Pyricularia are Mostly Host-Specialized with Limited Reciprocal Cross-Infection Between Wheat and Endemic Grasses in Minas Gerais, Brazil

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Abstract

Wheat blast, caused by *Pyricularia oryzae* Triticum (PoT), is an emerging threat to global wheat production. Current understanding of the population biology of the pathogen and epidemiology of the disease has been based on phylogenomic studies that compared the wheat blast pathogen with isolates collected from grasses that were invasive to Brazilian wheat fields. In this study, we performed a comprehensive sampling of blast lesions in wheat crops and endemic grasses found in and away from wheat fields in Minas Gerais. A total 1,368 diseased samples were collected (976 leaves of wheat and grasses and 392 wheat heads) which yielded a working collection of 564 *Pyricularia* isolates. We show that, contrary to earlier implications, PoT was rarely found on endemic grasses and, conversely, members of grass-adapted lineages were rarely found on wheat. Instead, most lineages were host-specialized with constituent isolates usually grouping according to their host-of-origin. With regard to the dominant role proposed for signalgrass in wheat blast epidemiology, we found only one PoT member in 67 isolates collected from signalgrass grown away from wheat fields, and only three members of *Urochloa*-adapted lineages among hundreds of isolates from wheat. Cross-inoculation assays on wheat and a signalgrass used in pastures (*U. brizantha*) suggested that the limited cross-infection observed in the field may be due to innate compatibility differences. Whether or not the observed level of cross-infection would be sufficient to provide an inoculum reservoir, or

serve as a bridge between wheat growing regions, is questionable and, therefore, deserves further investigation.

The ascomycete *Pyricularia oryzae* (anamorph), formerly *Magnaporthe oryzae*, and sometimes confused with *Magnaporthe grisea,* is one of the most studied and economically important fungal plant pathogens worldwide (Dean et al. 2012). It is the cause of diseases in commercial crops including rice blast (Valent and Chumley, 1991), wheat blast (Couch and Kohn, 2002) and gray leaf spot in annual and perennial ryegrasses (Farman, 2002). The disease is a current threat to the cultivation of wheat on three continents: South America, Asia, and Africa. First reported in 1985 in the state of Paraná, Brazil (Igarashi et al. 1986), wheat blast spread to all major Brazilian wheat-producing regions (Ceresini et al. 2018; Goulart et al. 1990), and to neighboring countries including Paraguay, Bolivia, and Argentina (Barea and Toledo, 1996; Cabrera and Gutierrez, 2007; Cazal-Martínez et al. 2021). International attention has been raised after the discovery of wheat blast in Bangladesh, south Asia (Malaker et al. 2016) and, more recently, in Zambia, Eastern Africa (Tembo et al. 2020).

Wheat blast epidemics occur more frequently in the tropics where significant yield losses have been associated more often with symptoms on the heads than on the leaves (Cruz and Valent, 2017). In fact, leaf blast sporadically occurs in Brazilian wheat fields when warm and wet weather during the early season might favor infection and inoculum build-up on young leaves (Cruz et al. 2015). The first detailed report of yield losses due to wheat blast was estimated at around 27% in Brazil (Goulart et al. 1990), but greater losses, nearing 100%, have also been reported (Coelho et al. 2016; Dianese et al. 2021; Goulart and Paiva, 2000; Santos et al. 2022; Trindade et al. 2006). In the first major epidemics in Bangladesh, in 2016, the disease caused losses in more than 15,000 ha, which resulted in complete destruction of some affected fields (Islam et al. 2016; Malaker et al. 2016).

P. oryzae as a species comprises a large number of phylogenetically distinct groups, or lineages, some of which exhibit fairly strict host-specificity, with very little evidence of cross-infection by non-member isolates. These include the lineages found on *Oryza (P. oryzae Oryza*, PoO*), Setaria* (PoS*), Stenotaphrum* (PoSt*),* and *Eleusine* (PoE) (Gladieux et al. 2018; Latorre

et al. 2020). In contrast, the *Triticum*-specialized lineage (PoT), and the related *Lolium* pathogens (PoL1), among others, should be more accurately defined as host-specialized because, although these are mostly found in association with their eponymous hosts, they can also be found on other *Poaceae* species (Kato et al. 2000; Tosa et al. 2004; Tosa and Chuma, 2014; Urashima et al. 1993). The ability of the wheat blast pathogen to infect additional cereal crops, as well as forage and turf grasses, is important when thinking about disease development and epidemic spread. This is because alternative hosts often occur in proximity to wheat fields and may occupy large geographical areas, either due to their invasive nature, or their widespread use as forage.

There have been some studies on the population structure and genetic diversity of wheat blast in Brazil and its relationship to isolates found on surrounding grasses (Castroagudín et al. 2017; Castroagudín et al. 2016; Maciel et al. 2014, 2023). In 2012, Ceresini and coworkers collected a large number of wheat blast isolates from more than ten locations in seven states of Brazil. At the same time, they sampled *P. oryzae* from grasses bordering the wheat fields, as well as isolates from rice production areas (Castroagudín et al. 2016). These studies revealed a strong phylogenetic relationship between isolates from wheat and certain grasses, and a distinct separation from PoO, which led to the proposition of a new species, *Pyricularia graminis tritici* (Pygt) (Castroagudín et al. 2016), although this has been questioned (Valent et al. 2019). Subsequent studies implied there was significant evidence of gene flow between the wheat blast and grass-infecting isolates (Castroagudín et al. 2017), prompting speculation that wheat blast undergoes mating on endemic grass species, thereby increasing genetic diversity within the blast population (Ceresini et al. 2018, 2019). Lastly, it was suggested that isolates causing wheat blast showed a particularly close taxonomic affinity with isolates from signalgrass (*Urochloa* spp.) - a widely grown forage crop in Brazil - promoting the hypotheses that wheat blast evolved via a host jump from *Urochloa* (Stukenbrock and McDonald, 2008); and that *Urochloa* serves as a key inoculum reservoir, and a "bridge" facilitating gene flow between separate wheat growing regions (Ceresini et al. 2018, 2019).

However, as noted in the accompanying paper (Farman et al. 2022), when the fungal isolates used by Ceresini and colleagues were analyzed in a broader phylogenetic framework, this revealed that the foregoing studies had not actually sampled the endemic grass-infecting pathogens because the isolates from grasses were PoT and PoL1 lineage members - probably from opportunistic infections on grasses invasive to wheat crops. Moreover, a preliminary

survey based on genome sequencing of a sample of grass-infecting isolates collected at varying distances away from wheat fields suggested that PoT is rarely found on endemic grasses. This latter finding motivated the present study where we sought to characterize the endemic grass-infecting lineages in the Cerrado region of Minas Gerais (MG) state with the specific goals of testing the following hypotheses: 1) Infection of endemic grasses, and especially signalgrass, by the wheat-infecting (PoT) lineage is mostly restricted to plants in and around wheat fields, where wheat blast inoculum densities are highest; 2) fungal isolates that typically infect native grasses are rarely found on wheat; and 3) signalgrass/wheat does not support effective colonization of plant tissue by PoT/non-PoT lineage members. To test these hypotheses, we comprehensively sampled *P. oryzae* from wheat fields and from grasses growing at varying distances from wheat-growing locations. PCR assays and genotyping-by-sequencing were then performed to identify isolates down to species and lineage levels, thereby providing an accurate insight into the relationship between fungal populations infecting wheat and grasses. A particular focus was placed on populations infecting signalgrass to re-evaluate the hypothesis that they play a major role in wheat blast epidemiology.

Materials and Methods

Study area and sampling

Surveys were conducted in wheat-growing regions and natural landscapes of MG state during the 2018 and 2019 growing seasons. While wheat blast was found only on the heads of wheat crops in 2018 (n = 4 wheat fields), it occurred both on leaves and heads of wheat in 2019 (n = 11 wheat fields). The sampling target and design varied according to the timing of sampling and whether the site was a wheat or non-wheat area (Fig. 1). The pre-season sampling in mid-February (summer season in MG) targeted grass weed hosts which were collected randomly by visiting natural landscapes along roadsides and in off-season wheat areas (Fig. S1). Each sample comprised five to ten leaves, which were placed into paper bags and placed at room temperature (23°C ±4°C) to dry for one week before being stored at 10°C. Mid-season sampling in mid to late May (Fall season) focused on collecting: a) blast-symptomatic leaves (only in 2019) and heads (2018 and 2019) in wheat fields and b) blast-symptomatic leaves of grass weeds either within or near to wheat growing areas. For the wheat blast samples, five to 10 (depending on field size) 50-m transects placed 200 m apart were randomly defined. At least

one sample (five to ten leaves or five heads) was collected at each transect, similar to a previous study (Maciel et al. 2014). Weed species were identified morphologically based on the literature (taxonomic guides) (Lorenzi, 2014). The wheat varieties could not be identified. All natural landscapes, wheat commercial fields, and individual plants in the field were photographed using a smartphone camera (72 dpi resolution). In the laboratory, photographs of the symptoms, on leaves or heads (in the case of wheat), were obtained using a smartphone camera and a digital magnifying miniscope (10X, 96 dpi resolution) (Fig. 2S).

Culturing, purification and storage

Wheat heads (one per sample) and leaves (five per sample) of wheat or grass weeds, were cut into small pieces and placed within a 9 cm-plastic dish filled with moistened filter paper, and incubated for 24 h at 25°C ±5 under a 12/12 h photoperiod (light/darkness) to induce sporulation (Urashima et al. 2017). Under the stereomicroscope light, conidiophores and associated sparkling crystal-clear spore mass on leaf and head-rachis could be visualized. A sterilized sealed Pasteur pipette was scraped over the sporulating mass and streaked across the water agar supplemented with chloramphenicol and streptomycin, each at 100 µg/ml. Plates were incubated at 25±5°C for 24 h (12/12 h fluorescent light/darkness) (Farman et al. 2017). For each culture, a single biseptate, pyriform conidium (Klaubauf et al. 2014; Murata et al. 2014) with a visible germ tube was transferred to oatmeal agar (OA) (30 g oats, 20 g agar, 1 L distilled water), and pieces of sterilized filter paper (10 mm x 0.4 mm) were placed nearby. The dishes were incubated as above for 7 d until the mycelium fully covered the filter paper. The papers were then transferred to a new Petri plate filled with blue silica crystals and left to dry at room temperature (25°C±5) for 5 d. Dried paper pieces were transferred to a 2 ml-microtube half-filled with fresh sterile blue silica and stored in a -10°C freezer (Farman et al. 2017). Isolates were stored in duplicates as a backup of the entire collection.

Growth of *Pyricularia* spp. and DNA extraction

A single filter paper of each isolate was placed on a potato dextrose agar and incubated at 25±5°C under 12/12 h photoperiod (fluorescent light/darkness). A 6 mm mycelial block from a 5-day-old colony was then transferred to a 50 mL falcon tube filled with 20 mL of liquid complete medium (6 g casamino-acids, 6 g yeast extract, and 10 g sucrose per 1 liter). The tubes were shaken for 7 days at 150 rpm under room temperature (23-26°C) and ambient light. The mycelium was recovered through two layers of cheesecloth and let drying at an ambient

161 temperature for 3 h, and freeze-dried in 2 mL microtubes for 24 h (M. Farman et al. 2017;
162 Urashima et al. 2017) using a CoolSafe Freeze Dryer (SCANVAC). The mycelium ball was
163 manually crushed against the microtube wall until it formed a powder, which was then
164 resuspended in 1 mL lysis buffer (100 mM Tris-HCl, pH8; 0.5 M NaCl, 10 mM EDTA; 1 %
165 SDS) and heated 65°C for 30 min. Adding 700 µl phenol:chloroform:isoamyl alcohol (25:24:1)
166 and heated 65°C for 30 min. Subsequently, centrifuged at 14000 rpm for 15 min, and carefully
167 transferred 0.8 µl of aqueous phase to a new identified microtube, where was added 450 µl of
168 cool isopropanol and centrifuged at 14,000 rpm for 10 min to pellet the DNA. The supernatant
169 was carefully discarded and the pellet was washed with 1 mL of 70% ethanol, and re-pelleted
170 by centrifuging for 5 min at 14,000 rpm. The supernatant was discarded and the DNA was
171 dried at room temperature for 60 min, redissolved in 100 µl TE + 2 µl RNAse A (1 µg/ml), and
172 stored at 4°C overnight, before being placed in the -20°C freezer (Farman et al. 2017). The
173 DNA concentration was estimated using a spectrophotometer NanoDrop 2000 (Thermo
174 Scientific™) and adjusted to 100 ng/µl using TE buffer.

175 **PCR assays targeting *P. oryzae***

176 The entire collection of 572 isolates was first screened by using PCR to amplify the CH7-
177 BAC9 locus, which is present in *P. oryzae* (Po) but absent in other *Pyricularia* (non-Po),
178 including *P. grisea, P. pennisetigena*, or *P. urashimae* (Couch et al. 2005). The primers used
179 were F: TGTAAGAAGCTCGGTGACTGAT and R: AGTGTTGCTTGAACGGCTAA and
180 produce products of ~300 bp depending on microsatellite length. The assays were performed
181 using 1 µl of genomic DNA (100 ng/µl) and primer concentrations of 10 µM, with the GoTaq®
182 Colorless Master Mix, according to the manufacturer's specifications (Promega). Reactions
183 were carried out in a MyGene™ thermal cycler (Model MG96G), with the following
184 parameters: an initial denaturation at 95° for 8 min, followed by 35 cycles of 95°C for 15 sec,
185 55°C for 20 sec, 72°C for 60 sec, and a final extension at 72°C for 5 min (Couch et al. 2005).
186 To confirm the accuracy of CH7-BAC9 for discriminating Po from non-Po, the MPG1 locus
187 was amplified and sequenced for all PCR-negatives and select positives. . PCR assays were
188 performed using the primers F: AGATCCCCATCGACGTTCTC; and R:
189 TCCCTCACAGAAACTCCAAAC (product length, ~380 bp), and 1 µl of genomic DNA
190 template (100 ng/µl) The same GoTaq® Mix and thermal cycler were used for amplification
191 of MPG1 with the following parameters: initial denaturation at 95° for 8 min, followed by 35
192 cycles of 95°C for 15 sec, 55°C for 20 sec, 72°C for 60 sec, and a final extension at 72°C for

5 min (Couch et al. 2005). PCR products were cleaned up with ExoSAP-ITTM according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA) and 1 µl was used for sequencing with BigDye v3.1 (Thermo Fisher) in a total volume of 10 µl (2 µl 5X buffer; 1 µl primer (3.2 pmol); 0.5 µl BigDye mix; 5.5 µl H2O). The sequence of this gene was used for phylogeny analysis to identify Pyricularia at the species level (Couch et al. 2005).

