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

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▶ **To cite this version:**

Adel Pordel, Sebastien Ravel, Florian Charriat, Pierre P. Gladieux, Sandrine Cros-Arteil, et al.. Tracing the Origin and Evolutionary History of Pyricularia oryzae Infecting Maize and Barnyard Grass. Phytopathology, 2021, 111 (1), pp.128-136. 10.1094/PHYTO-09-20-0423-R . hal-03468375

HAL Id: hal-03468375
https://hal.inrae.fr/hal-03468375v1

Submitted on 22 Oct 2024

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3 # Tracing the origin and evolutionary history of *Pyricularia oryzae* infecting
4 maize and barnyard grass
5
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20

21 **Keywords**: *Magnaporthe oryzae*, emergence, new disease, blast, maize, host range.

22

23 **Funding:** University of Tehran, CIRAD, ANR Grant MagMAX (ANR-18-CE20-0016) awarded
24 to PG.

25

26

27 **Abstract**

28 Blast disease is a notorious fungal disease leading to dramatic yield losses on major food crops
29 such as rice and wheat. The causal agent, *Pyricularia oryzae*, encompasses different lineages,
30 each having a different host range. Host shifts are suspected to have occurred in this species from
31 *Setaria* sp. to rice and from *Lolium* sp. to wheat. The emergence of blast disease on maize in Iran
32 was observed for the first time in the North of the country in 2012. We later identified blast
33 disease in two additional regions of Iran, Gilan in 2013, and Golestan in 2016. Epidemics on the
34 weed barnyard grass (*Echinochloa* spp.) were also observed in the same maize fields. Here, we
35 showed that *P. oryzae* is the causal agent of this disease on both hosts. Pathogenicity assays in
36 the greenhouse revealed that strains from maize can infect barnyard grass and conversely.
37 However, genotyping with SSR markers and comparative genomics showed that strains causing
38 field epidemics on maize and on barnyard grass are different, although they belong to the same
39 previously undescribed clade of *P. oryzae*. Phylogenetic analyses including these strains and a
40 maize strain collected in Gabon in 1985, revealed two independent host-range expansion events
41 from barnyard grass to maize. Comparative genomics between maize and barnyard grass strains
42 revealed the presence/absence of five candidate genes associated with host specificity on maize,
43 with the deletion of a small genomic region possibly responsible for adaptation to maize. This
44 recent emergence of *P. oryzae* on maize provides a case study to understand host range
45 expansion. Epidemics on maize raise concerns about potential yield losses on this crop in Iran
46 and potential geographic expansion of the disease.

47 **Keywords**: *Magnaporthe oryzae*, emergence, new disease, blast, maize, host range.

48 **Introduction**

49 Like for human diseases, the emergence of new plant diseases is favored by global changes and
50 is an increasing matter of concern. In particular, epidemics caused by fungal pathogens have
51 increased in frequency and are a recurrent threat to global food security (Fisher et al. 2012). The
52 emergence of new plant diseases can be caused by human-driven or aerial dispersal of
53 pathogens, increase in virulence or infection of a novel host (Giraud et al. 2010). Emblematic
54 examples are the introductions of *Cryphonecteria parasitica* and *Phytophthora cinnamomi* into
55 the USA from Japanese chestnut trees (*Castanea crenata*), which were imported, and sold

56 throughout the United States resulting in the devastation of the American chestnut (*C. dentata*)
57 (Milgroom et al. 1966). The causal agent of ergot disease was also introduced on Sorghum to
58 American and Australian countries from Asian and African countries (Bandyopadhyay and
59 Frederiksen 1999). The introduction of *Phytophthora infestans* from South America in Europe,
60 resulted in epidemics of late blight of potato, which caused a famine in Ireland in the nineteenth
61 century (Bandyopadhyay and Frederiksen 1999). The blast disease was endemic to South
62 America but recently emerged in Bangladesh (Islam et al. 2016) and it is now spreading to India
63 (Chaudhuri 2017) and Africa (Tembo et al. 2020).
64 The fungal species *Pyricularia oryzae* causes blast diseases of staple crops such as rice and
65 wheat, or other cereals of local importance (e.g. millets). Epidemics caused by *P. oryzae*
66 annually destroy enough food supply to sustain millions of people (Pennisi 2010). The blast also
67 has an economic impact through the cost of control methods to prevent or limit epidemics in
68 recreation areas such as golf courses or stadiums (Uddin et al. 1999). Blast epidemics are highly
69 destructive leading to 100% yield loss on rice in some extreme cases, 40 to 100% on wheat in
70 Brazil (Skamnioti and Gurr 2009), and over 90% destruction on turfgrass in several golf courses
71 (Uddin et al. 1999). The blast pathosystem is a model not only for studying plant-pathogen
72 interactions (Valent 1990; Talbot 2003; Dean et al. 2012) but it is also emerging for investigating
73 the host shifts, host-range expansions, and disease spillovers that occur among grasses crops
74 (Gladieux et al. 2018). The rice blast fungus likely emerged on rice following a host shift from
75 *Setaria* sp. (Couch et al. 2005). A host shift from ryegrass (*Lolium* sp.) to wheat was recently
76 hypothesized following genomic studies of field strains (Farman et al. 2017; Inoue et al 2017;
77 Gladieux et al. 2018). Epidemics of blast on maize were also previously observed in Gabon
78 (Nottéghem 1990) and Japan (Yamanaka 1982) but seem to have been limited in time and space.
79 During the epidemic in Gabon in 1985, three strains were isolated. One of these strains (GA1,
80 also named GN0001 hereafter) was characterized and assigned to *P. oryzae* by phylogenetic
81 analysis of multiple gene genealogies (Klaubauf et al. 2014). GN0001 was inoculated by syringe
82 injection on Asian rice (*Oryza sativa*), finger millet (*Eleusine coracana*), wheat (*Triticum*
83 *aestivum*), pearl millet (*Pennisetum glaucum*), crabgrass (*Digitaria horizontalis*), and maize (*Zea*
84 *mays*) and was pathogenic only on maize (Biju-Duval 1994).
85 The emergence of blast disease on maize was recently observed in Iran. Blast symptoms on
86 maize were first observed in 2012 in Mazandaran province (Pordel et al. 2016) and spread to two

