012	6	–	1	1	1
IR0012	<i>Z. mays</i>	Mazandaran, Gharakhil	2012	6	1	1	3	1
IR0013	<i>Z. mays</i>	Mazandaran, Gharakhil	2012	6	6	1	1	1
IR0014	<i>Z. mays</i>	Mazandaran, Gharakhil	2012	6	6	1	2	2
IR0015	<i>Z. mays</i>	Mazandaran, Gharakhil	2012	6	–	1	1	1
IR0016	<i>Z. mays</i>	Mazandaran, Gharakhil	2012	6	–	1	3	2
IR0093	<i>Z. mays</i>	Golestan, Agh-Ghala	2016	6	1	1	1	1
IR0094	<i>Z. mays</i>	Golestan, Agh-Ghala	2016	6	–	1	1	1
IR0095	<i>Z. mays</i>	Golestan, Agh-Ghala	2016	6	1	1	1	1
IR0096	<i>Z. mays</i>	Golestan, Agh-Ghala	2016	6	–	1	1	1
IR0084	<i>E. crus-galli</i>	Golestan, Agh-Ghala	2016	6	6	1	1	–
IR0083	<i>E. crus-galli</i>	Golestan, Agh-Ghala	2016	6	6	1	1	–
IR0102	<i>E. crus-galli</i>	Golestan, Azad shahr	2016	6	6	1	1	–
IR0088	<i>E. crus-galli</i>	Golestan, Agh-Ghala	2016	5	1	1	1	–
GN0001	<i>Z. mays</i>	Gabon	1985	6	–	1	1	3
GY0011	<i>O. sativa</i>	French Guyana	1988	1	–	6	3	1
BR0032	<i>T. aestivum</i>	Brazil	1991	3	–	2	6	1
US0071	<i>S. viridis</i>	United States	ND ^b	3	–	1	1	6

^a Lesion types are as follows: – indicates not tested, 1 = no signs of infection, 2 = small brown lesions, 3 = small lesions with yellow centers and brown margins, and 6 = large diamond-shaped lesions (Silué et al. 1992).

^b ND = no data.

were scraped and washed with 3 to 5 ml of sterile distilled water. Conidia concentration was quantified using a hemocytometer and adjusted to 2×10^5 spores ml^{-1} and 0.5% gelatin for inoculation. The conidia suspension (10 ml) was sprayed on four pots (one per plant species). Subsequently, the plants were incubated for 24 h in a controlled climatic chamber at 24°C with 95% relative humidity. Inoculated plants were then transferred back to the greenhouse and scored 7 days after inoculation (Silué et al. 1992). Pathogenicity tests were repeated five times.

RESULTS

First report of epidemics of blast on maize and barnyard grass in Iran. Blast epidemics in maize fields were observed in three geographical regions of Iran. Blast symptoms on maize were first observed in August 2012 in Mazandaran (Pordel et al. 2016), then in August 2013 in Gilan Province and September 2016 in Golestan Province (Fig. 1). Blast symptoms on plants of barnyard grass growing as weeds in the maize fields were also observed. Leaf spot symptoms on maize initially appeared as gray lesions with a light margin, then expanded rapidly to several centimeters in length and became lighter in color with a distinct brown margin. Typical diamond-shaped symptoms with a brown margin and yellow center

were observed on barnyard grass. In all, 12 and 32 *Pyricularia* sp. monospore strains were isolated from infected leaves of maize and barnyard grass, respectively, and characterized for some morphological criteria commonly used for *Pyricularia* taxonomy (Ellis 1971; Klaubauf et al. 2014). Mycelium consisted of smooth, hyaline, branched, septate hyphae of 2 to 3 μm in diameter. Conidiophores were solitary, erect, straight, or curved, septate, pale brown, 130 to 150 by 3 to 4 μm in size. Conidiogenous cells were sympodial, denticulate. Conidia were pale brown, pyriform, two-septate, 16 to 24 by 6 to 8 μm . Together, these morphological characteristics are diagnostic of *P. oryzae* (Ellis 1971; Klaubauf et al. 2014).