PCR assays targeting *P. oryzae* Triticum lineage

To distinguish *P. oryzae* Triticum lineage members (PoT) from non-PoT, we used the MoT3 primer set (F: GTCGTCATCAACGTGACCAG; R: ACTTGACCCAAGCCTCGAAT) that yields a 362 bp amplicon (Pieck et al. 2017). For C17 diagnostics (Thierry et al. 2020), we designed a new primer set for a modified (standard PCR) assay that identifies PoT based on the positive amplification of a 500 bp fragment (F: GAGGAAGATCAAGTAAGTGG; R: GGTAGATGTCATGATTTCAC). Here, it is important to note that while these two loci were selected for the specific purpose of identifying PoT (MoT), neither is truly diagnostic because both loci were contributed to the PoT lineage via admixture (Rahnama et al. 2021). MoT3 was donated by a *Urochloa* pathogen from the PoU3 clade (a subgroup of the PoSt lineage) and, therefore, tests positive with certain isolates from *Urochloa.* Likewise, C17 was contributed to PoT by a lineage that is related to rice pathogens, but has not yet been sampled from the field ("PoX"). For this reason, tentative lineage designations (PoT or non-PoT) were made according to a specific schema (Table 1) and, where necessary, sequencing of the CH7-BAC9 and MPG1 loci, and genotyping by sequencing were performed to validate the assignments.

Assays were performed using 10 ng template and the same GoTaq® mix. Cycling conditions were as follows: MoT - initial denaturation at 95°C for 8 min, followed by 35 cycles of 95°C for 15 sec, 55°C for 20 sec, 72°C for 60 sec, and a final extension at 72°C for 5 min; C17 - initial denaturation at 94°C for 2 min, 35 cycles of 95°C for 10 sec, 54°C for 30 sec, 72°C for 30 sec, and a final extension at 72°C for 5 min. PCR products were separatedby electrophoresis in a 1%-agarose gel for 100 min at 80 Volts, 100 mA, and 80 watts, using a 1 Kb DNA ladder (Cellco®). The DNA was stained with GelRed® and the gel was visualized and photographed under ultraviolet (UV) light.

Phylogenetics of CH7BAC9 and MPG1 sequences.

Forward and reverse reads were assembled into single sequences using CAP3 and ambiguous nucleotide positions were either manually clipped off (at the end of sequences), or converted

to unknown bases. Equivalent sequences were mined from genome assemblies (Table S1) using a custom script. Briefly, CH7BAC9 and MPG1 were aligned with each genome using BLAST, SNPs were called using a module from iSNPcaller, and the query sequence was converted to match the SNP calls for each genome. The two datasets were concatenated, lineage assignments were added to the header lines, and the sequences were then aligned using MUSCLE. Phylogenetic analysis was then performed using RAxML-NG (Kozlov et al. 2019) with the GTR gamma model and 1,000 bootstrap replications. The "best" tree, as determined by RAxML, was then plotted using ggtree (Yu et al. 2017) using a custom script.

Targeted-sequencing using the Hi-Plex approach

Genomic DNA at a volume of 20 µl and roughly 20 ng/ul was sent to Floodlight Genomics LLC (Knoxville, USA) for amplification using a custom panel of PCR primers. A total of 84 target regions were originally designed for detection of a pandemic wheat blast lineage Latorre et al. (2023) and the organismal primers were designed using the online Batch Primer 3 at the default settings for 'general' primers and pooled in equal amounts and amplified and processed as described previously (Nguyen-Dumont et al. 2012). The Hi-Plex process adds a unique 12bp barcode during the amplifications and thus all PCR products can be pooled for sequencing using a single Illumina dual-indexed library. Floodlight Genomics provided the amplifications and coordinated the library construction and sequencing of the resulting pooled barcoded amplicons on an Illumina Hi-SeqX device at Admera Health LLC (Plainfield, NJ) for free as part of its Educational and Research Outreach Program (EROP). Library construction and sequencing were accomplished according to the manufacturer's specifications. The sample-specific raw data was made available via a secure link as FASTQ files and processed further for mapping, identification of polymorphic loci and assessment of genotypes.

Phylogenetic analysis using genotyping-by-sequencing data

Raw sequence reads (one dataset per isolate) were aligned with the rice blast reference genome (strain 70-15, version 8; NCBI accession GCA_000002495.2) using bowtie2 (Langmead and Salzberg 2012). Variants were called using the Genome Analysis Toolkit (GATK, version 4.1.4.1, McKenna et al 2010) and fasta files were then generated using a custom script which

called variants using the following criteria: the alternate (alt) allele was called if it was supported by a minimum of 7 reads, with no reads supporting the reference (ref) allele, or if the alt/ref ratio was > 7. Ref alleles were called if no variants were identified but read coverage across the targeted sites was ≥ 5. Novel variants not previously targeted in the original assay but identified in the present study were added to the dataset if they had > 100,000-fold coverage across all samples. Next, we used a custom script to retrieve the relevant nucleotide positions from each genome assembly. Briefly, we generated an alignment map between the 70-15 and B71 genome (NCBI accession GCA_004785725.2) assemblies. Then we used a custom script to interrogate existing SNP data generated using comparisons between B71 and the other genomes to determine if each genome had the B71 allele or an alternate allele at each of the relevant nucleotide positions. The two datasets were concatenated, lineage assignments were added to the header lines, and phylogenetic analysis was then performed using RAxML with the GTRgamma model and 1,000 bootstrap replications. The "best" tree, as determined by RAxML, was plotted using ggtree with a custom script.

Lineage assignment

Consensus lineage assignments were made using different criteria and took into account the host-of-origin, established patterns of marker distribution among the different host-specialized lineages, as well as known population structure. For example, if an isolate came from a host that typically harbors one or more specific lineages, and those lineages have not yet shown any evidence of admixture, sequence data for a single marker (CH7BAC9 or MPG1) allowed a confident assignment. For others - especially isolates from lineages with higher than normal cross-infection behavior, or known admixture - multi-locus genotyping was necessary.

Cross-inoculation assays

A subcollection of 20 strains isolated from wheat (n = 11, being all PoT) or signalgrass (n = 9, being six non-PoT [three PoU and two Pu] and three PoT) was studied with regards to aggressiveness towards leaves and heads of two wheat cultivars of varying resistance spectrum to wheat blast (BR 18-Terena = moderately resistant and BRS Guamirim = susceptible) and leaves of one *Urochloa brizantha* cultivar (cv. Marandu) (Table 2). Among the PoT isolates, a reference isolate (16MoT001), used as standard for aggressiveness in screening for host resistance (Cruppe et al. 2020), was included for comparison. The inoculations on the leaves were conducted on 35-day-old plants exhibiting three to four completely expanded leaves,

growth stage 15 (Zadoks et al. 1974). Inoculations on the heads were performed in 60-day-old plants at early anthesis, growth stage 60 (Zadoks et al. 1974). Each experiment (inoculation on leaves or heads) was conducted twice under greenhouse conditions between March and September 2020.

Inoculum production. For each isolate, a piece of filter paper containing the fungus was removed from the -10°C storage and re-activated on Potato Dextrose Agar (PDA). A 5-day-old mycelial plug was transferred to oatmeal-agar (OA) (replicated in five 9 cm-dishes per isolate). The fungus was cultured for seven days. To induce fungal sporulation, plates were scraped out using a Drigalski spatula and 5 ml of sterilized-distilled water. The dishes were incubated for a further seven days. Spores were harvested by adding 10 ml of distilled-sterilized water amended with 0.01% Tween-20, and carefully scraped using a Drigalski spatula. Spore suspension was filtered through two layers of cheesecloth. Spore concentration was adjusted to $1x10^5$ spores/mL using a Neubauer counting chamber. PDA and OA dishes were both supplemented with chloramphenicol and streptomycin at 100 µg/ml. Incubation was performed in a grown chamber with controlled temperature of 25°C (±2°), and photoperiod of 12/12 hours (fluorescent light/darkness) (Cruz et al. 2016; Urashima et al. 2017).

Plant growth conditions. The plants were sown in 2-L plastic pots filled with substrate (Tropstrato - Vida Verde) which was a mixture of pine bark, peat, and expanded vermiculite. Basal fertilization was performed with monoammonium phosphate (12% N and 50% P_2O_5). The number of plants per pot was reduced to eight and ten for wheat and signalgrass, respectively. Plants were kept in the greenhouse under controlled environmental conditions (±11 hour of light and 25°C ±4°C) and watered daily until inoculation time. Side-dressing fertilization were conducted weekly adding to each pot 30ml of nutritive solution prepared with 6.4mg/L KCl, 3.48mg/L K_2SO_4, 5.01mg/L $MgSO_4.7H_2O$, 2.03mg/L $(NH_2)2CO$, 0.009mg/L $NH_4MO_7O_{24}.4H_2O$, 0.054mg/L H_3BO_3, 0.222mg/L $ZnSO_4.7H_2O$, 0.058mg/L $CuSO_4.5H_2O$, 0.137mg/L $MnCl_2.4H_2O$, 0.27g/L $FeSO_4.7H_2O$ and 0.37g/L disodium-EDTA prepared with distilled water (Xavier Filha et al. 2011).

Inoculation procedures. Plants (leaves or heads) on each pot were sprayed-inoculated (15 mL) with the spore suspension using a 0.5L manual plastic sprayer (Guarany® - Gifor). The plants were placed in the dark in a chamber adjusted to 25°C (±2%) and >90% humidity for 20 h. The potted plants were moved to a growth chamber with controlled temperature at 28°C (±2°), humidity >80%, and 12/12 hours of fluorescent light/darkness during seven days, until performing the disease assessments.

318 *Disease assessment and data analysis*. The assessment of leaf blast severity (percentage
319 area affected) in wheat and signalgrass, and severity on wheat heads (percent of spikelets with
320 symptoms), was conducted seven days post-inoculation (dpi). Severity on the leaves was
321 measured on ten leaves randomly selected from each pot. These were removed from the plant
322 and imaged against a white background, using a flatbed scanner (HP - LaserJet M1132 MFP)
323 at 600-dpi resolution and JPEG file format. Images were analyzed in ImageJ (Schneider et al.
324 2012) to threshold the symptomatic and asymptomatic area, and then calculate severity (%
325 symptomatic area). The severity on wheat heads was assessed visually. The means and 95%
326 confidence interval of the percent severity of leaf and head blast were estimated for each isolate
327 after pooling data from two replicates of the experiment because the experiment effect was not
328 significant (data not shown).
329
330 **Data and code availability**
331 Data and custom R codes (R Core Team 2022) used for data analyses are available at
332 https://github.com/emdelponte/paper-wheat-blast-MG and
333 https://github.com/drdna/PyriculariaMG.
334
335 # Results

336 **Recovery of *Pyricularia* from blast-like lesions**
337 A large collection of isolates from wheat and grasses, both nearby and away (dozens to
338 hundreds of km) from wheat crops, were obtained during the two-year, multi-location, survey
339 conducted across the state of MG. A total of four surveys were conducted - prior to and during
340 the wheat growing seasons - in 2018 and 2019, which experienced typical and severe wheat
341 blast outbreaks, respectively. Poaceae with blast-like symptoms (diamond-shaped lesions)
342 were sampled during the visits, but with a focus on signalgrass (*Urochloa* spp.) growing near
343 to wheat fields, and in natural landscapes farther away. A total of 1,368 diseased samples were
344 collected (976 for leaves and 392 for wheat heads) from 20 Poaceae genera (31 species) (Table
345 3). Symptomatic plants collected at the non-wheat regions comprised mostly weeds and
346 included *Cenchrus*, *Cynodon*, *Digitaria*, *Eleusine*, *Hordeum*, *Melinis*, and *Panicum*. Symptoms
347 were found on six species of signalgrass (*U. brizantha, U. humidicola, U. plantaginea, U.*
348 *ruziziensis*, *U. arrecta, U. decumbens*), with *U. brizantha* being the most prevalent. In total,
349 pure cultures that were morphologically similar to *Pyricularia* spp. were successfully

recovered from approximately 68% of samples, resulting in a total of 932 monoconidial isolates (Table 3).

PCR-based diagnostics identified minimal cross-infection between isolates adapted to wheat versus endemic grasses

A subcollection of 564 isolates (including all of those from endemic grasses) were pre-screened using CH7BAC9 PCR (Table S1) which yields positive amplification for *P. oryzae* and *P. urashimae* but no products for *P. grisea* or *P. pennisetigena*. The 483 samples that were CH7BAC9-positive (85.6%) came from 16 plant species, with the predominant hosts being wheat, followed by *Urochloa*, *Eleusine, Melinis, and Panicum* (Table 4). The CH7BAC9-negative isolates, suspected to be other *Pyricularia* species, mostly came from *Cenchrus echinatus*, *Digitaria* spp., and *Panicum maximum*.

Among the 483 *P. oryzae* isolates analyzed by PCR, 313 (64.8%) were identified as PoT based on the successful amplification of both C17 and MoT3. Only nine of these (2.7%) came from non-wheat hosts, with only three coming from *Urochloa* (Table 4). The other grasses found to be harboring PoT were *Cenchrus echinatus* (n = 1 isolate)*, Eleusine indica* (n = 2)*, Melinis repens* (n = 1), *Panicum maximum* (n = 1)*,* and *Pennisetum sp.* (n = 1). Five of the nine PoT cross-infections on other grasses were for plants collected within, or adjacent to, wheat fields. The four other cases were in locations more than 30 km away from wheat fields, and only one of these remote cross infections was on *Urochloa* (Table 5).

PoT isolates lacking both MoT3 and C17 are occasionally found (e.g., Islam *et al.* 2016). However, because C17 and MoT3 actually target loci in native grass-infecting populations (see discussion), absences of either marker (or both) yield inconclusive assignments (Table 1). For this reason, we sought to verify equivocal lineage designations by sequencing CH7BAC9 PCR products and/or genotyping-by-sequencing. CH7BAC9 sequences were obtained for a total of 102 isolates from *Urochloa* sp. (n = 35), *Eleusine* (n = 42), *Melinis* (n = 8), *Cenchrus* (n = 2), *Panicum* (n = 10) and *Digitaria* (n = 5). These sequences were combined with a comprehensive dataset acquired by mining CH7BAC9 alleles from genome assemblies and the phylogenetic relationships between the MG isolates and previously-established lineages were determined using maximum likelihood. This revealed a clear pattern of host specialization because most of the above-mentioned isolates grouped in clades whose constituent members were usually from the same host (note co-clustering of like-colored tips in Fig. 2)). Here, it should be emphasized that some patterns of host-specialization are not

obvious in the figure because several lineages share similar/identical CH7BAC9 alleles (e.g. PoO/PoS/PoP & PoT/PoL1), while others are admixtures and possess multiple alleles (e.g. PoL1, PoT, and PoU3). With this being said, many of the hidden patterns were resolved after analyzing multiple loci (Table 6, and see below).

Four different CH7BAC9 alleles were found among the *Urochloa*-infecting isolates with two being predominant. One matched the PoU3 lineage, the other was identical to an allele found in a different species - *P. urashimae,* while the minor alleles matched those found in PoM and PoO/Le/P/S (the latter being indistinguishable because they share the same sequence). Most of the isolates from *Eleusine* (80%) grouped with the *Eleusine*-infecting lineages, PoE1 and PoE2, which share the same allele (n = 28), or with PoE3 (n = 8) (Fig. 2A and Table 6). The only examples of cross-infection on *Eleusine*, were two isolates from PoT, and three from the *P. oryzae Echinochloa* lineage (PoEc), which is represented by isolates from various hosts including *Digitaria, Echinochloa*, *Lolium*, *Zea*, and now *Eleusine* (Table 6, Table S1). The CH9BAC9 sequences for the isolates from *Melinis* all grouped with the sequence present in the reference genome of a previous strain from this host, MrJA49 and, therefore, appear to identify a new, phylogenetically-distinct, *Melinis*-adapted lineage.