87 additional provinces in the following years. The fungus *Pyricularia* sp. was systematically
88 isolated from lesions. Our observations also revealed that barnyard grass (*Echinochloa* spp.),
89 present as a weed in maize fields or nearby, was infected by *P. oryzae* and had severe blast
90 symptoms as well. Here, we report on a new epidemic caused by *P. oryzae* on maize and present
91 evidence that the emergence of blast disease on maize in Gabon and Iran was caused by
92 independent events of host range expansion from the common weed barnyard grass to maize.
93
94 **Materials and methods**
95 **Fungal isolation**
96 Leaves of maize (*Zea mays*) with blast symptoms were collected in the Mazandaran, Gilan, and
97 Golestan Provinces of Iran, in August 2012 and 2013, and September 2016. All *Pyricularia*
98 isolates were obtained by isolating single hyphal tips emerging from germinating conidia (Pordel
99 et al. 2016). Newly sequenced genomes from seven isolates from Iran and one isolate from
100 Gabon were analyzed together with 56 published genomes and 19 additional unpublished
101 genomes from strains isolated from rice, ryegrass, and *Setaria* spp. In total, genomic data for
102 eighty-five strains from 14 different hosts were included in the study (Supplementary Table 1).
103
104 **DNA extraction, and microsatellite amplification**
105 For each strain, a paper stock of sporulating mycelium was deposited on rice flour medium
106 maintained in a climatic chamber for four to five days to obtain actively growing mycelium.
107 Freshly grown mycelia were inoculated in 6 ml of 2 YEG liquid medium (2 g of yeast extract, 10
108 g of glucose, 3 g KNO3, 2 g KH2PO4, 500,000 units of Penicillin, and 1 L of water). Cultures
109 were maintained four days in the dark at 25°C, before being collected for genomic DNA
110 extraction following a previously published protocol (Adreit et al. 2007). Strains were genotyped
111 using ten Single Sequence Repeat (SSR) markers (Supplementary Table 2), previously
112 developed for population genetics studies of *P. oryzae* (Kaye et al. 2003; Adreit et al. 2007). SSR
113 markers were amplified by PCR (QIAGEN Multiplex PCR kit) in a total volume of 5 µL,
114 including 2.5 µL of Master Mix, 0.5 µL of 10x Mix primers, 0.5 µL of 5xQ solution, and 1.5 µL
115 of genomic DNA (10 ng/µL). The PCR program was as follows: i) predenaturation at 95°C for
116 15 min, ii) denaturation at 94°C for the 30s, iii) hybridization at 57 to 63°C for 90s, iv) extension
117 at 72°C for 60s, v) repeat step 1 through 4 for 40 cycles, and vi) final extension at 95°C for 30

118 min. Amplicons were separated and analyzed on a 16-capillary ABI Prism 3130XL machine
119 (Applied Biosystems, Foster City, CA, USA) and the size of the amplicons was evaluated by
120 fluorescence measurement. For this analysis, 1.5 µL of amplified products (1/70 dilution) was
121 mixed with 15 µL formamide HiDi and GeneScan-500LIZ size marker (Applied Biosystems).
122 The raw data collected were then analyzed and converted to allele size with GENEMAPPER 4
123 software (Applied Biosystems). A network of the different multilocus genotypes was constructed
124 with the goeBURST algorithm implemented in Phyloviz 2.0 v (Nascimento et al. 2012).
125

126 **Whole-genome sequencing, and assembly**

127 Sequencing was performed on Illumina HiSeq 3000 sequencers and produced 150 nucleotide
128 paired-end reads with >50X depth. Low-quality reads were removed using the software Cutadapt
129 (Martin 2011). De novo assembly was performed with ABySS software (Simpson et al. 2019).
130 We evaluated the N50 value of the assembled sequences following different K-mers values and
131 chose the assembled sequences having the highest N50 value for further analyses.
132

133 **Protein-coding genes identification**

134 Genes of each assembled sequence of *P. oryzae* isolates were predicted using the BRAKER1 and
135 AUGUSTUS v 3.0.3 softwares (Hoff et al. 2015). Gene prediction with BRAKER1 used RNA-
136 seq data from different sources (Supplementary Table 3). Homology relationships among genes
137 predicted in the 85 genomes were identified using OrthoFinder v2.4.0 (Emms and Kelly 2019).
138 Population genomic analysis was based on a set of 7466 single-copy orthologs. Sequences for
139 each single-copy ortholog were aligned and cleaned with TranslatorX (Abascal et al. 2010) using
140 default parameters.
141

142 **Population genomic analysis**

143 Maximum likelihood trees were constructed using the RAxML v8.2.12 (Stamatakis 2014) with
144 the general time-reversible-gamma model and bootstrap support was estimated based on 100
145 replicates. SplitsTree v4.13 (Huson and Bryant 2006) analysis was conducted on the
146 concatenation of sequences at single-copy orthologs, excluding sites with missing data, sites with
147 gaps, singletons, and monomorphic sites, to infer a neighbor-net phylogenetic network and test
148 the null hypothesis of clonality using the PHI test. Population subdivision was analyzed using

149 Structure v2.3.4 (Falush et al. 2003; Hubisz et al. 2009), based on multilocus haplotype profiles
150 identified from ortholog alignments. Structure was run with 20,000 steps, following 10,000
151 burnin, with 12 repeats. The presence of one single clustering solution (i.e., a single clustering
152 mode) was checked visually across all 12 repeats for all *K* values.
153

154 **Comparative genomics of effectors**

155 To identify candidate genes potentially involved in host expansion, we used the table of
156 homology relationships produced using OrthoFinder (Emms and Kelly 2019). We searched for
157 groups of orthologs that are present in one lineage and absent or pseudogenized in another
158 lineage. To reduce the number of orthogroups to be analyzed, we first removed groups of
159 orthologs that were present in all lineages. Then, for the remaining groups of orthologs, we used
160 Blastn to confirm that the absence of a given ortholog in the assembly of a given isolate was not
161 caused by annotation error. All Blastn hits with an intron of less than 1000 bp, coverage greater
162 than 90% of the gene length and 80% identity were counted as present. Once this correction was
163 made, we looked for sequences that would have been pseudogenized by the insertion of a
164 transposable element. We used RepeatMasker and the Repbase database to generate an
165 annotation file of transposable elements and we used these annotation files to identify sequences
166 with insertion of transposable elements. Finally, we used the orthology table corrected for
167 annotation errors and pseudogenization by transposable elements to identify candidate genes as
168 genes being present in the lineage and absent or inactivated by insertion of transposable elements
169 in the other lineage.
170

171 **Mating type determination and attempts to induce the formation of the teleomorph**

172 The two primer sets designed by Tredway et al. (2005) for *MAT1-1* and *MAT1-2* amplification,
173 were used in a multiplex PCR to determine the mating type of maize and barnyard grass strains.
174 The reaction mixture had a total volume of 20 µL and contained 3 µL of genomic DNA (5–20
175 ng.µL^{-1}), 2/25X PCR buffer, 0.2 mM of each dNTP, 5 pmol of each primer, 5 mM MgCl2, and
176 2.5 units Taq polymerase. The initial denaturation step was done at 95°C for 3 min, followed by
177 35 cycles of 94°C (60 s), 60°C (60 s) and 72°C (60 s); a final elongation step at 72°C (10 min)
178 was included. The products were separated on a 0.8% (w: v) agarose gel and visualized by
179 ethidium bromide staining.