***P. oryzae* strains from maize and barnyard grass can cross infect.** Host ranges of strains isolated from maize and barnyard grass (hereafter named maize strains and barnyard grass strains, respectively) were assessed by spray inoculation of spore suspensions on crops and weeds known to be hosts to *P. oryzae*. Control strains from wheat, rice, and foxtail millet showed severe symptoms on their original host. In all pathogenicity tests, the strains isolated from maize were highly pathogenic toward maize and the strains isolated from barnyard grass (except IR0088) were highly pathogenic on barnyard grass (Table 1; Fig. 2). Four and three strains from maize did produce some lesions (three to seven)

Fig. 2. Blast symptoms caused by **A**, maize strain IR0013 on maize and **B**, barnyard grass strain IR0083 on barnyard grass.

Maize and barnyard grass strains belong to a new clade of *P. oryzae*. We used a population genomic approach to determine how the fungal pathogens isolated from maize and barnyard grass relate to *P. oryzae* lineages infecting different cereals and grasses. We extracted the predicted gene sequences from the assembled genomic sequences of eight *P. oryzae* strains isolated from maize

To evaluate potential gene flow between and within clades and visualize evolutionary relationships, we used phylogenetic network analysis, as implemented in the SplitsTree4 software (Huson 1998; Huson and Bryant 2006). The network inferred from haplotypes identified using the 7,466 single-copy orthologs in the 85 *P. oryzae*

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strains showed limited reticulations along branches connecting this clade with other clades (Fig. 3C), suggesting limited gene flow between the maize or barnyard grass lineage and other lineages.

To further evaluate the possibility of admixture between *P. oryzae* strains from different hosts, we conducted a Bayesian clustering analysis using the program Structure 2.3.4 (Falush et al. 2003), based on haplotypes identified at 7,466 single-copy orthologs and modeling $K = 2$ to $K = 20$ clusters. The log probability of data showed two modes at $K = 8$ and $K = 14$, and the ΔK statistic presented modes at $K = 2$ and $K = 6$ (Supplementary Fig. S1). Models with $K > 15$ did not show new clearly defined clusters (i.e., clusters in which some individuals have membership proportions > 0.8); therefore, only models with $K = 2$ to $K = 15$ clusters are presented (Fig. 3B). Strain IR0088 isolated from barnyard grass separated from other strains isolated from maize and barnyard grass at $K = 4$. Strain GN0001 isolated from maize in Gabon displayed high membership proportions ($q > 0.6$) in the cluster grouping most strains from maize and barnyard grass at all K values but it also had substantial membership proportion ($q > 0.35$) at higher K values ($K > 12$) in a cluster that was not represented in our dataset.

Iranian *P. oryzae* populations from maize and barnyard grass are single mating type and female-sterile despite signatures of recombination. *P. oryzae* is a heterothallic fungus whose sexual compatibility is controlled by genes at the *MAT1* locus, with sexual reproduction being possible only between strains of the opposite mating type (Mat1-1 and Mat1-2). Three strains (IR0013, IR0015, and IR0095) from maize and four strains (IR0088, IR0084, IR0083, and IR0102) from barnyard grass were of the same mating type. For all of these strains, PCR on genomic DNA amplified the 390-bp-specific fragment of the *MAT1-2* locus but did not amplify the *MAT1-1*-specific marker. All maize and barnyard grass strains induced formation of perithecia when crossed with the Mat1-1 tester and, thus, they were considered as male-fertile. None of the maize and barnyard grass strains produced perithecia when crossed with the Mat1-1 tester and, thus, they were considered as female-sterile. Although these mating type and fertility experiments

suggest that these populations are asexual, PHI tests based on levels of homoplasy rejected the null hypothesis of clonality in the Iranian *P. oryzae* populations from maize and barnyard grass ($P < 0.0001$). The GN0001 strain from Gabon was Mat1-2 and female-fertile, and the null hypothesis was also rejected with the PHI test when this strain was included in the dataset. A neighbor-net network estimated from the dataset including the Iranian *P. oryzae* populations from maize and barnyard grass and the Gabon strain from maize displayed reticulations (Supplementary Fig. S2).