Host-specialization in other *Pyricularia* species

No CH7BAC9 PCR products were obtained for most of the isolates (73/84) from *Cenchrus*, *Digitaria*, and *Pennisetum*, which was consistent with the absence of this locus in the genome assemblies of representative isolates of *P. grisea* and *P. pennisetigena* (Table S1). For the few isolates that did yield amplicons, sequencing revealed loci that were related to those found in PoE1/2 (4 isolates from *Digitaria*; 1 from *Cenchrus*, 1 from *Pennisetum*) and PoO (one isolate from *Digitaria*) (Fig. 2). Given the rarity of hybridization between different *Pyricularia* species (unpublished data), these presumably were cases of cross-infection.

Isolates from *Cenchrus*, *Digitaria*, and *Pennisetum* that failed to yield CH7BAC9 amplicons were characterized by amplifying and sequencing the MPG1 locus. Phylogenetic analysis of the resulting data, along with sequences mined from genomes of reference isolates, including *P. oryzae*, indicated that isolates from *Digitaria* (n = 45) were *P. grisea*, while those from *Cenchrus* (n = 23) and *Pennisetum* (n= 1) grouped with *P. pennisetigena* isolates (Fig. 3). The taxonomic assignment of *Cenchrus* isolates to *P. pennisetigena* is supported by phylogenomic analyses which grouped another Cenchrus (Ce88424) pathogen with this species (Farman et al. 2023) *P. pennisetigena* is named because the type isolate came from *Pennisetum*

(Klaubauf et al. 2014). However, the present data suggest that *Cenchrus* is also a canonical host for this species.

GBS confirmed that most *P. oryzae* lineages are host-specialized

The combined results of the MoT3/C17 assays and CH7BAC9 sequencing identified very few PoT isolates on endemic grasses and, conversely, very few grass-adapted isolates on wheat (Table 5). However, because a small proportion of PoT isolates are known to lack MoT3, we considered it important to rule out the possibility that shifts in the PoT population had produced isolates that lack C17, or both MoT3 and C17. Therefore, to validate the lineage assignments made with MoT3, C17, and CH7BAC9, we used "MonsterPlex" - Floodlight Genomics's variation of the Hi-Plex2 assay (Hammet et al. 2019) - to perform genotyping-by-sequencing (GBS) on a selection of isolates from wheat (n = 66), *Urochloa* (n = 38), *Eleusine* (n = 6), *Hordeum* (n = 3), *Melinis* (n = 6) and *Panicum* (n = 11). We then examined their phylogenetic relationships to *in silico*-mined genotypes from a set of 232 reference isolates whose lineage affiliations were already well established (e.g., Gladieux et al. 2018).

Although the multiplex assay was originally designed to target a single SNP at each of 84 loci dispersed throughout the genome, we identified a total of 228 variant sites within the targeted loci. Together, these SNPs were capable of resolving all 34 of the PoT haplotypes known to exist prior to this study (Rahnama et al. 2021). Sixty-two of the 64 MG isolates identified as PoT using PCR-based diagnostics grouped with one of the two established PoT/PoL clades (Fig. 4). For the remaining pair of isolates, the GBS data revealed that they had been mis-characterized as PoT based on a $MoT3^{-}/C17^{+}$ amplification profile (see below). Also analyzed were two isolates from wheat for which the original PCR tests failed altogether. One was found to be a PoU3 member, and the other grouped with other *Urochloa* pathogens in the PoU4 clade, which is phylogenetically related to *Panicum* pathogens (PoP).

GBS was also performed for $MoT3^{-}$ and/or $C17^{-}$ isolates from non-wheat hosts (*Eleusine*, *Melinis*, *Panicum,* and *Urochloa*) to test for possible cross-infections by PoT members with atypical genotypes. No such evidence was obtained because all isolates analyzed grouped outside of the PoT clades (Fig. 4). The only potential cross-infections identified involved isolates from *Hordeum vulgare* (UFVPY247, 248, 249), which grouped with PoL1. Isolates from *Eleusine* and *Melinis* grouped strictly according to their respective hosts of origin, with the *Eleusine* pathogens belonging to PoE1/2 (n = 4) and PoE3 (n = 1), and the *Melinis* isolates to PoM (n = 6). Only eleven of the 38 isolates from *Urochloa* belonged to one of the

previously defined PoU lineages - this being PoU3. The remainder formed two novel *Urochloa*-associated clades, one related to torpedograss (*Panicum repens*) pathogens (PoU4, n = 3 isolates), and the other, defines a lineage that appears to fall under the umbrella of the sister species, *P. urashimae* (Pu, n = 24) because it houses PmJA1 and PmJA115 (Fig. 4), and these isolates possess Pu alleles for a number of reference genes (data not shown). It should be noted that there were a large number of missing datapoints for isolates within the Pu lineage, presumably due to significant sequence divergence at the target loci affecting primer binding, (~10%) but sequence variation at successfully amplified sites confirms the presence of high variation (data not shown). Lastly, it should be emphasized that strain isolations, PCR assays and GBS were performed only one time, so it is possible that a small number of sample mix-ups were made. Given the large number of isolates processed from wheat versus any of the non-wheat hosts, mistakes are more likely to have artificially inflated the number of wheat ← → non-wheat cross-infections than reducing them and, therefore, would not affect our overall conclusion that cross-infection was uncommon.

Identification of false positives for the MoT3 and C17 diagnostic markers

A large fraction of the isolates from *Urochloa* and *P. maximum* exhibited a $MoT3^{+}/C17^{-}$ genotype, which implied that these isolates are false positives for MoT3. This was confirmed using GBS (and genome sequencing, see Farman et al. 2022) which revealed that all of the $MoT3^{+}$ *Urochloa* pathogens are PoU3 and the positive *Panicum* pathogens belong to the *P. urashimae* lineage (Fig. 4). Conversely, isolate UFVPY183 from *Eleusine* (PoE3) and UFVPY578 from *Melinis* (PoM) reproducibly tested positive for C17 and negative for MoT3 and, therefore, are the first examples of non-PoT isolates that have given positive results for C17.

***Triticum-Urochloa* specificity observed in the field may be due to inherent differences in infection capability on canonical versus non-canonical hosts**

Molecular analysis of field isolates collected from wheat and *Urochloa* revealed that cross-infection between the two hosts is uncommon. To explore whether this is due to inherent differences in relative aggressiveness toward the respective hosts, we performed reciprocal infection assays. In a first experiment, 14 PoT isolates (11 from wheat and three from *Urochloa*) and six isolates that were obtained from *Urochloa* (three from the PoU3 lineage; two from Pu and one from PoU4 - hereafter non-PoT) were inoculated on leaves of two wheat

478 cultivars (Guamirim and BRS18- Terena) and leaves of one signalgrass cultivar (Marandu). In
479 general, the isolates within each lineage were consistently and significantly more aggressive
480 on their primary host of origin (with the exception of the PoTs obtained from *Urochloa)* than
481 on the alternative host, although there were differences among individual isolates (Figs. 4, 5, 6
482 and 7).

483 Mean severity induced by PoT isolates ranged from 20 to 70% (group mean = 44.1%) on
484 BR18 Terena wheat and from 40 to 80% (mean = 63.1%) on BRS Guamirim wheat, across
485 isolates. Mean severity induced by the non-PoT isolates on leaves of wheat was only 1.39%
486 and 0.73% on BR18 Terena and BRS Guamirim, respectively. Mean severity induced by PoT
487 and non-PoT on Marandu signalgrass was 0.51% and 14.26%, respectively (Fig. 5).

488 In a separate experiment, wheat heads of the same two cultivars were inoculated with the same
489 set of PoT and non-PoT isolates. The PoT isolates were generally more aggressive than the
490 non-PoT isolates on the wheat hosts (Fig. 6 and 8). The percent of infected spikelets were, on
491 average, 89% and 93% on BRS Guamirim and BR18 Terena, respectively, when challenged
492 with PoT isolates, including those three isolates obtained from *Urochloa* (Fig.). Contrarily,
493 percent infected spikelets by the non-PoT isolates were on average 54.2% and 50.9% across
494 the isolates (Fig. 6). It is worth noting that lesions caused by the non-PoT isolates on the
495 affected spikelet were small and scattered, not affecting the entire spikelet (Fig. 8). On the other
496 hand, most PoT isolates were highly aggressive, producing the typical bleaching of the affected
497 spikelets (Fig. 8).

498
499 ## Discussion
500
501 Over four separate sampling trips spanning two years (prior to and during the wheat growing
502 season), we generated a comprehensive collection of *Pyricularia* isolates obtained from blast
503 lesions on endemic grasses grown near to or away from wheat fields. The grass genera from
504 which we recovered non-PoT *Pyricularia* were *Cenchrus*, *Cynodon, Digitaria*, *Eleusine*,
505 *Hordeum*, *Melinis*, *Panicum*, *Pennisetum,* and *Urochloa*. At the same time, we established the
506 first extensive collection of several hundreds of isolates obtained from wheat cultivated in both
507 southern and western regions of MG, Brazil.
508 A large majority of isolates could be reliably identified down to species/lineage through
509 amplification/sequencing of just three PCR-based markers. Successful amplification of

CH7BAC9 by itself distinguished *P. oryzae* from the other species, and positive amplification for both MoT3 and C17 (Pieck et al. 2017; Thierry et al. 2020) proved to be definitive for PoT. Amplification of either MoT3 or C17 alone, however, yielded equivocal results. Although MoT3 showed early promise as a PoT diagnostic, occasional exceptions (false positives/negatives) have been reported (Pieck et al. 2017; Yasuhara-Bell et al. 2018). In the past, the most common exceptions involved wheat-infecting members of the related PoL1 lineage which are MoT3$^-$/C17$^-$. We found that 2.5% (9/394) of the wheat blast isolates from MG wheat fell into this category. More concerningly, however, we found an extremely high frequency of false positives, with 41% (64/157) of non-PoT isolates yielding a MoT3$^+$ reaction. This result is largely attributable to the fact that PoT/PoL1 and acquired the MoT3 locus from PoU3 (Rahnama et al. 2021), one of the main lineages found on *Urochloa*. Additionally, a highly similar, and amplifiable, MoT3 sequence was ubiquitously present in *P. urashimae* isolates from *Panicum maximum* (14/14) (Table S1). Consequently, assays that survey MoT3 alone are unreliable for PoT detection. This throws into question conclusions from recent studies which used MoT3 amplification to assess the presence of PoT on Brazilian grasses (Maciel et al. 2023) or in the air (Vicentini et al. 2023). Because all of the MoT3$^+$ isolates in that Maciel and collaborators (Maciel et al. 2023) study came from *Urochloa* (13/58; 22.4%), it is quite possible that none of the sampled isolates were PoT, especially considering the low frequency of PoT we found on *Urochloa*. It would, therefore, be instructive to reassess PoT prevalence after performing C17 assays.

Here, it should also be stressed that the specificity of the C17 assay was also not perfect. Although C17 yielded positive results for the nine PoT isolates that were MoT3$^-$, we recorded the first examples of false positives in isolates from *Eleusine* (PoE3) and *Melinis* (PoM). This, again, is not surprising because PoT inherited the C17 locus from another - in this case, unknown - *P. oryzae* lineage (PoX) (Rahnama et al 2021), which means that this locus, too, will yield false positive results when isolates from the relevant host(s) are sampled. Therefore, for reliable PoT detection, at a minimum we recommend that both MoT3 and C17 be surveyed in parallel. Finally, it is also important to note that the current formulation of the MonsterPlex assay, while being highly effective at lineage assignment for most isolates, and identifying new lineages, is also not capable of positively identifying PoT. This is because several known PoT isolates group with PoL1, while others such as PoT6 and PoT29, group with neither PoT, nor PoL1 (Fig. 4). This is not surprising because the assay was originally designed with the main goal of distinguishing the B71 lineage from all other PoT (Latorre et al. 2023).

Overall, among the 572 *Pyricularia* spp. isolates, *P. oryzae* dominated the collection (87%) and this likely reflected the fact that other *Pyricularia* were much more host-restricted, with *P. pennisetigena* being found almost exclusively on *Cenchrus*, *P. grisea* on *Digitaria,* and *P. urashimae* on *Panicum* and *Urochloa*. By way of contrast, *P. oryzae* was recovered from all genera that yielded *Pyricularia*, except *Cenchrus*; and accounted for all but two of the isolates sampled from wheat heads (n = 333). Thus, species outside of the *P. oryzae* clade are very unlikely to cause wheat blast. This is contrary to what has been suggested following inoculation in controlled environment studies, which reported pathogenicity and high aggressiveness of *P. pennisetigena* and *P. zingibericola* - from grasses in Brazil - to Anahuac 75, a wheat cultivar regarded as universally susceptible cultivar to PoT (Reges et al. 2016).

The *P. oryzae* isolates we collected from non-wheat/*Lolium* hosts grouped into nine distinct lineages/species variously specialized on eight different grass genera, most of which were previously known hosts, including *Cynodon*, *Echinochloa*, *Eleusine*, *Urochloa* (Borromeo et al. 1993), and *Hordeum* (Urashima et al. 2004). Although we identified members of previously known lineages (PoE1/2) in association with the expected hosts, a majority of isolates belonged to new phylogenetic lineages. These also showed evidence of host-specialization because constituent members were usually isolated from the same genus/species. Examples included PoE3 specialized on *Eleusine*, PoM (from *Melinis*), PoU3 and PoU4 (both on *Urochloa*). One additional lineage was identified (PoC2 from *Cynodon*) but was only represented by one isolate, so its host-specialization status is unclear.

We also report *P. maximum* as a new host for *P. urashimae* (Pu), the type isolate of which originally came from *Urochloa brizantha* (Crous et al. 2016). This seems to be quite a specific interaction from *P. maximum*'s perspective because 12/16 isolates from this grass were in the Pu phylogenetic group, with limited cross-infection by PoM, and PoT having been observed. However, most Pu members came from *Urochloa*, indicating that the lineage has dual specificity. This property might be partially explained by the GBS data, which suggests that the Pu species is highly diverse, such that it too, like *P. oryzae*, might comprise a number of genetically-distinct sub-lineages, with some being specialized on *P. maximum*, and others on *Urochloa*.

With the discovery of eight non-PoT/PoL1 lineages on endemic grasses, our study greatly expands understanding on native *Pyricularia* populations in Brazil. In an accompanying paper (Farman et al 2023), we show that prior efforts to characterize grass-infecting

populations (Castroagudín et al. 2017; Castroagudín et al. 2016; Ceresini et al. 2018, 2019) mostly sampled PoT, with just one isolate from a *bona fide* grass-adapted lineage (Farman et al, 202) having been recovered. That isolate, Ds555i (a.k.a. 12.1.555i, from *Digitaria*), is a member of the PoEc (*Echinochloa*) lineage which distinguishes itself by a distinct absence of host-specialization among its constituent members. In line with this trend, we found two PoEc members on a previously unknown host of this lineage - *Eleusine*.