180 Male and female fertility was determined by *in vitro* crossing experiments. Sexual
181 competence assays consisted in pairing actively growing mycelia of field strains and fertile tester
182 strains (Br48, Mat1-1; Guy11, Mat1-2) on rice flour-agar medium. The plates were incubated at
183 25°C under 12 h dark/light cycles for three weeks. The mating type of field strains was
184 designated as the opposite of the tester strain with which perithecia were produced and female
185 fertility was declared when field strains formed perithecia (Nottéghem and Silue 1992). Male
186 fertility was declared when field strains induced formation of perithecia (Nottéghem and Silue
187 1992).
188
189 **Pathogenicity test**
190 Eleven maize strains and four barnyard grass strains were tested in greenhouse assays for
191 pathogenicity on wheat, (*Triticum aestivum* variety Thésée), rice (*Oryza sativa* variety
192 Maratelli), wild foxtail millet (*Setaria viridis*, Red-green foliage variety, from Graines Voltz
193 international, Colmar, France), barnyard grass (*Echinochloa crus-galli*) and maize (*Zea mays*
194 varieties B73, M017, and one hybrid line). Seeds of the different hosts were planted in 10-cm-
195 diameter plastic pots filled with compost. Six seeds were planted per pot. Pots were kept in the
196 greenhouse for 21 days and watered as needed to maintain the substrate wet. Plants were
197 fertilized with NPK 15:10:15 liquid fertilizer once a week. Strains were cultured on rice flour-
198 agar medium for 15 days at 25°C under a 12 h dark /12 h fluorescent light regime. Conidia were
199 scraped and washed with 3–5 ml of sterile distilled water. Conidia concentration was quantified
200 using a hemocytometer and adjusted to 2×10^5 spores.ml^{-1} and 0.5% gelatin for inoculation. Ten
201 ml of the conidia suspension were sprayed on four pots (one per plant species). Subsequently, the
202 plants were incubated for 24 h in a controlled climatic chamber at 24°C with 95% relative
203 humidity. Inoculated plants were then transferred back to the greenhouse and scored 7 days after
204 inoculation (Silue et al. 1992). Pathogenicity tests were repeated five times.
205
206 **Results**
207 **First report of epidemics of blast on maize and barnyard grass in Iran**
208 Blast epidemics in maize fields were observed in three geographical regions of Iran. Blast
209 symptoms on maize were first observed in August 2012 in Mazandaran (Pordel et al. 2016), then
210 in August 2013 in Gilan, and in September 2016 in Golestan provinces (Fig. 1). Blast symptoms

211 on plants of barnyard grass growing as weeds in the maize fields were also observed. Leaf spot
212 symptoms on maize initially appeared as grey lesions with a light margin and then expanded
213 rapidly to several centimeters in length and became lighter in color with a distinct brown margin.
214 Typical diamond shape symptoms with brown margin and yellow center were observed on
215 barnyard grass. Twelve and thirty-two *Pyricularia* sp. monosporic strains were isolated from
216 infected leaves of maize and barnyard grass, respectively, and characterized for some
217 morphological criteria commonly used for *Pyricularia* taxonomy (Ellis 1971; Klaubauf et al.
218 2014). Mycelium consisted of smooth, hyaline, branched, septate hyphae of 2–3 µm in diameter.
219 Conidiophores were solitary, erect, straight, or curved, septate, pale brown, 130-150 µm × 3-4
220 µm in size. Conidiogenous cells were sympodial, denticulate. Conidia were pale brown,
221 pyriform, 2-septate, 16-24 × 6-8 µm. Together, these morphological characteristics are
222 diagnostic of *Pyricularia oryzae* (Ellis 1971; Klaubauf et al. 2014).
223
224 ***P. oryzae* strains from maize and barnyard grass can cross infect**
225 Host range of strains isolated from maize and barnyard grass (hereafter named maize strains and
226 barnyard grass strains respectively) were assessed by spray inoculation of spore suspensions on
227 crops and weeds known to be host to *P. oryzae*. Control strains from wheat, rice, and foxtail
228 millet showed severe symptoms on their original host. In all pathogenicity tests, the strains
229 isolated from maize were highly pathogenic toward maize and the strains isolated from barnyard
230 grass (except IR0088) were highly pathogenic on barnyard grass (Table 1; Figure 2). Four and
231 three strains from maize did produce some lesions (three to seven) on one or two leaves of wheat
232 and foxtail millet, respectively. Maize strains did not produce any lesions on rice. Rice strains
233 were non-pathogenic on maize. Only two strains not originating from maize did produce lesions
234 on maize but these lesions were of a less severe type or fewer in number than those produced by
235 strains isolated from maize. Strains US0071 from foxtail millet and BR0032 from wheat
236 produced, respectively type 3 and type 2 lesions on maize (Table 1), but the lesions did not
237 produce spores when they were placed in conditions conducive to sporulation. The four isolates
238 from barnyard grass produced susceptible-type lesions on maize and two maize strains out of
239 three produced susceptible-type lesions on *Echinochloa crus-galli* (Table 1).
240
241