Maize and barnyard grass strains belong to two differentiated populations. Genotyping with 10 SSR markers showed that *P. oryzae* strains from maize and barnyard grass belong to different populations. Genotyping of 11 maize strains from Golestan and Mazandaran Provinces and 26 barnyard grass strains from Golestan and Gilan Provinces generated 16 multilocus genotypes (MLGs). Strains isolated from maize and barnyard grass had different MLGs. Maize strains were distributed in three MLGs (numbered 14 to 16) and barnyard grass strains were distributed in the 13 other MLGs (Fig. 4). The five maize strains collected in 2016 in Agh Ghala in Golestan Province had the same MLG (MLG 14) whereas the 13 barnyard grass strains collected in the same area at the same time were distributed in seven other MLGs.

Candidate genes for host specificity. Comparative genomics between maize and barnyard grass strains was conducted to search for candidate genes that could be responsible for host specificity. Effectors that are present in one group and absent or putatively not functional in the other were searched for in annotated genomes. We found four candidate genes (Table 2) that were present in the maize strains and absent or putatively nonfunctional in the barnyard grass strains. At least three of them were physically closely linked in the reference genome of *P. oryzae* (70-15 strain). These three genes were mapped on chromosome 1 between positions 392,967 and 417,691. The comparison of maize and barnyard grass isolates to isolates from other hosts did not reveal groups of orthologs specific to the combination of maize and barnyard grass isolates (Table 2).

Fig. 3. (Continued)

DISCUSSION

The emergence of *P. oryzae* on maize in Iran raises the question of the origin of maize strains. Either the pathogen was introduced in Iran from foreign epidemic areas or it emerged locally from another host species, through events of disease spillover, host-range expansion, or host shift. Phylogenomic and population genomic analyses revealed that the recent emergence of wheat blast in Bangladesh and in Zambia was caused by introductions of the pathogen from South America (Islam et al. 2016) and from an as-

yet-undetermined origin (Tembo et al. 2020), respectively. Because the blast disease of maize was previously reported in Gabon and Japan, a hypothesis is that maize blast was imported to Iran from another country. This hypothesis can only be tested for Gabon because genotypic, mating type, and fertility data for maize strains from Japan are not available and these strains were not conserved. The fact that GN0001 is female-fertile and displays shared ancestry with an unsampled group in clustering analyses suggest that *P. oryzae* populations on maize in Gabon could have been reproducing sexually. Unlike the maize strain from Gabon,

Fig. 4. Genotype network of *Pyricularia oryzae* strains isolated from maize and barnyard grass in Iran. Multilocus genotypes were identified based on 10 simple sequence repeat markers.

TABLE 2. Candidate genes for host specificity identified by comparative genomics between maize and barnyard grass strains of *Pyricularia oryzae* and with other hosts^a

OG ^b	Gene ^c	Position ^d	Putative function	Maize				Barnyard grass				Setaria	Rice	Wheat
				IR0013	IR0015	IR0095	GN0001	IR0083	IR0084	IR0102	IR0088	US71	GY11	BR0032
OG0000055	MGG_02051T0	1:392,967–395,203	CAMK protein kinase	+	+	+	+	–	–	–	INS G	–	–	–
OG0010704	...	...	Kinase domain, secreted P	+	+	+	–	–	–	–	–	INS G	+	INS G
OG0010727	MGG_16022T0	1:410,855–411,153	...	+	+	+	+	–	–	–	INS P	+	+	–
OG0011065	MGG_02056T0	1:414,777–417,691	Secreted P	–	–	–	INS G	+	+	+	+	+	+	–

^a Symbols: – indicates absent, + indicates present, and INS indicates present but with insertion of a transposable element or a simple sequence repeat in the gene (G) or the 500 upstream region (P).

^b Orthologous group.

^c Gene in reference genome.

^d Position in reference genome.

P. oryzae populations infecting maize in Iran do not show biological features consistent with sexual reproduction (only one mating type and no female-fertile strains). Iranian *P. oryzae* populations infecting maize show a signature of recombination according to the PHI test for clonality but the test does not allow a conclusion about the timing of recombination (recombination can be contemporary or historical). If maize-infecting strains migrated from Gabon to Iran, either only Mat1-2 strains were introduced or strains of both mating types were introduced but only Mat1-2 strains were established on maize as asexual populations. The loss of sexual reproduction following migrations was already documented for rice-infecting populations of *P. oryzae* (Gladieux et al. 2018b; Saleh et al. 2014; Thierry et al. 2020).