Although our primary motivation for sampling wheat blast was to examine the extent of cross-infection by grass-adapted lineages, the GBS data also provided key insights into the MG wheat blast population. A recent phylogenomic analysis suggested that wheat blast and gray leaf spot co-evolved very recently through a series of admixtures involving *P. oryzae* isolates from five different host-specialized lineages. Examination of the specific chromosome segments that were inherited from the various donor isolates identified 37 distinct chromosomal haplotypes among the isolates from wheat (34 PoT; 3 PoL1) (Rahnama et al. 2021). The MG wheat blast isolates defined eight phylogenetic groups, none of which perfectly matched previously established haplotypes; and because so few SNPs have arisen in the population since wheat blast/gray leaf spot evolved (Rahnama et al 2021), the MG blast population most likely comprises new chromosomal haplotypes. All of the PoT isolates analyzed previously came from other states, namely Paraná, Rio Grande do Sul, Mato Grosso do Sul, Goiás, and São Paulo, which suggests that there may be regional differences in the genetic composition of the South American wheat blast population.

Whereas sampling of non-wheat hosts in, or near, wheat fields mostly resulted in the recovery of PoT/PoL1 (Maciel et al. 2014; Castroagudín et al. 2016), our sampling of the same grasses away from wheat usually yielded isolates whose phylogenetic affinities implied adaptation to the host of origin. Indeed, most of the weeds we found to be harboring PoT were collected near to heavily infected wheat plots, where inoculum densities would be the highest. There appears to be no obvious pattern to PoT's cross-infectivity, because we identified it on six different host genera. Together with the data from prior studies (Castroagudín et al. 2016; Maciel et al. 2014), this expands the list of surrogate hosts for PoT to ten (*Bromus*, *Cenchrus*, *Digitaria*, *Echinochloa*, *Eleusine*, *Lolium*, *Melinis*, *Panicum*, *Pennisetum*, and *Urochloa*). Further, if we include PoL1 isolates, based on the fact that some of its members can also infect wheat, this also adds *Avena*, and *Hordeum* as potential surrogates.

606 The discovery of seven non-PoT/PoL isolates on wheat was rather surprising because,
607 with the exception of PoL1 lineage members, cross-infection of wheat by other host-adapted
608 forms of *P. oryzae* has never been shown beforehand. Four of the isolates were collected in the
609 same wheat field in the Triângulo Mineiro region, along with 40 PoT strains. It is possible that
610 we were successful in identifying these rare cases due to extensive sampling from the same
611 field, and because we specifically screened for fungal isolates that were MoT3^{-}/C17^{-}.
612 For the samples collected away from wheat fields, isolates found on non-canonical
613 hosts were fairly evenly distributed among the different lineages. This, along with the discovery
614 that certain host genera are susceptible to multiple genetically-distinct lineages (e.g., *Cynodon*,
615 *Eleusine*, *Hordeum*, *Panicum*, *Urochloa*) implies that host-specificity barriers to *P. oryzae,* as
616 a rule, are somewhat fluid and reinforces the notion that most lineages are "host-adapted" or
617 "host-specialized," as opposed to "host-specific."
618 A main focus of our study was to characterize the fungal population(s) found on
619 *Urochloa* because we suspected that prior studies implicating this host as a central player in
620 the evolution, inoculum development and epidemic spread of wheat blast (Ceresini et al. 2018,
621 2019; Maciel et al. 2014; Stukenbrock and McDonald, 2008) hadn't actually sampled the
622 endemic *Urochloa*-infecting population (see Farman et al. 2022). Previous studies only
623 sampled two *P. oryzae* lineages from *Urochloa* - PoT and PoL1 (Castroagudín et al. 2017;
624 Castroagudín et al. 2016; Ceresini et al. 2018, 2019). Here, we identified an additional seven
625 lineages on the genus (PoU1, PoU2, PoU3, PoU4, PoE3, PoL, PoM, PoSt, and PoT), as well
626 as *P. urashimae*. At first, this might imply that *Urochloa* is a "universally susceptible" host.
627 However, most isolates were placed in the *Urochloa*-adapted lineages, PoU3 and PoU4, or the
628 highly diverse *P. urashimae* (Pu) clade, which is dually specialized on *Urochloa* and *P.*
629 *maximum*. Thus, the small number of isolates from various other lineages found on *Urochloa*,
630 probably reflects a low level of inherent, base-line cross-infectivity across the species.
631 The low frequency of wheat ← → *Urochloa* cross-infection found in nature seems to
632 be correlated with innate compatibility differences because, using cross-inoculation
633 experiments, we found that PoT members were consistently more aggressive on wheat leaves,
634 and the isolates from *Urochloa* (PoU3/4 and Pu) were more aggressive on signalgrass. Thus,
635 our findings hold to the general pattern that PoT is usually more aggressive on wheat, while
636 non-PoT/non-*P. oryzae* isolates tend to be less aggressive, even under favorable, controlled
637 environments (Chung et al. 2020; Kato et al. 2000; Reges et al. 2016, 2019). And, even though
638 the *Urochloa* pathogens showed incidences on the spikelets up to 50%, after spraying spikelets,

the percent diseased area was apparently rather low, and the symptoms were mild, consisting of small, reddish-brown to dark-gray spots, or even hypersensitive reactions. This was in striking contrast to the symptoms caused by PoT isolates, which were characterized by bleaching of the heads. It seems doubtful that inconspicuous lesions on spikes caused by non-PoT isolates are likely to cause significant yield loss, although this should be further confirmed using polycyclic infection assays. It should also be noted that *Urochloa* leaves collected from the field often showed an abundance of blast-like symptoms but the vast majority of lesions failed to produce the profuse sporulation characteristic of *Pyricularia* infection after overnight humidification. True blast infections typically show rapid and abundant sporulation from all lesions and, therefore, it appears that not only was PoT rarely recovered from *Urochloa* but the incidence of blast was also a lot lower than was initially apparent based on macroscopic symptoms.

Prior studies have implied that cross infection of *Urochloa* by PoT is significant and widespread (Castroagudín et al. 2017; Ceresini et al. 2018, 2019; Maciel et al. 2023), and a major concern for wheat blast management. Overall, our data challenge this idea because most *Pyricularia* isolates from signalgrass plants - even ones collected in proximity to wheat fields - belonged to *Urochloa*-specific lineages; and members of these lineages were recovered from wheat even less frequently than was PoT from *Urochloa*. In addition, blast-like lesions were rarely observed on signalgrass plants growing at remote distances from wheat fields, and were sometimes even hard to find on signalgrass plants immediately adjacent to devastated wheat (personal observations). Thus, we feel we can propose an equally viable hypothesis that infected wheat more often serves as a source of inoculum for the occasional cross-infection of nearby *Urochloa*. Of course, we cannot rule out the possibility that the low prevalence of PoT on *Urochloa,* and the low sporulation capacity, might still be sufficient to produce an inoculum reservoir to trigger seasonal epidemics, and facilitate long-range movement. However, it should be noted that PoT was found as often on other weedy grasses, as it was on *Urochloa* and, therefore, the proportional contributions of these different grasses, if any, to wheat blast epidemiology remains an open question.

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Literature Cited

Barea, G., and Toledo, J. 1996. Identificación y zonificación de Pyricularia o brusone (*Pyricularia oryzae*) en el cutivo de trigo en el departamento de Santa Cruz (Informe Tecnico Proyecto de Investigacion Trigo, pp. 76–86). Centro de Investigación Agrícola Tropical.

Borromeo, E. S., Nelson, R. J., Bonman, J. M., and Leung, H. 1993. Genetic differentiation among isolates of *Pyricularia* infecting rice and weed hosts. Phytopathology *83*:393–399.

Cabrera, M. G., and Gutierrez, S. A. 2007. Primer registro de *Pyricularia oryzae* en cultivos de trigo del NE de Argentina. In Jornada de Actualización en Enfermedades de Trigo pp. 18–180. Instituto Fitotécnico de Santa Catalina.

Castroagudín, V., Danelli, A., Intra Moreira, S., Teodora de Assis Reges, J., Carvalho, G., Maciel, J. L. N., Bonato, A. L. V., Forcelini, C. A., Alves, E., McDonald, B., Croll, D., and Ceresini, P. 2017. The wheat blast pathogen *Pyricularia graminis-tritici* has complex origins and a disease cycle spanning multiple grass hosts. BioRxiv. https://doi.org/10.1101/203455

Castroagudín, V. L., Moreira, S. I., Pereira, D. A. S., Moreira, S. S., Brunner, P. C., Maciel, J. L. N., Crous, P. W., McDonald, B. A., Alves, E., and Ceresini, P. C. 2016. *Pyricularia graminis-tritici*, a new *Pyricularia* species causing wheat blast. Persoonia - Mol. Phylogeny Evol. Fungi 37:199–216.

Cazal-Martínez, C. C., Reyes-Caballero, Y. M., Chávez, A., Pérez-Estigarribia, P. E., Rojas, A., Arrua, A., Moura-Mendes, J., Lopez-Nicora, H. D., and Kohli, M. M. 2021. First report of a leaf blight caused by *Pyricularia pennisetigena* on *Cenchrus echinatus* in Paraguay. Plant Dis. 105:3758.

Ceresini, P. C., Castroagudín, V. L., Rodrigues, F. Á., Rios, J. A., Aucique-Pérez, C. E., Moreira, S. I., Croll, D., Alves, E., de Carvalho, G., Maciel, J. L. N., and McDonald, B. A. 2019. Wheat blast: From its origins in South America to its emergence as a global threat. Molecular Plant Pathol., 20:155–172.

Ceresini, P. C., Castroagudín, V. L., Rodrigues, F. Á., Rios, J. A., Eduardo Aucique-Pérez, C., Moreira, S. I., Alves, E., Croll, D., and Maciel, J. L. N. 2018. Wheat blast: past, present, and puture. Annu. Rev. Phytopathol. 56:427–456.

Chung, H., Goh, J., Han, S.-S., Roh, J.-H., Kim, Y., Heu, S., Shim, H.-K., Jeong, D. G., Kang, I. J., and Yang, J.-W. 2020. Comparative pathogenicity and host ranges of *Magnaporthe oryzae* and related species. Plant Pathol. J. 36:305–313.

Coelho, M. A. de O., Torres, G. A. M., Cecon, P. R., and Santana, F. M. 2016. Sowing date reduces the incidence of wheat blast disease. Pesq. Agropec. Bras. 51:631–637.

Couch, B. C., Fudal, I., Lebrun, M.-H., Tharreau, D., Valent, B., van Kim, P., Nottéghem, J.-L., and Kohn, L. M. 2005. Origins of host-specific populations of the blast pathogen *Magnaporthe oryzae* in crop domestication with subsequent expansion of pandemic clones on rice and weeds of rice. Genetics 170:613–630.

Couch, B. C., and Kohn, L. M. 2002. A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae,* from *M. grisea*. Mycologia 94:683–693

719 Crous, P. W., Wingfield, M. J., Burgess, T. I., Hardy, G. E. St. J., Crane, C., Barrett, S., Cano-
720 Lira, J. F., Le Roux, J. J., Thangavel, R., Guarro, J., Stchigel, A. M., Martín, M. P., Alfredo,
721 D. S., Barber, P. A., Barreto, R. W., Baseia, I. G., Cano-Canals, J., Cheewangkoon, R.,
722 Ferreira, R. J., … Groenewald, J. Z. 2016. Fungal Planet description sheets: 469–557.
723 Persoonia: Mol. Phylogeny Evol. Fungi 37:218–403.
724 Cruppe, G., Cruz, C. D., Peterson, G., Pedley, K., Asif, M., Fritz, A., Calderon, L., Lemes da
725 Silva, C., Todd, T., Kuhnem, P., Singh, P. K., Singh, R. P., Braun, H.-J., Barma, N. C. D.,
726 and Valent, B. 2020. Novel sources of wheat head blast resistance in modern breeding lines
727 and wheat wild relatives. Plant Dis. 104:35–43.
728 Cruz, C. D., Bockus, W. W., Stack, J. P., Valent, B., Maciel, J. N., and Peterson, G. L. 2016.
729 A standardized inoculation protocol to test wheat cultivars for reaction to head blast caused
730 by *Magnaporthe oryzae* (*Triticum* pathotype). Plant Health Prog. 17:186–187.
731 Cruz, C. D., Kiyuna, J., Bockus, W. W., Todd, T. C., Stack, J. P., and Valent, B. 2015.
732 *Magnaporthe oryzae* conidia on basal wheat leaves as a potential source of wheat blast
733 inoculum. Plant Pathol. 64:1491–1498.
734 Cruz, C. D., and Valent, B. (2017). Wheat blast disease: Danger on the move. Trop. Plant
735 Pathol., 42:210–222.
736 Dean, R., Kan, J. a. L. V., Pretorius, Z. A., Hammond-Kosack, K. E., Pietro, A. D., Spanu, P.
737 D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., and Foster, G. D. 2012. The Top 10
738 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13:414–430.
739 Dianese, A. D. C., Zacaroni, A. B., de Souza, B. C. P., da Silva Pagani, A. P., Pinheiro, N. O.,
740 de Castro Gomes, E. M., Torres, G. A. M., Consoli, L., and Café-Filho, A. C. 2021.
741 Evaluation of wheat genotypes for field resistance to wheat blast caused by *Magnaporthe*
742 *oryzae* pathotype Triticum (MoT) and correlation between yield loss and disease incidence
743 in the Brazilian Cerrado. Euphytica 217:1-13.
744 Santos, G. B., de Oliveira Coelho, M. A., and Del Ponte, E. M. 2022. Critical-point yield loss
745 models based on incidence and severity of wheat head blast epidemics in the Brazilian
746 Cerrado. Eur. J Plant Pathol. Early view at https://doi.org/10.1007/s10658-022-02614-7
747 Farman, M. L. 2002. *Pyricularia grisea* isolates causing gray leaf spot on perennial ryegrass
748 (*Lolium perenne*) in the United States: Relationship to *P. grisea* isolates from other host
749 plants. Phytopathology 92:245–254.
750 Farman, M., Peterson, G., Chen, L., Starnes, J., Valent, B., Bachi, P., Murdock, L., Hershman,
751 D., Pedley, K., Fernandes, J. M., and Bavaresco, J. 2017. The *Lolium* pathotype of
752 *Magnaporthe oryzae* recovered from a single blasted wheat plant in the United States. Plant
753 Dis. 101:684–692.
754 Farman, M. L. Ascari, J. P., Rahnama, M., Del Ponte, E. M., Pedley, K. F., Martinez, S.,
755 Fernandes, J. M. C., and Valent, B. 2022. A re-evaluation of phylogenomic data reveals
756 that current understanding in wheat blast population biology and epidemiology is
757 obfuscated by oversights in population sampling (p. 2022.12.19.521104). bioRxiv.
758 https://doi.org/10.1101/2022.12.19.521104
759 Gladieux, P., Condon, B., Ravel, S., Soanes, D., Maciel, J. L. N., Nhani, A., Chen, L., Terauchi,
760 R., Lebrun, M.-H., Tharreau, D., Mitchell, T., Pedley, K. F., Valent, B., Talbot, N. J.,