242 **Maize and barnyard grass strains belong to a new clade of *P. oryzae***
243 We used a population genomic approach to determine how the fungal pathogen isolated from
244 maize and barnyard grass relate to *P. oryzae* lineages infecting different cereals and grasses. We
245 extracted the predicted gene sequences from the assembled genomic sequences of eight *P. oryzae*
246 strains isolated from maize and barnyard grass and combined these data with previously
247 published (Gladieux et al. 2018) and newly generated (this study) genomic data from strains
248 originating from other cereal hosts. We identified 7466 groups of single-copy orthologous genes.
249 A maximum likelihood genealogy was inferred based on the concatenated sequences of single-
250 copy orthologs (292,056 bp in total) using RAxML v8.2.12 (Stamatakis 2014). In the inferred
251 genome genealogy, all maize and barnyard grass strains grouped together with maximum
252 statistical support (Figure 3A and B; Maximum likelihood bootstrap proportion, MLBP = 100%).
253 In addition, maize and barnyard grass strains formed a lineage that was distinct from the lineages
254 formed by strains isolated from other hosts (Figure 3A and B; MLBP = 89%).
255 To evaluate potential gene flow between and within clades and visualize evolutionary
256 relationships, we used phylogenetic network analysis, as implemented in the SplitsTree4
257 software (Huson 1998; Huson and Bryant 2006). The network inferred from haplotypes
258 identified using the 7466 single-copy orthologs in the 85 *P. oryzae* strains showed limited
259 reticulations along branches connecting this clade with other clades (Figure 3C), suggesting
260 limited gene flow between the maize/barnyard grass lineage and other lineages.
261 To further evaluate the possibility of admixture between *P. oryzae* strains from different
262 hosts, we conducted a Bayesian clustering analysis using the program Structure 2.3.4 (Falush et
263 al., 2003), based on haplotypes identified at 7466 single-copy orthologs, and modeling K=2 to
264 K=20 clusters. The log probability of data showed two modes at K=8 and K=14, and the ΔK
265 statistic presented modes at K=2 and K=6 (Supplementary Figure 1). Models with K>15 did not
266 show new clearly defined clusters (i.e. clusters in which some individuals have membership
267 proportions >0.8), therefore only models with K=2 to K=15 clusters are presented (Figure 3B).
268 Strain IR0088 isolated from barnyard grass separated from other strains isolated from maize and
269 barnyard grass at K=4. Strain GN0001 isolated from maize in Gabon displayed high membership
270 proportions (q>0.6) in the cluster grouping most strains from maize and barnyard grass at all K
271 values, but it also had substantial membership proportion (q>0.35) at higher K values (K>12) in a
272 cluster that was not represented in our dataset.

273 **Iranian *P. oryzae* populations from maize and barnyard grass are single mating type and**
274 **female-sterile despite signatures of recombination**
275 *P. oryzae* is a heterothallic fungus whose sexual compatibility is controlled by genes at the *MAT1*
276 locus, with sexual reproduction being possible only between strains of the opposite mating type
277 (Mat1-1 and Mat1-2). Three strains (IR0013, IR0015, and IR0095) from maize and four strains
278 (IR0088, IR0084, IR0083, and IR0102) from barnyard grass were of the same mating type. For
279 all these strains, PCR on genomic DNA amplified the 390-bp specific fragment of the *MAT1-2*
280 locus but did not amplify the *MAT1-1* specific marker. All maize and barnyard grass strains
281 induced formation of perithecia when crossed with Mat1-1 tester and they were thus considered
282 as male fertile. None of the maize and barnyard grass strains produced perithecia when crossed
283 with Mat1-1 tester and they were thus considered as female-sterile. Although these mating type
284 and fertility experiments suggest that these populations are asexual, PHI-tests based on levels of
285 homoplasy rejected the null hypothesis of clonality in the Iranian *P. oryzae* populations from
286 maize and barnyard grass ($P<0.0001$). The GN0001 strain from Gabon was Mat1-2 and female-
287 fertile, and the null hypothesis was also rejected with the PHI test when this strain was included
288 in the dataset. A neighbor-net network estimated from the dataset including the Iranian *P. oryzae*
289 populations from maize and barnyard grass and the Gabon strain from maize displayed
290 reticulations (Supplementary Figure 2).
291

292 **Maize and barnyard grass strains belong to two differentiated populations**
293 Genotyping with ten Simple Sequence Repeat markers showed that *P. oryzae* strains from maize
294 and barnyard grass belong to different populations. Genotyping of 11 maize strains from
295 Golestan and Mazandaran provinces and 26 barnyard grass strains from Golestan and Gilan
296 provinces generated 16 Multi-Locus Genotypes (MLGs). Strains isolated from maize and
297 barnyard grass had different MLGs. Maize strains were distributed in three MLGs (numbered 14
298 to 16) and barnyard grass strains were distributed in the 13 other MLGs (Figure 4). The five
299 maize strains collected in 2016 in Agh ghala in Golestan province had the same MLG (MLG 14)
300 whereas the 13 barnyard grass strains collected in the same area at the same time were
301 distributed in seven other MLGs.
302
303

304 **Candidate genes for host specificity**

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

314 **Discussion**

315 The emergence of *P. oryzae* on maize in Iran raises the question of the origin of maize strains.
316 Either the pathogen was introduced in Iran from foreign epidemic areas or it emerged locally
317 from another host species, through events of disease spillover, host-range expansion, or host
318 shift. Phylogenomic and population genomic analyses revealed that the recent emergence of
319 wheat blast in Bangladesh and in Zambia were caused by introductions of the pathogen from
320 South America (Islam et al. 2016) and from a yet undetermined origin (Tembo et al. 2020),
321 respectively. Since the blast disease of maize was previously reported in Gabon and Japan, a
322 hypothesis is that maize blast was imported in Iran from another country. This hypothesis can
323 only be tested for Gabon because genotypic, mating type and fertility data for maize strains from
324 Japan are not available and these strains were not conserved. The fact that GN0001 is female-
325 fertile and displays shared ancestry with an unsampled group in clustering analyses suggest that
326 *P. oryzae* populations on maize in Gabon could have been reproducing sexually. Unlike the
327 maize strain from Gabon, *P. oryzae* populations infecting maize in Iran do not show biological
328 features consistent with sexual reproduction (only one mating type, no female-fertile strains).
329 Iranian *P oryzae* populations infecting maize show a signature of recombination according to the
330 PHI test for clonality, but the test does not allow to conclude about the timing of recombination
331 (recombination can be contemporary or historical). If maize-infecting strains migrated from
332 Gabon to Iran, either only Mat1-2 strains were introduced or strains of both mating types were
333 introduced but only Mat1-2 strains established on maize as asexual populations. The loss of

334 sexual reproduction following migrations was already documented for rice-infecting populations
335 of *P. oryzae* (Saleh et al. 2014; Gladieux et al. 2018; Thierry et al. 2020).