Previous reports of maize blast in Gabon and Japan date from 30 years ago and we are not aware of any contemporary blast epidemic of maize outside Iran. This observation supports the hypothesis of a recent event of host range expansion in Iran over the hypothesis of a recent introduction from another area. The clustering of Gabonese and Iranian isolates in the same group suggests, however, that maize-infecting strains emerged from a population of *P. oryzae* that is present in both regions. In *P. oryzae*, several events of infection of a novel host have been suspected or documented, such as the host shift from *Setaria* to rice (Couch et al. 2005) or the host shift from *Lolium* to wheat (Gladieux et al. 2018a; Inoue et al. 2017). Several lines of evidence support the hypothesis that the emergence of maize blast in Iran was caused by the adaptation to maize of strains present on barnyard grass. Analyses of population structure, including at a local scale, show that *P. oryzae* populations on maize and barnyard grass are differentiated while belonging to the same phylogenomic lineage. In addition, in our experiments in controlled conditions, barnyard grass strains could infect maize, and two maize strains could infect barnyard grass. Finally, blast epidemics on barnyard grass were observed in maize fields with blast symptoms in Iran, showing the cooccurrence of the potential source population (barnyard grass) and the potential recipient (maize) of the host range expansion event. Together, these results and observations support a scenario in which the epidemics in Gabon and Japan were due to independent events of infection of a novel host, with barnyard grass being the host of origin of populations infecting maize.

Recent events of infection of a novel host provide valuable materials to study the genetic determinants of host specificity. In such cases, comparative genomics is expected to lead to a limited number of candidate genes because populations diverged recently. Because barnyard grass strains are pathogenic to maize in controlled conditions, the adaptation to maize may be governed by a few genes and host range expansion may have a limited fitness cost. In this study, we identified four candidate genes that are differentially present or absent or potentially nonfunctional between the maize and barnyard grass strains. We can speculate that the adaptation to maize is due to the loss of an effector that was previously recognized by the plant and was triggering defense mechanisms (i.e., the loss of a so-called avirulence gene). Following this scenario, genes absent or nonfunctional in maize strains and present in barnyard grass strains are prime candidates as genetic determinants of adaptation to hosts. Among the four genes identified in our comparative genomic analysis, a putative secreted protein and a protein kinase represent interesting candidates. The vast majority of fungal avirulence genes cloned to date are secreted proteins (Giraldo and Valent 2013). In *P. oryzae*, several avirulence genes were already shown to control host specificity or are presumed to be involved in host shifts, with most of them having been identified by genetic crosses (*PWL1* and 2, *PWT1* to 5, *Pfml* and 2, and *eA1* to 5) (Asuke et al. 2020). A limited number of genes involved in host shifts were cloned. An allele of *PWL2* controls specificity on weeping lovegrass (*Eragrostis curvula*). It is absent in weeping lovegrass-infecting strains and triggers the plant defense mechanisms when present. *PWL2* encodes a glycin-rich, hydrophilic, and secreted protein. Similarly, *PWL1* is absent in strains

attacking weeping lovegrass whereas it is present in closely related nonpathogenic strains (Asuke et al. 2020). Molecular plant pathology experiments and comparative genomics suggest that the loss of *PWT3*, an avirulence gene recognized by the wheat resistance gene *Rwt3*, has favored the host shift from ryegrass (*Lolium* spp.) to wheat (Inoue et al. 2017). The roles in host specificity of the candidate genes we identified need to be investigated by functional analysis. Interestingly, all of the candidate genes that we could identify in silico are located in a narrow genomic area (25 kb) in the reference genome of *P. oryzae* (strain 70-15). This clustering of candidate genes could be indicative of a single event of insertion or deletion. Yoshida et al. (2016) identified the gain and loss of genes as a major evolutionary mechanism responsible for the specialization of *P. oryzae* to rice and *Setaria* spp.

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