761 Farman, M., and Fournier, E. 2018. Gene flow between divergent cereal- and grass-specific
762 lineages of the rice blast fungus *Magnaporthe oryzae*. MBio 9:e01219-17.
763 Goulart, A. C. P., and Paiva, F. de A. 2000. Avaliação de perdas no rendimento de grãos de
764 trigo causadas por *Pyricularia grisea*, no período de 1988 a 1992, em Mato Grosso do Sul
765 (7th ed.). Embrapa.
766 Goulart, A. C. P., Paiva, F., and Mesquita, A. N. 1990. Occurrence and losses caused by wheat
767 (*Triticum aestivum* L.) blast (*Pyricularia oryzae* Cav.) in the state of Mato Grosso do Sul,
768 in 1988. In Annual Wheat Newsletter (Vol. 36). Department of Agronomy, Colorado State
769 University.
770
771 Hammet, F., Mahmood, K., Green, T. R., Nguyen-Dumont, T., Southey, M. C., Buchanan, D.
772 D., Lonie, A., Nathanson, K. L., Couch, F. J., Pope, B. J., and Park, D. J. 2019. Hi-Plex2:
773 A simple and robust approach to targeted sequencing-based genetic screening.
774 BioTechniques, 67:118–122.
775 IBGE. 2017. Censo Agropecuário. Instituto Brasileiro de Geografia e Estatística. Online at
776 https://www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria/21814-2017-
777 censo-agropecuario.html?=andt=o-que-e
778 Igarashi, S., Utiamada, C. M., Igarashi, L. C., Kazuma, A. H., and Lopes, R. S. 1986.
779 Occurrence of *Pyricularia* sp. in wheat (*Triticum aestivum* L.) in the State of Paraná, Brazil.
780 Fitopatol. Bras. 11:351–352.
781 Islam, M. T., Croll, D., Gladieux, P., Soanes, D. M., Persoons, A., Bhattacharjee, P., Hossain,
782 Md. S., Gupta, D. R., Rahman, Md. M., Mahboob, M. G., Cook, N., Salam, M. U., Surovy,
783 M. Z., Sancho, V. B., Maciel, J. L. N., NhaniJúnior, A., Castroagudín, V. L., Reges, J. T.
784 de A., Ceresini, P. C., Revel, S., Kellner, R. Fournier, E. Tharreau, D., Lebrun, M. H.,
785 McDonald, B. A., Stitt, T., Swan, D., Talbot, N. J., Saunders, D. G. G., Win, J., and
786 Kamoun, S. 2016. Emergence of wheat blast in Bangladesh was caused by a South
787 American lineage of *Magnaporthe oryzae*. BMC Biol. 14:1-11
788 Kato, H., Yamamoto, M., Yamaguchi-Ozaki, T., Kadouchi, H., Iwamoto, Y., Nakayashiki, H.,
789 Tosa, Y., Mayama, S., and Mori, N. 2000. Pathogenicity, mating ability and DNA
790 Restriction Fragment Length Polymorphisms of *Pyricularia* populations isolated from
791 gramineae, *Bambusoideae* and *Zingiberaceae* plants. J. Gen. Plant Pathol. 66:30–47.
792 Klaubauf, S., Tharreau, D., Fournier, E., Groenewald, J. Z., Crous, P. W., de Vries, R. P., and
793 Lebrun, M.-H. 2014. Resolving the polyphyletic nature of *Pyricularia* (Pyriculariaceae).
794 Stud. Mycol. 79:85–120.
795 Kozlov, A. M., Darriba, D., Flouri, T., Morel, B., Stamatakis, A. 2019. RAxML-NG: A fast,
796 scalable, and user-friendly tool for maximum likelihood phylogenetic inference.
797 Bioinformatics 35(21):4453-4455
798 Langmead, B., Salzberg, S. L. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods.
799 9(4):357-9.
800 Latorre, S. M., Reyes-Avila, C. S., Malmgren, A., Win, J., Kamoun, S., and Burbano, H. A.
801 2020. Differential loss of effector genes in three recently expanded pandemic clonal
802 lineages of the rice blast fungus. BMC Biol. 18:1-15

Latorre, S. M., Were, V. M., Foster, A. J., Langner, T., Malmgren, A., Harant, A., Asuke, S., Reyes-Avila, S., Gupta, D. R., Jensen, C., Ma, W., Mahmud, N. U., Mehebub, Md. S., Mulenga, R. M., Muzahid, A. N. Md., Paul, S. K., Rabby, S. M. F., Rahat, A. A. M., Ryder, L., Shrestha, R. K., Sichilima. S., Soanes, D. M., Singh, P. K., Bentley, A. R., Saunders, D. G. O., Tosa, Y., Croll, D., Lamour, K. H., Islam, T., Tembo, B., Win, J., Talbot, N. J., Burbano, H. A., Kamoun, S. (2023). Genomic surveillance uncovers a pandemic clonal lineage of the wheat blast fungus. PLOS Biology 21(4):e3002052

Lorenzi, H. 2014. Manual de Identificação e Controle de Plantas Daninhas (7th ed.). Plantarum.

Maciel, J. L. N., Ceresini, P. C., Castroagudín, V. L., Zala, M., Kema, G. H. J., and McDonald, B. A. 2014. Population structure and pathotype diversity of the wheat blast pathogen *Magnaporthe oryzae* 25 years after its emergence in Brazil. Phytopathology 104:95–107.

Maciel, J. L. N., Kovaleski, M., Silva, A. N., Bonato, A. L. V., and Costa, I. F. D. da. 2023. Occurrence of *Pyricularia oryzae* Triticum in plants of the genus *Urochloa* in Brazil. Cienc. Rural 53:e20210839.

Malaker, P. K., Barma, N. C. D., Tiwari, T. P., Collis, W. J., Duveiller, E., Singh, P. K., Joshi, A. K., Singh, R. P., Braun, H.-J., Peterson, G. L., Pedley, K. F., Farman, M. L., and Valent, B. 2016. First Report of wheat blast caused by *Magnaporthe oryzae* pathotype Triticum in Bangladesh. Plant Dis. 100:2330.

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M. and DePristo, M.A. 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20(9):1297-1303.

Murata, N., Aoki, T., Kusaba, M., Tosa, Y., and Chuma, I. 2014. Various species of *Pyricularia* constitute a robust clade distinct from *Magnaporthe salvinii* and its relatives in Magnaporthaceae. Journal of General Plant Pathology 80:66–72.

Nguyen-Dumont, T., Pope, B. J., Hammet, F., Southey, M. C. and Park, D. J. 2013. A high-plex PCR approach for massively parallel sequencing. Biotechniques 55(2):69-74.

Pieck, M. L., Ruck, A., Farman, M. L., Peterson, G. L., Stack, J. P., Valent, B., and Pedley, K. F. 2017. Genomics-based marker discovery and diagnostic assay development for wheat blast. Plant Dis. 101:103–109.

Rahnama, M., Condon, B., Ascari J. P., Dupuis, J. Del Ponte, E. M., Pedley, K. F., Martinez S., Valent, B., Farman, M. L. 2021. Recombination of standing variation in a multi-hybrid swarm drove adaptive radiation in a fungal pathogen and gave rise to two pandemic plant diseases. bioRxiv 2021.11.24.469688; doi: https://doi.org/10.1101/2021.11.24.469688

Reges, J. T. de A., Jesus, M. N., Silva, S. D. R. da, Souza, M. H., and Rodrigues, J. W. 2019. Teste de patogenicidade dos isolados de *Pyricularia oryzae* nos hospedeiros de trigo, cevada, arroz e braquiária. Rev. Cult. Agron. 28:*19*

Reges, J. T. de A., Negrisoli, M. M., Dorigan, A. F., Castroagudín, V. L., Maciel, J. L. N., and

Ceresini, P. C. 2016. *Pyricularia pennisetigena* and *P. zingibericola* from invasive grasses infect signal grass, barley and wheat. Pesq. Agropec. Trop. 46:206–214. Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods, 9:671-675

Stukenbrock, E. H., and McDonald, B. A. 2008. The origins of plant pathogens in agro-ecosystems. Annu. Rev. Phytopathol. 46:75–100.

Talbot, N. J., Salch, Y. P., Ma, M., and Hamer, J. E. 1993. Karyotypic variation within clonal lineages of the rice blast fungus, *Magnaporthe grisea*. Appl. Environ. Microbiol. 59:585–593.

Tembo, B., Mulenga, R. M., Sichilima, S., M'siska, K. K., Mwale, M., Chikoti, P. C., Singh, P. K., He, X., Pedley, K. F., Peterson, G. L., Singh, R. P., and Braun, H. J. 2020. Detection and characterization of fungus (*Magnaporthe oryzae* pathotype Triticum) causing wheat blast disease on rain-fed grown wheat (*Triticum aestivum* L.) in Zambia. PloS one, 15:e0238724

Tembo, B., Mahmud, N. U., Paul, S. K., Asuke, S., Harant, A., Langner, T., Reyes-Avila, C. S., Chanclud, E., Were, V., Sichilima, S., Mulenga, R. M., Gupta, D. R., Mehebub, M. S., Muzahid, A. N. M., Rabby, M. F., Singh, P. K., Bentley, A., Tosa, Y., Croll, D., Kurt, L., Islam, T., Talbot, N. J., Kamoun, S., and Win, J. 2021. Multiplex amplicon sequencing dataset for genotyping pandemic populations of the wheat blast fungus [Data set]. Zenodo. https://doi.org/10.5281/zenodo.4605959

Thierry, M., Gladieux, P., Fournier, E., Tharreau, D., and Ioos, R. 2020. A Genomic approach to develop a new qPCR test enabling detection of the *Pyricularia oryzae* lineage causing wheat blast. Plant Dis. 104:60–70.

Tosa, Y., and Chuma, I. 2014. Classification and parasitic specialization of blast fungi. J. Gen. Plant Pathol. *80:*202–209.

Tosa, Y., Hirata, K., Tamba, H., Nakagawa, S., Chuma, I., Isobe, C., Osue, J., Urashima, A. S., Don, L. D., Kusaba, M., Nakayashiki, H., Tanaka, A., Tani, T., Mori, N., and Mayama, S. 2004. Genetic constitution and pathogenicity of *Lolium* isolates of *Magnaporthe oryzae* in comparison with host species-specific pathotypes of the blast fungus. Phytopathology 94:454–462.

Trindade, M., Siqueira, M., Silva, H., and Prabhu, A. 2006. Danos causados por *Magnaporthe grisea* em trigo (Comunicado Técnico Online 202). Online at: http://trigo.cnpt.embrapa.br/biblio/co/p_co202.htm

Urashima, A. S., Alves, A. F., Silva, F. N., Oliveira, D., and Gazaffi, R. 2017. Host range, mating type and population structure of *Magnaporthe* sp. of a single barley field in São Paulo state. Brazil. J. Phytopathol. 165:414–424.

Urashima, A. S., Igarashi, S., and Kato, H. 1993) Host range, mating type, and fertility of *Pyricularia grisea* from wheat in Brazil. Plant Dis. 77:1211–1216.

Urashima, A. S., Martins, T. D., Bueno, C. R. N. C., Favaro, D. B., Arruda, M. A., and Mehta, Y. R. 2004. Triticale and barley: new hosts of *Magnaporthe grisea* in São Paulo, Brazil - Relationship with blast of rice and wheat. In S. Kawasaki (Ed.), Rice Blast: Interaction with Rice and Control (pp. 251–260). Springer Netherlands.

Valent, B., and Chumley, F. G. 1991. Molecular genetic analysis of the rice blast fungus, *Magnaporthe grisea*. Annu. Rev. Phytopathol. 29:443–467.

Valent, B., Farman, M., Tosa, Y., Begerow, D., Fournier, E., Gladieux, P., Islam, M. T., Kamoun, S., Kemler, M., Kohn, L. M., Lebrun, M.-H., Stajich, J. E., Talbot, N. J., Terauchi, R., Tharreau, D., and Zhang, N. 2019. *Pyricularia graminis-tritici* is not the correct species name for the wheat blast fungus: Response to Ceresini et al. (MPP 20:2). Mol. Plant Pathol. 20:173–179.

Vicentini, S. N. C., Hawkins, N. J., King, K. M., Moreira, S. I., de Paiva Custódio, A. A., Leite Júnior, R. P., Portalanza, D., Garcés-Fiallos, F. R., Krug, L. D., West, J. S., and Fraaije, B.A., Jesus-Junior, W. C., Cereini, P. C. 2023. Aerobiology of the wheat blast pathogen: inoculum monitoring and detection of fungicide resistance alleles. Agronomy 13:1238.

Xavier Filha, M. S., Rodrigues, F. A., Domiciano, G. P., Oliveira, H. V., Silveira, P. R., and Moreira, W. R. 2011. Wheat resistance to leaf blast mediated by silicon. Aust. Plant Pathol. 40:28–38.

Yasuhara-Bell, J., Pedley, K. F., Farman, M., Valent, B., and Stack, J. P. 2018. Specific detection of the wheat blast pathogen (*Magnaporthe oryzae* Triticum) by loop-mediated isothermal amplification. Plant Dis. 102:2550–2559.

Yu, G., Smith, D. K., Zhu, H., Guan, Y., and Lam, T. T.-Y. 2017. ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol. Evol. 8:28–36.

Zadoks, J. C., Chang, T. T., and Konzak, C. F. 1974. A decimal code for the growth stages of cereals. Weed Res. 14:415–421.

TABLE 1. Tentative identification of *Pyricularia oryzae* lineages, as well as *P. grisea*, *P. pennisetigena*, and *P. urashimae* based on MoT3/C17, CH7BAC9, MPG1 amplification[a]

MoT3	C17	CH7BAC9	MPG1	Possible lineages
+	+	+	+	PoT
-	+	+	+	PoE3, PoM, PoT, PoX[a]
+	-	+	+	PoU3, *P. urashimae*[a]
-	-	+	+	PoL1, other *P. oryzae*[a]
-	-	-	+	*P. grisea*, *P. pennisetigena*

[a] Definitive identification of lineage will require sequencing of additional loci, genotyping-by-sequencing, or whole genome sequencing.