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

355 Recent events of infection of a novel host provide valuable materials to study the genetic
356 determinants of host specificity. In such cases, comparative genomics is expected to lead to a
357 limited number of candidate genes because populations diverged recently. Since barnyard grass
358 strains are pathogenic to maize in controlled conditions, the adaptation to maize may be
359 governed by a few genes and host range expansion may have a limited fitness cost. In this study,
360 we identified four candidate genes that are differentially present/absent or potentially non-
361 functional between the maize and barnyard grass strains. We can speculate that the adaptation to
362 maize is due to the loss of an effector that was previously recognized by the plant and was
363 triggering defense mechanisms, i.e. to the loss of a so-called avirulence gene. Following this

364 scenario, genes absent/non-functional in maize strains and present in barnyard grass strains are
365 prime candidates as genetic determinants of adaptation to hosts. Among the four genes identified
366 in our comparative genomic analysis, a putative secreted protein and a protein kinase represent
367 interesting candidates. The vast majority of fungal avirulence cloned to date are secreted proteins
368 (Giraldo and Valent 2013). In *P. oryzae*, several avirulence genes were already shown to control
369 host specificity or are presumed to be involved in host shifts, with most of them having been
370 identified by genetic crosses (*PWL1* and *2*, *PWT1* to *5, Pfm1* and *2, eA1 to 5*) (Asuke et al.
371 2020). A limited number of genes involved in host shifts were cloned. An allele of *PWL2*
372 controls specificity on weeping lovegrass (*Eragrostis curvula*). It is absent in weeping lovegrass
373 infecting strains and triggers the plant defense mechanisms when present. *PWL2* encodes a
374 glycin-rich, hydrophilic, and secreted protein. Similarly, *PWL1* is absent in strains attacking
375 weeping lovegrass whereas it is present from closely related non-pathogenic strains (Asuke et al.
376 2020). Molecular plant pathology experiments and comparative genomics suggest that the loss of
377 *PWT3*, an avirulence gene recognized by the wheat resistance gene *Rwt3*, has favored the host
378 shift from ryegrass (*Lolium sp.*) to wheat (Inoue et al. 2017). The role in host specificity of the
379 candidate genes we identified need to be investigated by functional analysis. Interestingly, all the
380 candidate genes that we could identify *in silico*, are located in a narrow genomic area (25 kb) in
381 the reference genome of *P. oryzae* (strain 70-15). This clustering of candidate genes could be
382 indicative of a single event of insertion/deletion. Yoshida et al. (2016) identified the gain and
383 loss of genes as a major evolutionary mechanism responsible for the specialization of *P. oryzae*
384 to rice and *Setaria*.

385 **Acknowledgments**

386 We gratefully acknowledge the High Council for Research of the University of Tehran and
387 CIRAD institute for financially supporting and providing materials. SSR data used in this work
388 were produced through the GenSeq technical facilities of the « Institut des Sciences de
389 l'Evolution de Montpellier » With the support of LabEx CeMEB, an ANR "Investissements
390 d'avenir" program (ANR-10-LABX-04-01). This work was also funded by ANR Grant
391 MagMAX (ANR-18-CE20-0016) awarded to PG.

392

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547

548 **Figure Legends**

549 **Figure 1**: Schematic representation of sampling locations of *Pyricularia oryzae* on maize and
550 barnyard grass in Iran.

551 **Figure 2:** Blast symptoms caused by maize strain IR0013 on maize (A), and caused by barnyard
552 grass strain IR0083 on barnyard grass (B).

553 **Figure 3:** Population subdivision in *P. oryzae*. (A) Maximum likelihood tree based on the
554 concatenation of 7466 orthologous coding sequences extracted from 85 *P. oryzae* genome.
555 Nodes with bootstrap support >50% are indicated by dots (100 bootstrap replicates). (B)
556 Bayesian clustering of 85 *P. oryzae* strains isolated from different hosts (for *K* clusters between 2
557 and 15). Each strain is represented by a vertical bar divided in *K* segments of different colors,
558 indicating membership proportions in *K* clusters. (C) Neighbor-net network of haplotypes
559 identified based SNPs identified in 7466 single-copy orthologs in 85 *P. oryzae* genomes.

560 **Figure 4:** Genotype network of *Pyricularia oryzae* strains isolated from maize and barnyard
561 grass in Iran. Multi-locus genotypes were identified based on 10 SSR markers.

562

563 Supplementary Table 1: list of *Pyricularia* strains.
564 Supplementary Table 2: list of SSR markers and primers.
565 Supplementary Table 3: list of RNA-seq libraries used for genome annotation.
566 Supplementary Figure 1: Ln Probability of data (A) and ΔK statistic (B) as a function of the
567 number of clusters *K* estimated with the Structure software.
568 Supplementary Figure 2: neighbor-net network estimated with Splitstree4 from the dataset
569 including the Iranian *P. oryzae* populations from maize and barnyard grass and the Gabon strain
570 from maize.
571