925

926 TABLE 2**.** Information for isolates obtained from wheat or signalgrass in Minas Gerais state,
927 Brazil, and which were used in replicated cross-inoculation experiments.
928

Code	Host of Origin	Municipality	Collection date	IDa
UFVPY108	*Urochloa brizantha*	Patos de Minas	Feb. 2018	PoU3
UFVPY110	*U. brizantha*	Patos de Minas	Feb. 2018	PoU3
UFVPY112	*U. brizantha*	Patos de Minas	Feb. 2018	PoU3
UFVPY166	*U. brizantha*	Uberaba	May 2018	PoU4
UFVPY209	*U. brizantha*	Formiga	Feb. 2019	Pu
UFVPY656	*U. brizantha*	Catas Altas da Noruega	Feb. 2019	Pu
UFVPY742	*U. brizantha*	Madre de Deus	May 2019	PoT
UFVPY758	*U. brizantha*	Madre de Deus	May 2019	PoT
UFVPY213	*U. humidicola*	São Gonçalo do Pará	Feb. 2019	PoT
UFVPY167	*Triticum aestivum*	Patos de Minas	May 2018	PoT
UFVPY238	*T. aestivum*	Ibiá	May 2019	PoT
UFVPY239	*T. aestivum*	Ibiá	May 2019	PoT
UFVPY367	*T. aestivum*	Uberaba	May 2019	PoT
UFVPY375	*T. aestivum*	Santa Juliana	May 2019	PoT
UFVPY376	*T. aestivum*	Patrocínio	May 2019	PoT
UFVPY309	*T. aestivum*	Boa Esperança	May 2019	PoT
UFVPY311	*T. aestivum*	Boa Esperança	May 2019	PoT
UFVPY604	*T. aestivum*	Madre de Deus	May 2019	PoT
UFVPY813	*T. aestivum*	Boa Esperança	May 2019	PoT
MoT01	*T. aestivum*	Passo Fundo, RS	2019	PoT

929
930 a The separation between PoT *(Pyricularia oryzae Triticum* pathotype) and non-PoT isolates was performed using
931 MoT3 and C17 primers in a PCR assay (see table 1). The non-PoT lineages were identified based on genotyping
932 by sequencing data. PoU = *Pyricularia oryzae* lineage *Urochloa*; and Pu = *Pyricularia urashimae* (see table S1*)*

933

TABLE 3. Number of plant samples and isolates obtained from of Poaceae plants during visits to both wheat-producing regions (Triângulo Mineiro and Centro-Sul de Minas) and natural landscapes during summer (February, wheat off-season) and fall (May, wheat-growing season) 2018 and 2019, MG, Brazil.

Poaceae species[a]	N. of plant samples		N. of *Pyricularia* spp. isolates [d]
	Total [b]	Blast-infected [c]	
Cenchrus echinatus	21	15	25
Cynodon dactylon	6	1	1
C. plectostachyus	1	1	1
Digitaria horizontalis	37	14	21
D. insularis	46	15	20
D. sanguinalis	61	21	31
Echinochloa colonum	2	1	1
Eleusine indica	50	27	42
Melinis roseum	20	16	28
Panicum maximum	127	10	19
Pennisetum sp.	28	2	2
Triticum aestivum	505	377	670
U. brizantha	329	35	59
U. humidicola	16	3	7
U. plantaginea	24	3	3
U. ruziziensis	8	1	2
Total	1,281	542	932

[a] Additional plant species that were found with symptoms but from which no *Pyricularia* isolate was obtained: *Andropogon virginicus*, *Chloris polydactyla, Cynodon dictyoneura, Cyperus rotundus, Eragrostis ciliaris, Eragrostis pilosa, Imperata brasiliensis, Melinis minutiflora, P. miliaceum, Paspalum notatum, Setaria viridis, Sorghum arundinaceum, Urochloa arrecta and U. decumbens*

[b] Sample composed of 5 to 10 leaves of wheat or Poaceae weeds, or 1 to 3 wheat heads.

[c] At least one *Pyricularia* sp. isolate.

[d] Number of monoconidial isolates per sample. In cases more than one isolate was obtained from a sample, but not from the same tissue (leaf or head).

950 TABLE 4. Summary results of PCR assays targeting Pyricularia oryzae and P. oryzae Triticum
951 pathotype, in a subcollection of Pyricularia spp. strains from 16 Poaceae hosts recovered from wheat
952 (leaf and head) and grass weeds (leaves) grown at wheat-producing regions and natural landscapes
953 during summer (February, pre-season) and fall (May, wheat-growing season) of 2018 and 2019 seasons
954 in Minas Gerais, Brazil.
955

Host of isolation	*Pyricularia* sp.	*P. oryzae*	Lineages	
			PoT (proximity)[a]	Non-PoT
Cenchrus echinatus	20	2	1 (away)	1
Cynodon dactylon	1	0	-	-
Cynodon plectostachyus	1	1	-	1
Digitaria horizontalis	21	2	-	2
Digitaria insularis	20	1	-	1
Digitaria sanguinalis	18	5	-	5
Echinochloa colonum	1	0	-	-
Eleusine indica	34	32	2 (nearby)	30
Panicum maximum	16	16	1 (nearby)	15
Pennisetum sp.	2	1	1 (away)	-
Melinis roseum	28	28	1 (away)	27
Triticum aestivum	333	331	324	7
Leaf	127	127	126	1
Head	206	204	198	6
Urochloa brizantha	57	56	2 (nearby)	54
Urochloa humidicola	7	7	1 (away)	6
Urochloa plantaginea	3	2	-	2
Urochloa ruziziensis	2	2	-	2
Total	564	483	329	154

956 [a] distance of the isolates obtained from a grass plant relative to wheat fields. Nearby = less than 1km and away =
957 more than 50 km.

958

959 TABLE 5. Frequencies of positive (indicating a *Pyricularia oryzae Triticum* lineage - PoT) and negative
960 (indicating a non-PoT lineage) amplifications of the C17 for a set of 487 *Pyricularia* isolates obtained
961 from leaves of grass plants located away or nearby wheat fields or from leaves or heads of wheat plants
962 displaying the typical blast symptoms across several locations in MG state, Brazil.
963

Host	Proximity to wheat	C17+ (PoT) Count (%)	C17- (non-PoT) Count (%)	Sum
Wheat	-	322 (97.9%)	7 (2.1%)	329
Grass	Nearby (< 1 km)	7 (10.6%)	59 (89.3%)	66
	Away (> 30km)	2 (2.1%)	90 (98.1%)	92
Sum		331	156	487

964

TABLE 6. Phylogenetic lineage affiliations of Pyricularia isolates obtained from wheat, and endemic/cultivated grasses in MG, Brazil

Host of isolation	Phylogenetic Lineage Affiliation[a]									Canonical host (%)[b]
	PoC	PoE1/2/3	PoEc	PoM	PoT/L	PoU3/4	Pg	Pp	Pu	
Triticum/Lolium		2		1	304	4			1	97
Cenchrus					1			23		96
Cynodon	(1)[c]							1		(50)c
Digitaria		4					45			92
Eleusine		36	3		2					88
Melinis				7		1				88
Panicum					1	1			12	86
Pennisetum					1					0
Urochloa[d]				2	3	19		24		90

[a] Lineages were named according to the primary host from which most members were isolated: PoE = *Eleusine*, PoEc = *Echinochloa*, PoM = *Melinis*; PoU = *Urochloa*; Pg = *P. grisea*, Pp = *P. pennisetigena*; Pu = *P. urashimae*. Numerical suffixes identify phylogenetically distinct lineages found on a single host genus.

[b] Percentage of isolates belonging to a lineage whose members are normally associated with specified host-of-origin.

[c] Tentative assignment because the lone isolate defines a new lineage (PoC2) for which no other members have yet been identified.

d Host-specialized isolates from Urochloa belonged to two main lineages - one, being PoU3 and the other bearing haplotypes consistent with P. urashimae.

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Figure titles

Fig. 1. Map of Minas Gerais (MG) state, Brazil, depicting wheat area (in hectares) planted per municipality (color gradient) and the locations of the sampling sites where blast-symptomatic Poaceae (wheat and grasses) were collected (dots). Source: (IBGE, 2017).

Fig. 2. Maximum likelihood tree for CH7BAC9 tree showing phylogenetic placement of *P. oryzae*/*P. urashimae* isolates. Tip labels are provided only for isolates collected in this study and are colored according to the host-of-origin, as are the branches for the unlabeled reference isolate. Phylogenetic lineages showing obvious host-specialization are highlighted with gray boxes and named according to primary host as follows: PoEc, *Echinochloa*; PoE/PoE3, *Eleusine*, PoM, *Melinis*; PoO/PoS *Oryza/Setari*a; PoU3, *Urochloa*; and Pu, *Pyricularia urashimae*. Note that PoT and PoL1 are not highlighted because constituent isolates variously inherited CH7BAC9 from members of four other host-specialized populations. Nodes with bootstrap support ≥ 0.7 are highlighted with circles. The tree was drawn using ggtree with the branch.length = "none" option, with the intention to show grouping patterns among isolates from the same host. No inferences can be drawn from branch lengths.

Fig. 3. Maximum likelihood tree for MPG1 showing phylogenetic placement of *Pyricularia* isolates collected from *Cenchrus*, *Digitaria*, *Panicum* and *Urochloa*. For clarity, tip labels are shown only for the isolates from MG, and their prefixes are in lowercase and abbreviated. Bootstrap values are provided on key nodes. Tip labels are colored according to host-of-origin. The species designation for each phylogenetic clade is also shown. The tree was drawn using ggtree with the branch.length = "none" option to show grouping patterns among isolates from the same host. No inferences can be drawn from branch lengths.

Fig. 4. Maximum Likelihood tree showing phylogenetic placement of *Pyricularia* isolates from MG as determined using "MonsterPlex" genotyping-by-sequencing. Isolate names are colored according to host-of-origin and those from MG are identified with a UVFPY prefix. Phylogenetic lineages are highlighted with black lines and are labeled according to the primary host (as noted in Figure 2). PoU3 forms a subgroup of PoSt but is labeled separately to emphasize that it constitutes a key, *Urochloa*-infecting lineage. Nodes with bootstrap support ≥ 0.7 are highlighted with circles.

Fig. 5. Aggressiveness, expressed as percentage leaf area affected (% severity), evaluated in replicated greenhouse experiments (foliar inoculations on two wheat cv. [BR18 Terena and Guamirim and one signalgrass cv [Marandu]), for a set of 19 isolates, all with prefix UFVPY, (10 from wheat [*Triticum aestivum*], and 9 [six non-PoT] and three PoT = 742, 758 and 213] from signalgrass [*Urochloa* spp.]) of *Pyricularia oryzae* collected in MG state, Brazil and which showed a positive (indicating Triticum lineage) or a negative (indicating non-Triticum lineage) reaction when screened using the C17 primer set. The non-PoT isolates were further identified with genotype by sequencing: isolates 108, 110 and 112 = *Pyricularia oryzae* lineage *Urochloa3*; 166 = *P. oryzae* lineage *Urochloa4;* 209 and 656 = *P. urashimae*. The MoT01 strain is PoT used as a reference for an aggressive isolate collected in Passo Fundo, Brazil, and used (coded as 16MoT001) in screening for host resistance studies (Cruppe et al. 2020). The empty circles represent values of replicates (two experiments combined), the symbols represent the mean values and the error bar is the 95% confidence limit.

Fig. 6. Aggressiveness, expressed as percentage of affected spikelets (% severity), evaluated in replicated greenhouse experiments (head inoculations on two wheat cv., BR18 Terena and BRS Guamirim) for a set of 19 isolates, all with prefix UFVPY, (10 from wheat [*Triticum aestivum*], and 9 [six non-PoT and three PoT = 742, 758 and 213] from signalgrass [*Urochloa* spp.]) of *Pyricularia oryzae* collected in MG state, Brazil and which showed a positive (indicating Triticum lineage) or a negative (indicating non-Triticum lineage) reaction when screened using the C17 primer set. The non-POT isolates were further identified with genotype by sequencing: isolates 108, 110 and 112 = *Pyricularia oryzae* lineage *Urochloa3*; 166 = *P. oryzae* lineage *Urochloa4;* 209 and 656 = *P. urashimae*. The MoT01 strain is a reference for an aggressive isolate collected in Passo Fundo, Brazil, and used (coded as 16MoT001) in screening for host resistance studies (Cruppe et al. 2020). The empty circles

represent values of replicates (two experiments combined), the symbols represent the mean values and the error bar is the 95% confidence limit.

Fig. 7. Blast symptoms on wheat (Triticum aestivum) (A, B) and signalgrass (Urochloa brizantha) (C, D) leaves resulting from inoculation of *Pyricularia oryzae Triticum* lineage [isolate 16MoT001] isolated from wheat (B, D) and Pyricularia oryzae *Urochloa* lineage 3 [UFVPY112] isolated from signalgrass (A, C). The numbers below each section of leaf image represent the percent area covered by the symptoms.

Fig. 8. Wheat head blast symptoms resulting from spray-inoculation (during wheat anthesis) of *Pyricularia oryzae Urochloa* lineage 3 (UFVPY112), isolated from signalgrass (A) and *P. oryzae Triticum* lineage (16MoT001), isolated from wheat (B)