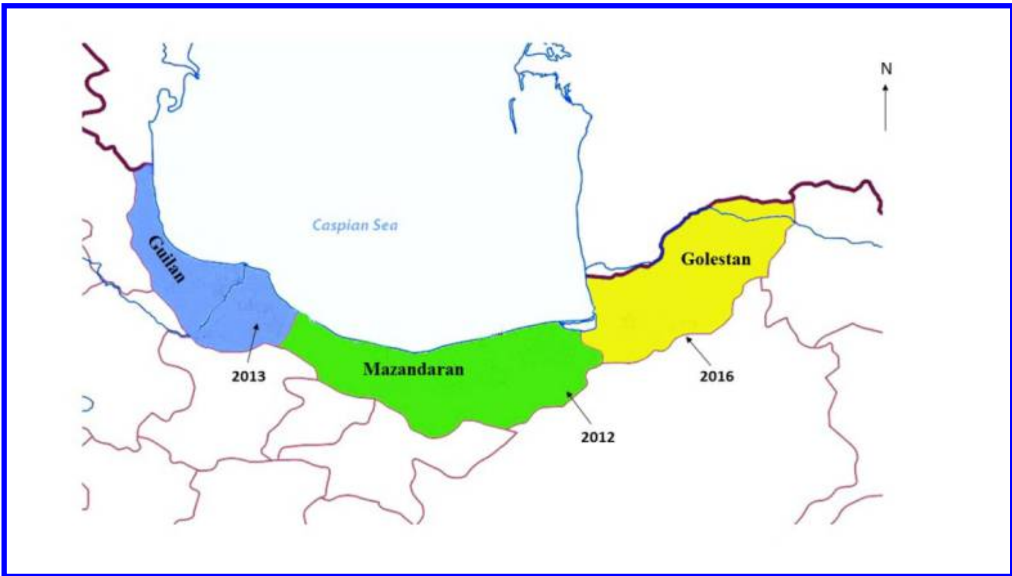
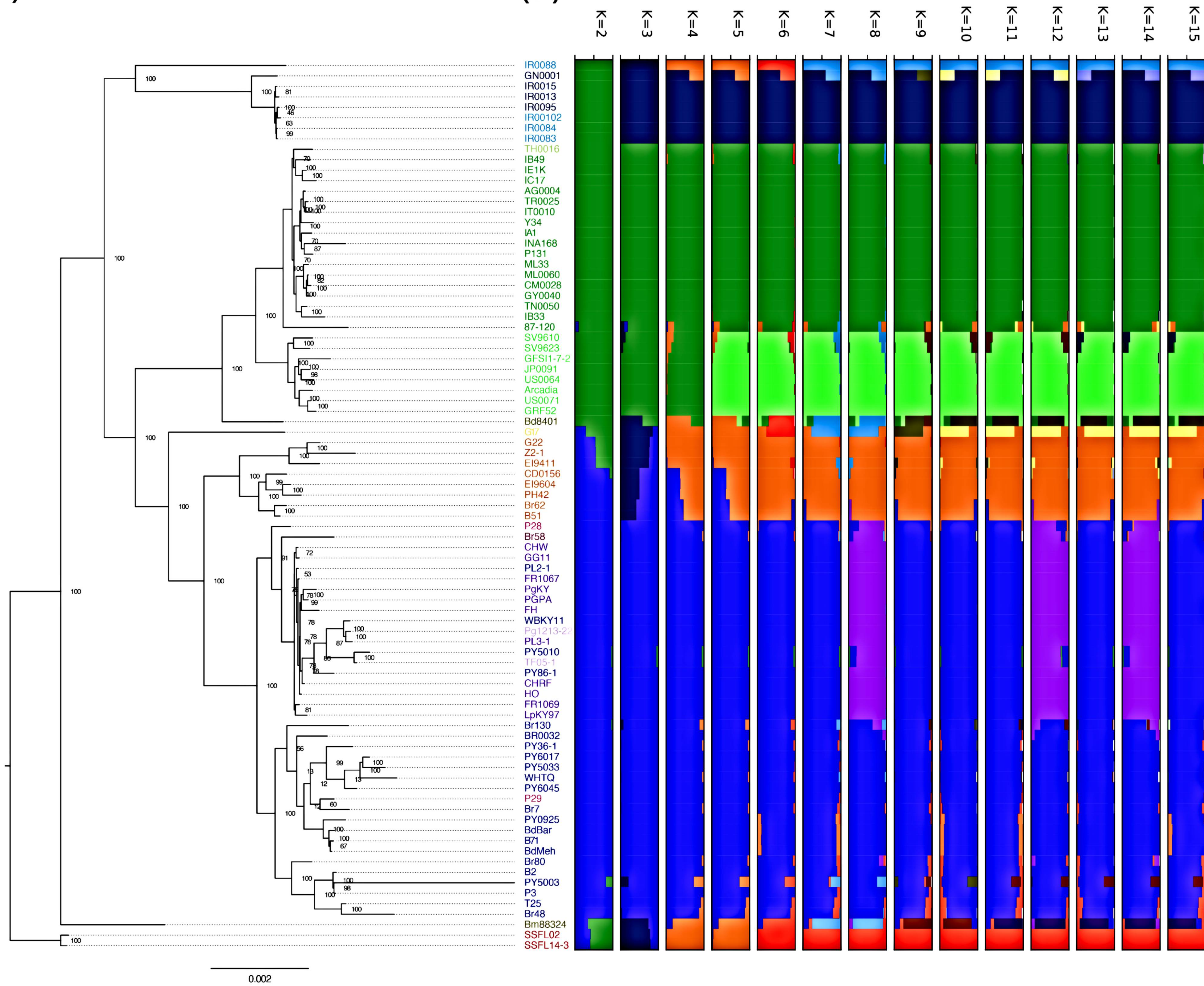


Figure 1: Schematic representation of sampling locations of Pyricularia oryzae on maize and barnyard grass in Iran.

338x190mm (96 x 96 DPI)

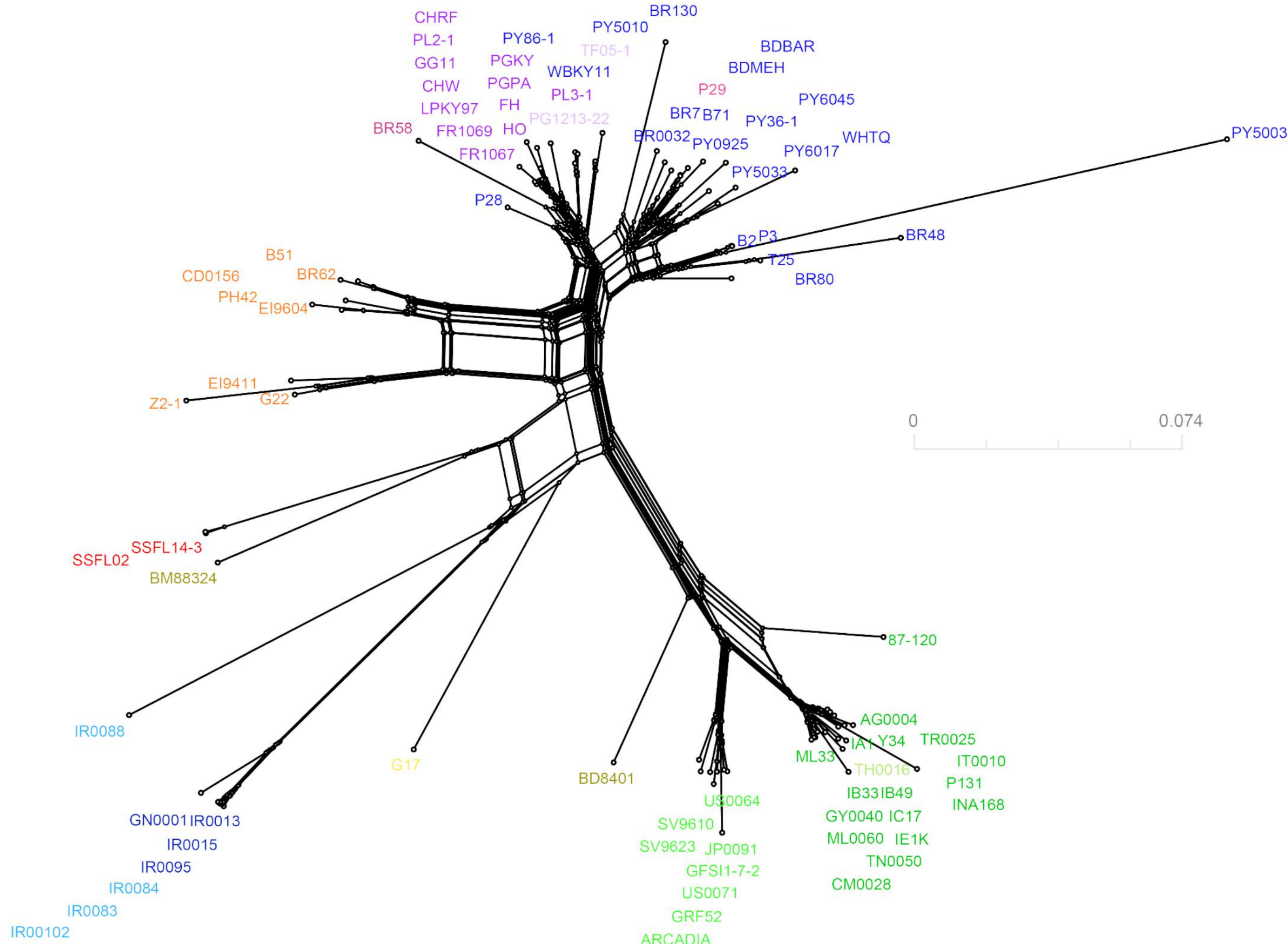
Figure 2: Blast symptoms caused by maize strain IR0013 on maize by (A), and caused by barnyard grass strain IR0083 on barnyard grass by (B).