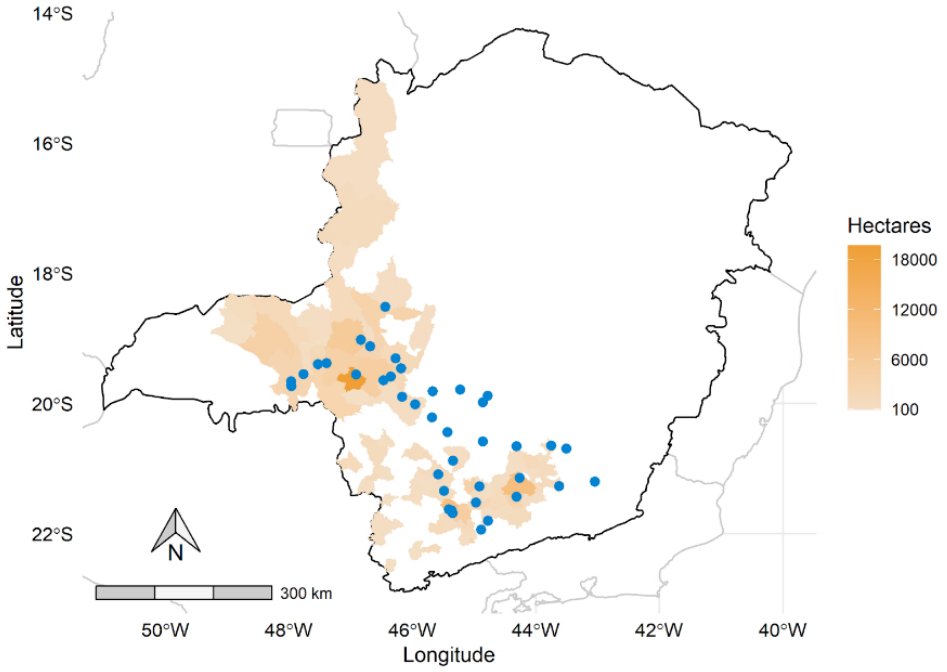
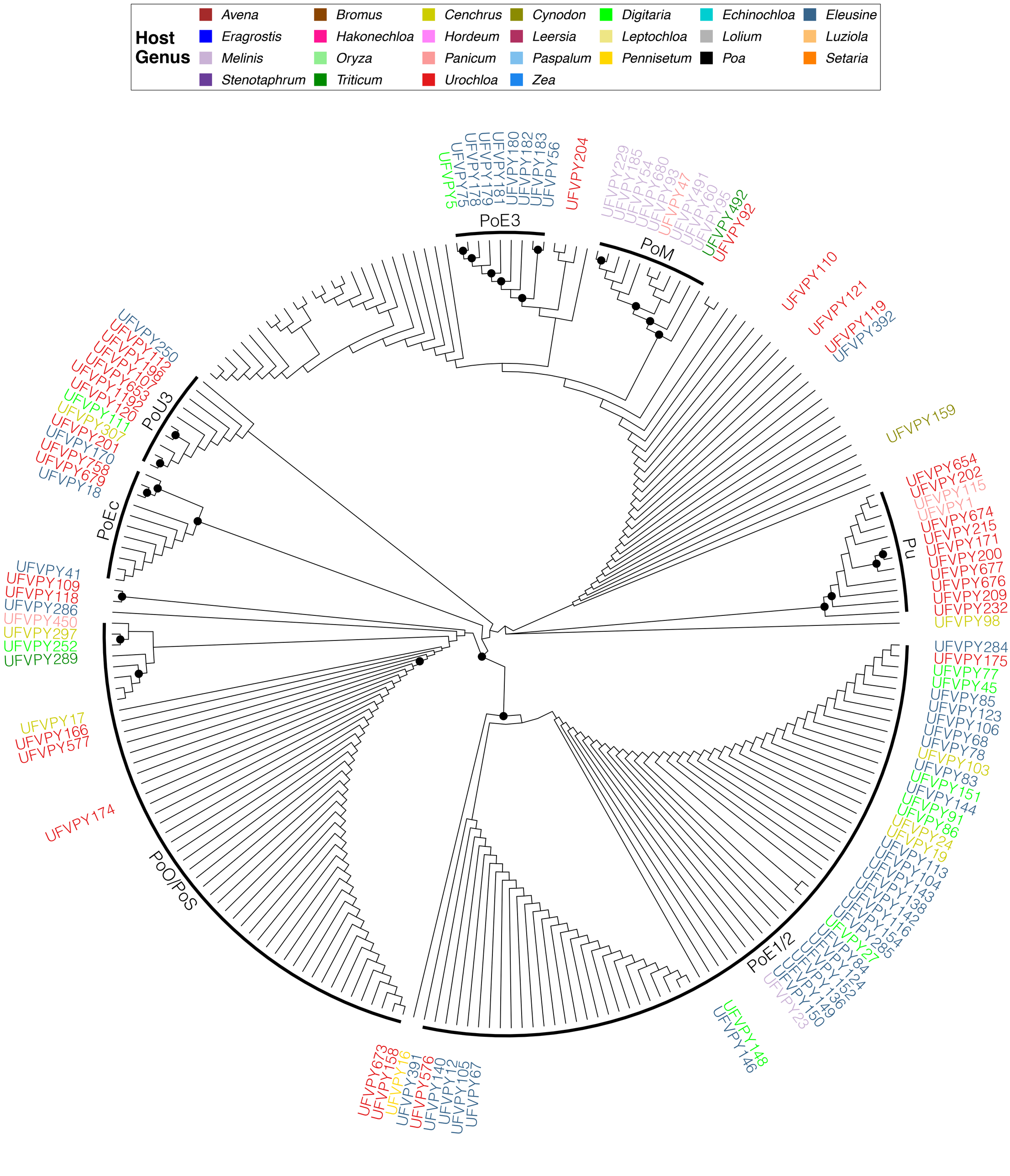


Fig. 1. Map of Minas Gerais (MG) state, Brazil, depicting wheat area (in hectares) planted per municipality (color gradient) and the locations of the sampling sites where blast-symptomatic Poaceae (wheat and grasses) were collected (dots). Source: (IBGE, 2017).

325x228mm (72 x 72 DPI)


Host Genus
Avena
Bromus
Cenchrus
Cynodon
Digitaria
Echinochloa
Eleusine
Eragrostis
Hakonechloa
Hordeum
Leersia
Leptochloa
Lolium
Luziola
Melinis
Oryza
Panicum
Paspalum
Pennisetum
Poa
Setaria
Stenotaphrum
Triticum
Urochloa
Zea
PoE3
PoM
PoU3
PoEc
Pu
PoO/PoS
PoE1/2
UFVPY5
UFVPY178
UFVPY179
UFVPY181
UFVPY180
UFVPY182
UFVPY183
UFVPY56
UFVPY204
UFVPY229
UFVPY185
UFVPY54
UFVPY680
UFVPY93
UFVPY47
UFVPY491
UFVPY60
UFVPY95
UFVPY492
UFVPY92
UFVPY110
UFVPY121
UFVPY119
UFVPY392
UFVPY159
UFVPY654
UFVPY202
UFVPY115
UFVPY1
UFVPY674
UFVPY215
UFVPY171
UFVPY200
UFVPY677
UFVPY676
UFVPY209
UFVPY232
UFVPY98
UFVPY284
UFVPY175
UFVPY77
UFVPY45
UFVPY85
UFVPY123
UFVPY106
UFVPY68
UFVPY78
UFVPY103
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UFVPY144
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UFVPY154
UFVPY285
UFVPY21
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UFVPY105
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UFVPY577
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UFVPY297
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UFVPY198
UFVPY107
UFVPY655
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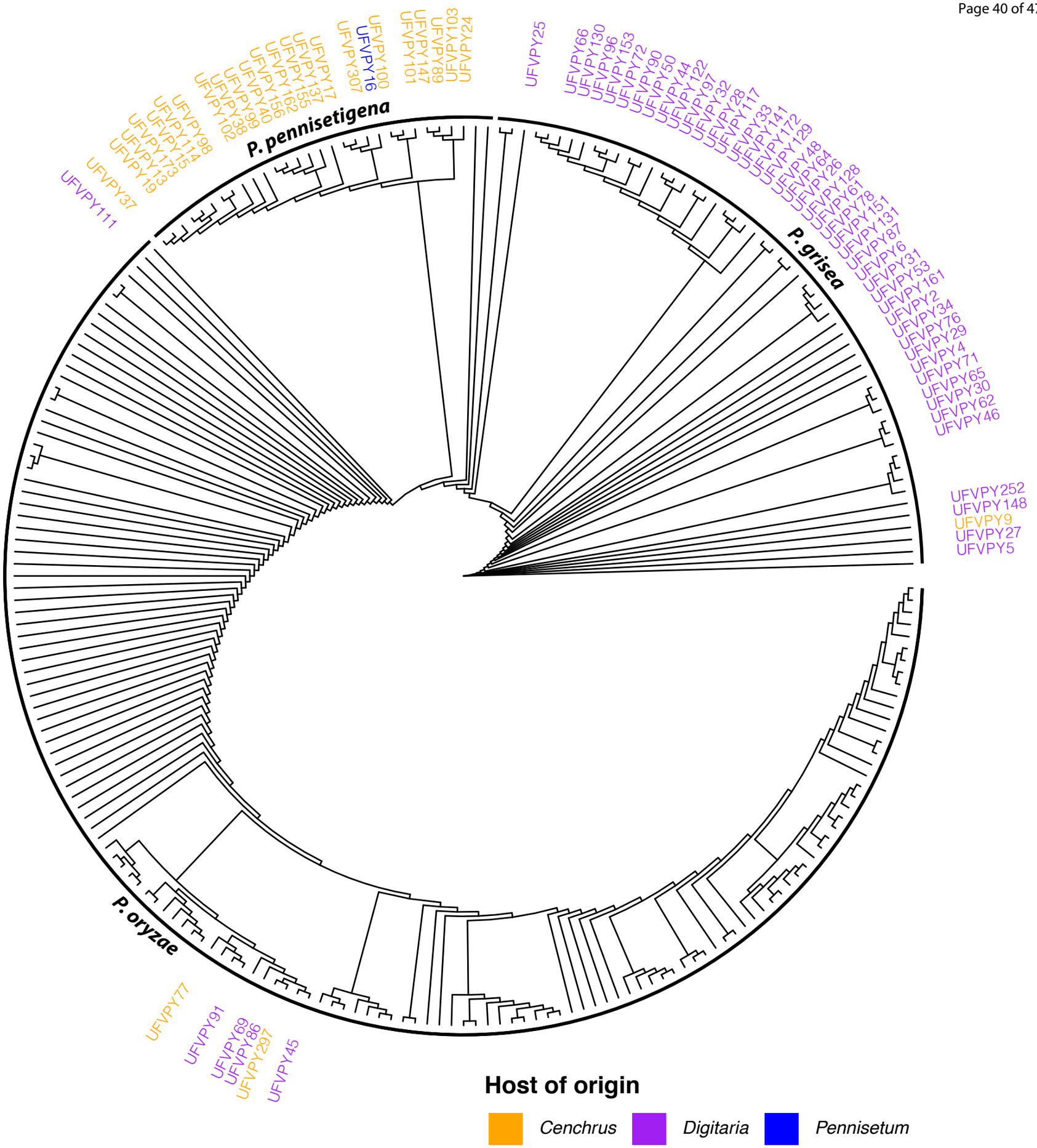
UFVPY170
UFVPY758
UFVPY679
UFVPY18


P. pennisetigena
P. grisea
P. oryzae
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UFVPY37
UFVPY19
UFVPY133
UFVPY173
UFVPY15
UFVPY114
UFVPY98
UFVPY102
UFVPY38
UFVPY99
UFVPY40
UFVPY156
UFVPY162
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UFVPY137
UFVPY17
UFVPY307
UFVPY16
UFVPY100
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UFVPY108
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UFVPY48
UFVPY64
UFVPY26
UFVPY128
UFVPY61
UFVPY78
UFVPY151
UFVPY131
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UFVPY53
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UFVPY2
UFVPY34
UFVPY76
UFVPY29
UFVPY4
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UFVPY46
UFVPY252
UFVPY148
UFVPY9
UFVPY27
UFVPY5
UFVPY77
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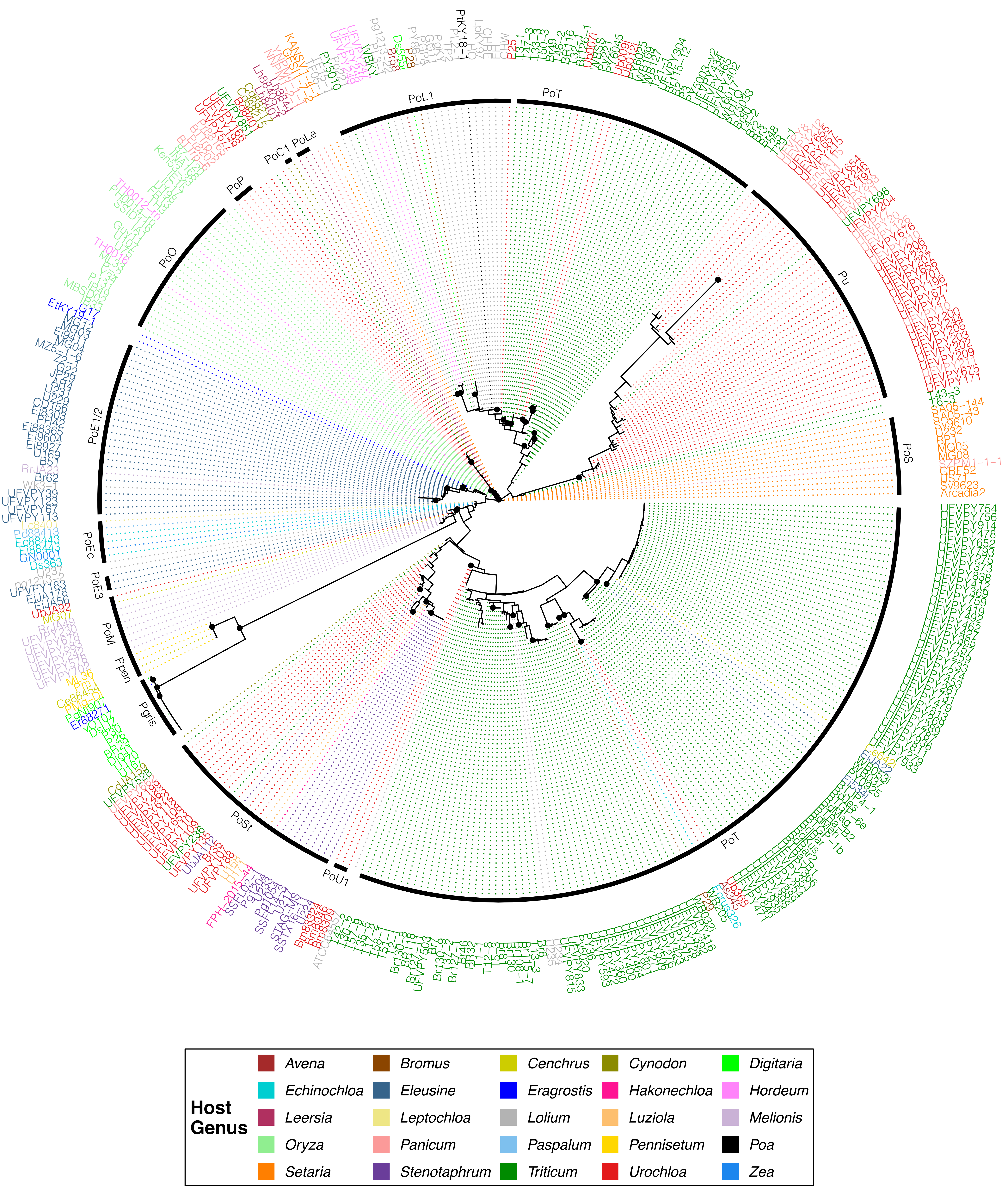
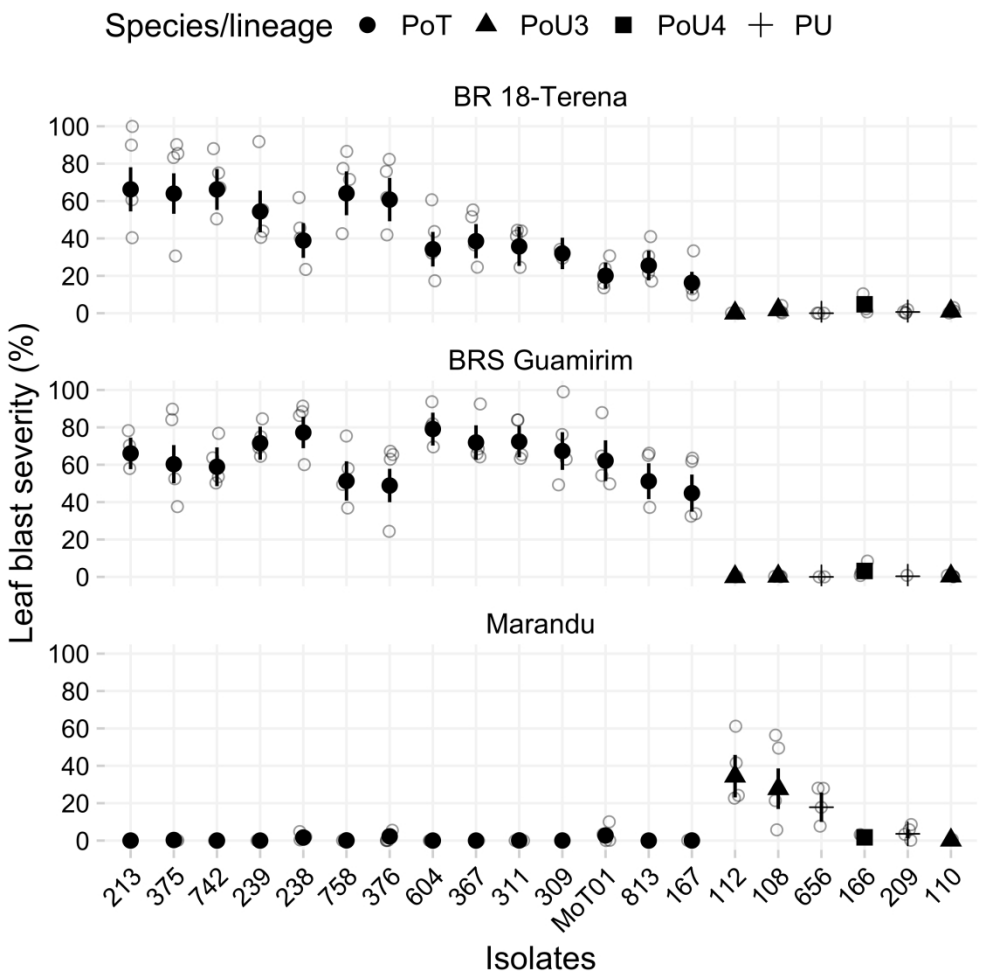
UFVPY69
UFVPY86
UFVPY297
UFVPY45
Host of origin
Cenchrus
Digitaria
Pennisetum


PoL1
PoT
Pu
PoS
PoT
PoU1
PoSt
Pgris
Ppen
PoM
PoE3
PoEc
PoE1/2
PoO
PoP
PoC1
PoLe
Host Genus
Avena
Bromus
Cenchrus
Cynodon
Digitaria
Echinochloa
Eleusine
Eragrostis
Hakonechloa
Hordeum
Leersia
Leptochloa
Lolium
Luziola
Melionis
Oryza
Panicum
Paspalum
Pennisetum
Poa
Setaria
Stenotaphrum
Triticum
Urochloa
Zea



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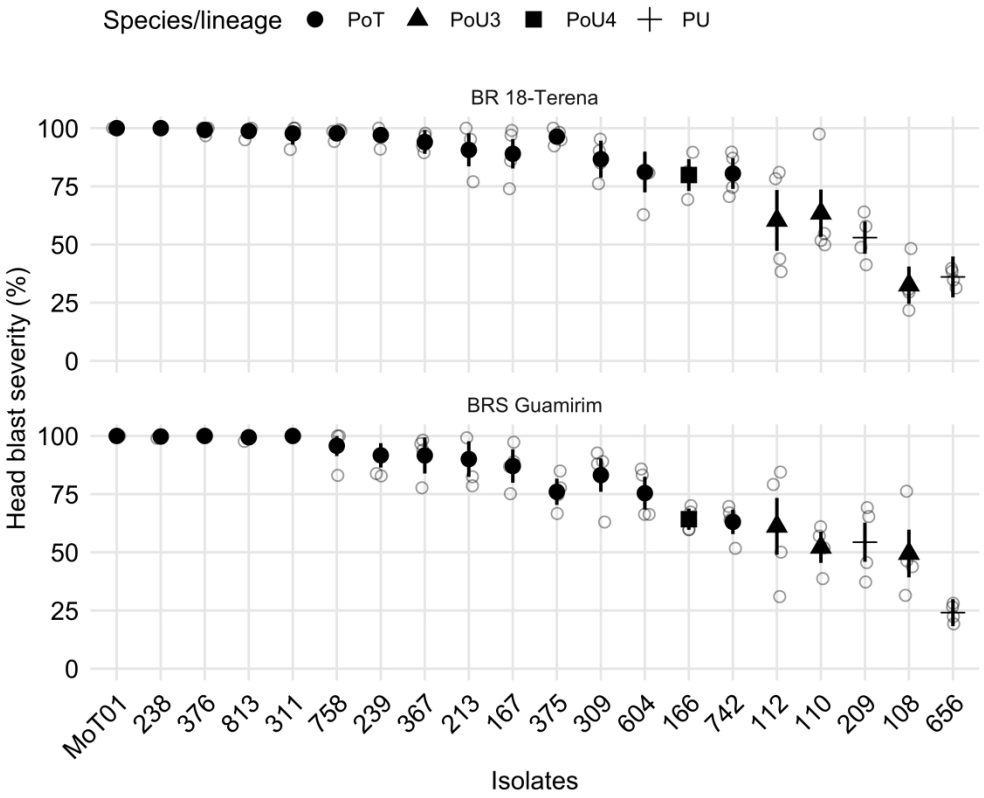

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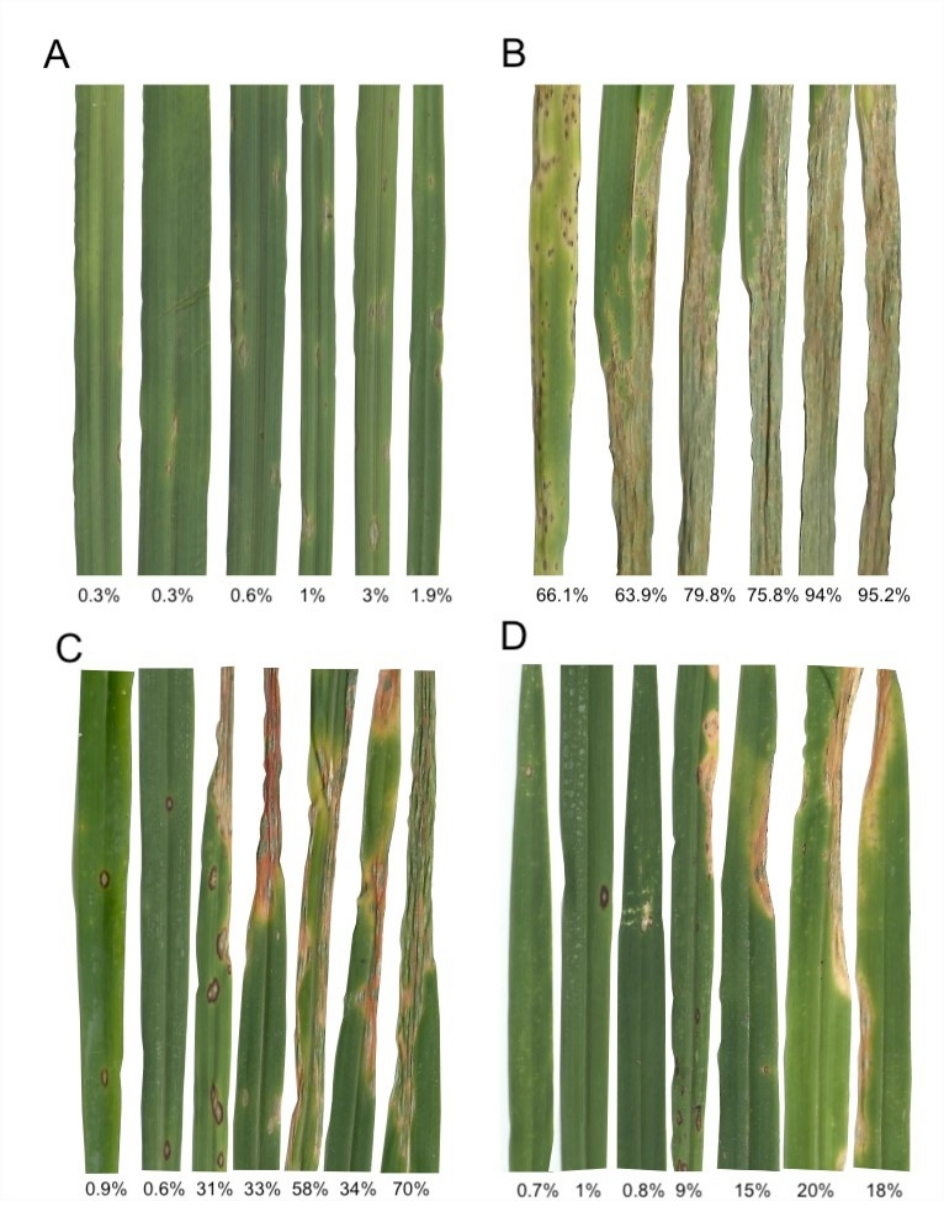

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194x247mm (100 x 100 DPI)

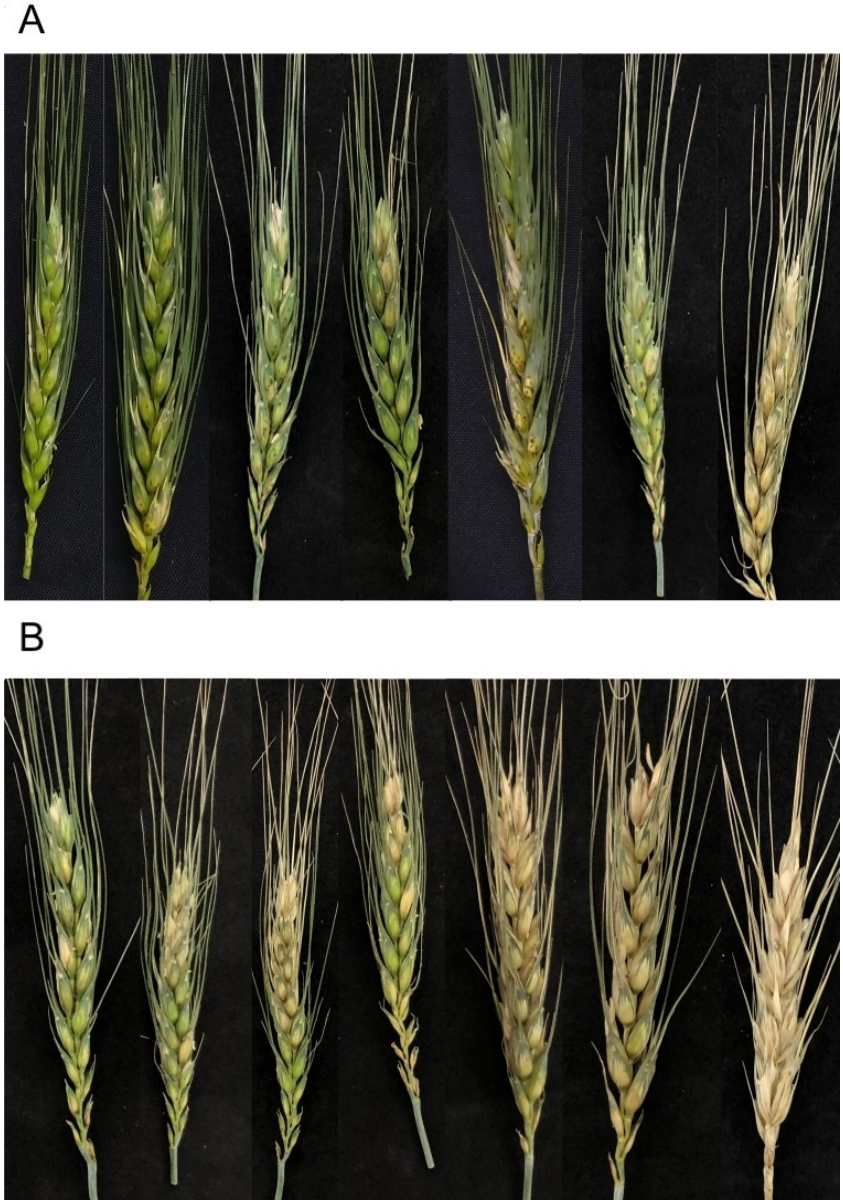


Fig. 8. Wheat head blast symptoms resulting from spray-inoculation (during wheat anthesis) of Pyricularia oryzae Urochloa lineage 3 (UFVPY112), isolated from signalgrass (A) and P. oryzae Triticum lineage (16MoT001), isolated from wheat (B)

192x276mm (100 x 100 DPI)

Grass weeds infestation in wheat off-season

Grass landscape at non-wheat region

Wheat field surrounded by grass weeds

Grass landscape at non-wheat region

Wheat experimental area infested by grass weeds

Grass weeds roadside in non-wheat region

Wheat plots surrounded by blasted grass weeds

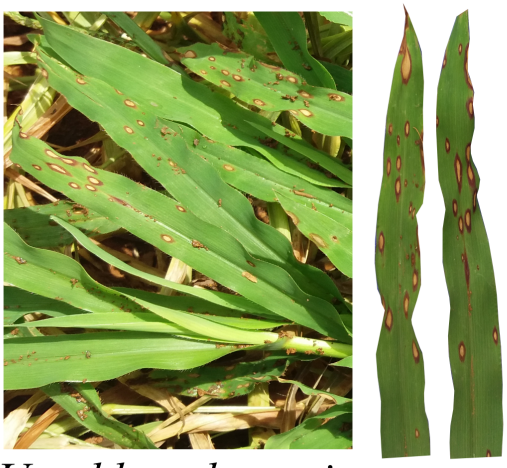
Natural landscape covered by many grass species

Senescent wheat leaves with active blast sporulation

Natural landscape covered by many grass species

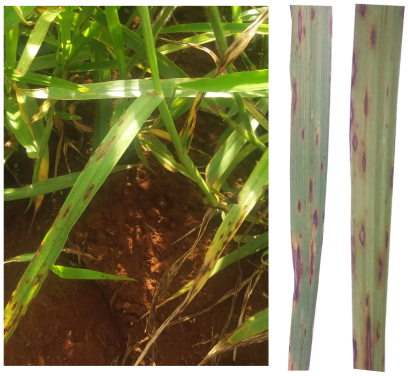
Panicum maximum

Eleusine indica

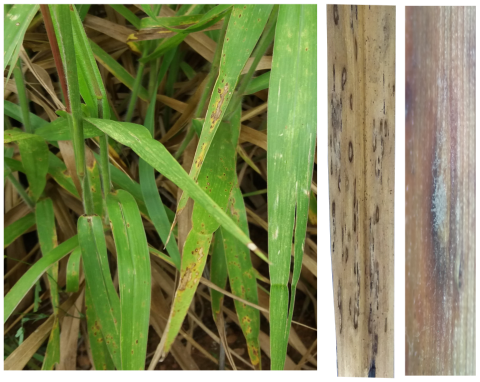


Urochloa plantaginea

Rhynchelytrum roseum

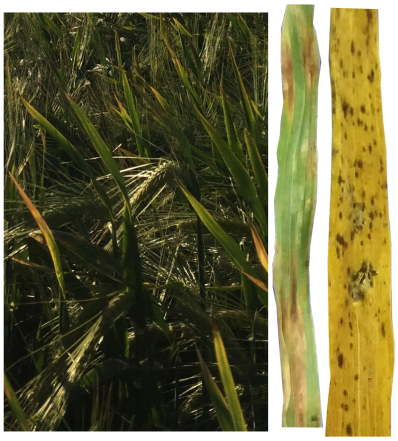


Cenchrus echinatus



Urochloa brizantha

Digitaria sanguinalis



Hordeum vulgare

Urochloa ruziziensis

Pennisetum sp.

Digitaria horizontalis

Urochloa humidicula