183x151mm (96 x 96 DPI)



(A)
(B)
K=2
K=3
K=4
K=5
K=6
K=7
K=8
K=9
K=10
K=11
K=12
K=13
K=14
K=15
IR0088
GN0001
IR0015
IR0013
IR0095
IR00102
IR0084
IR0083
TH0016
IB49
IE1K
IC17
AG0004
TR0025
IT0010
Y34
IA1
INA168
P131
ML33
ML0060
CM0028
GY0040
TN0050
IB33
87-120
SV9610
SV9623
GFSI1-7-2
JP0091
US0064
Arcadia
US0071
GRF52
Bd8401
G17
G22
Z2-1
EI9411
CD0156
EI9604
PH42
Br62
B51
P28
Br58
CHW
GG11
PL2-1
FR1067
PgKY
PGPA
FH
WBKY11
Pg1213-22
PL3-1
PY5010
TF05-1
PY86-1
CHRF
HO
FR1069
LpKY97
Br130
BR0032
PY36-1
PY6017
PY5033
WHTQ
PY6045
P29
Br7
PY0925
BdBar
B71
BdMeh
Br80
B2
PY5003
P3
T25
Br48
Bm88324
SSFL02
SSFL14-3
0.002

(C)



Label colors

- Echinochloa
- Zea
- Hordeum
- Oryza
- Setaria
- Brachiaria
- Eragrostis
- Eleusine
- Bromus
- Triticum
- Lolium
- Festuca
- Avena
- Stenotaphrum

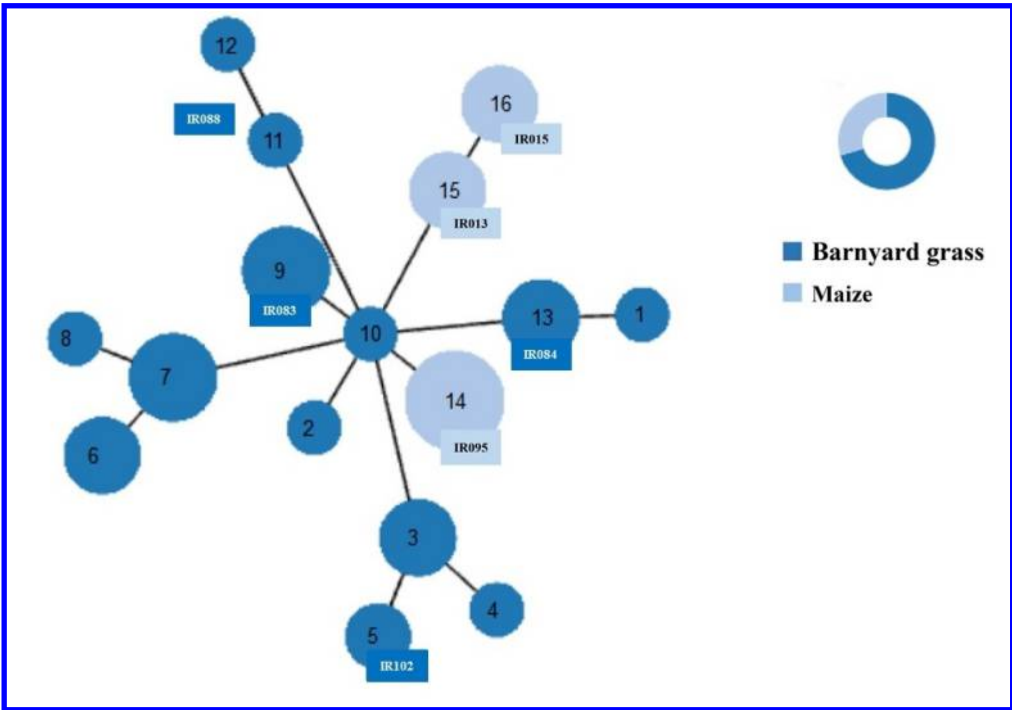


Figure 4: Genotype network of Pyricularia oryzae strains isolated from maize and barnyard grass in Iran. Multi-locus genotypes were identified based on 10 SSR markers.

271x188mm (96 x 96 DPI)

Table 1 Pathogenicity results of *P. oryzae* strains on maize, barnyard grass, rice, wheat, and wild foxtail millet

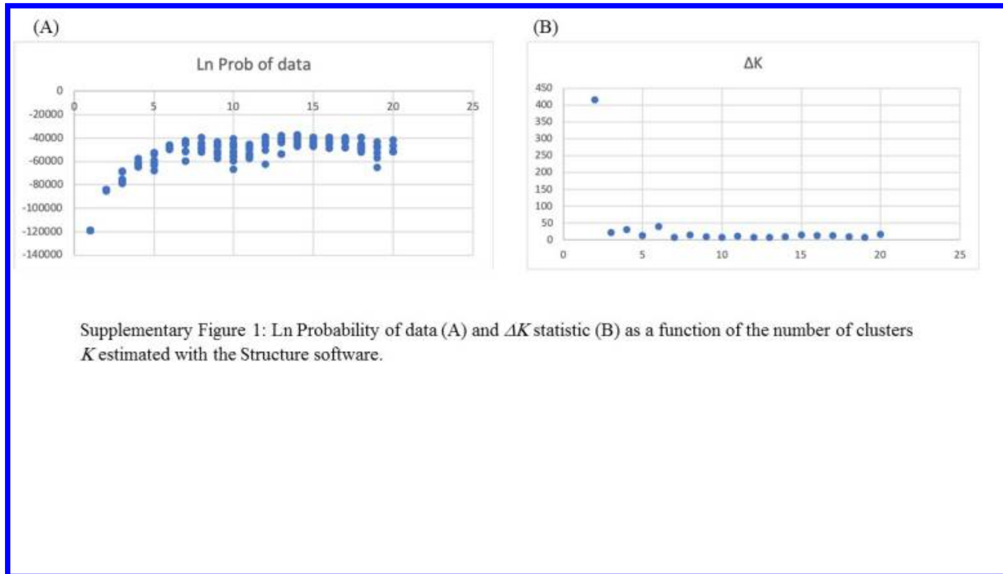
Fungal strain	Host of origin	Locality (Province/City)	Year	*Z. mays*	*E. crus-galli*	*O. sativa*	*T. aestivum*	*S. viridis*
IR0010	*Z. mays*	Mazandaran/ Gharakhil	2012	6	-	1	1	1
IR0012	*Z. mays*	Mazandaran/ Gharakhil	2012	6	1	1	3	1
IR0013	*Z. mays*	Mazandaran/ Gharakhil	2012	6	6	1	1	1
IR0014	*Z. mays*	Mazandaran/ Gharakhil	2012	6	6	1	2	2
IR0015	*Z. mays*	Mazandaran/ Gharakhil	2012	6	-	1	1	1
IR0016	*Z. mays*	Mazandaran/ Gharakhil	2012	6	-	1	3	2
IR0093	*Z. mays*	Golestan/ Agh-Ghala	2016	6	1	1	1	1
IR0094	*Z. mays*	Golestan/ Agh-Ghala	2016	6	-	1	1	1
IR0095	*Z. mays*	Golestan/ Agh-Ghala	2016	6	1	1	1	1
IR0096	*Z. mays*	Golestan/ Agh-Ghala	2016	6	-	1	1	1
IR0084	*E. crus-galli*	Golestan/ Agh-Ghala	2016	6	6	1	1	-
IR0083	*E. crus-galli*	Golestan/ Agh-Ghala	2016	6	6	1	1	-
IR0102	*E. crus-galli*	Golestan/ Azad shahr	2016	6	6	1	1	-
IR0088	*E. crus-galli*	Golestan/ Agh-Ghala	2016	5	1	1	1	-
GN0001	*Z. mays*	Gabon	1985	6	-	1	1	3
GY0011	*O. sativa*	French Guyana	1988	1	-	6	3	1
BR0032	*T. aestivum*	Brazil	1991	3	-	2	6	1
US0071	*S. viridis*	USA	ND*	3	-	1	1	6

Lesion types are as follows: -, not tested; 1, no signs of infection; 2, small brown lesions; 3, small lesions with yellow centers and brown margins; 6, large diamond-shaped lesions (Silue, et al., 1992). * ND, no data.

Table 2: Candidate genes for host specificity identified by comparative genomics between maize and barnyard grass strains of *P. oryzae* and with other hosts.

				maize				barnyard grass				Setaria	rice	wheat
Orthologous group	**Gene in reference genome**	**Position in ref. genome**	**Putative function**	**IR0013**	**IR0015**	**IR0095**	**GN0001**	**IR0083**	**IR0084**	**IR0102**	**IR0088**	**US71**	**GY11**	**BR0032**
OG0000055	MGG_02051T0	1:392967-395203	CAMK protein kinase	+	+	+	+	-	-	-	INS G	-	-	-
OG0010704			Kinase domain, Secreted P	+	+	+	-	-	-	-	-	INS G	+	INS G
OG0010727	MGG_16022T0	1:410855-411153		+	+	+	+	-	-	-	INS P	+	+	-
OG0011065	MGG_02056T0	1:414777-417691	Secreted P	-	-	-	INS G	+	+	+	+	+	+	-

- absent, + present, INS present but with insertion of a transposable element or a simple sequence repeat in the gene (G) or in 500 upstream region (P).



Supplementary Figure 1: Ln Probability of data (A) and ΔK statistic (B) as a function of the number of clusters K estimated with the Structure software.

338x190mm (96 x 96 DPI)

0.0010
IR00102
IR0083
IR0084
GN0001
IR0095
IR0015
IR0013

Supplementary Table 2. Primers and polymorphism of the 10 Simple Sequence Repeat (SSR) markers used for genotyping (Adreit, et al., 2007)

Name	Chromosome	Primers	Number of alleles[a]	Size (pb)[b]
Pyrms63	1	F:TTGGGATCTTCGGTAAGACG R: GCCGACAAGACACTGAATGA	3	149-153
Pyrms37	4	F:ACCCTACCCCCACTCATTTC R: AGGATCAGCCAATGCCAAGT	4	209-217
Pyrms657	6	F:ATCAGTCGAACCCACAAAGC R: ATGTGTGGACGAACCAGTCC	2	162-164
Pyrms607	3	F:CCCAAGCTCCATAATACGCTAC R:TCCGAGACTCTTTGGATAGCAC	4	242-296
Pyrms233	5	F:TGAGATGGACCGCATGATTA R: TTGATGGCAGAGACATGAGC	2	241-250
Pyrms47	4	F:TCACATTTGCTTGCTGGAGT R: AGACAGGGTTGACGGCTAAA	7	195-237
Pyrms77B	3	F:AGGCTCTCTGCCTACGAAGT R: GCTTTCGGCAAGCCTAATC	2	218-226
Pyrms83B	2	F:GTCTGCCTCGACTCCTTCAC R: GCAAAGTTGTTTGAGCAAGG	2	97-103
Pyrms99B	5	F:CACCACTTTATGGCGCAGT R: ACCTAGGTAGGTATACATGTTGTT	2	218-226
Pyrms409	4	F: TCCCAGTACTTGCCCATCTC R: ATCTCATATCCGTCGGTCGT	3	295-351

[a] Number of alleles and [b] size range of alleles in the sample.