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# Cautionary Notes on Use of the MoT3 Diagnostic Assay for *Magnaporthe oryzae* Wheat and Rice Blast Isolates

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**ABSTRACT.** The blast fungus *Magnaporthe oryzae* is comprised of lineages that exhibit 18 19 varying degrees of specificity on about 50 grass hosts, including rice, wheat and barley. 20 Reliable diagnostic tools are essential given that the pathogen has a propensity to jump to new 21 hosts and spread to new geographic regions. Of particular concern is wheat blast, which has 22 suddenly appeared in Bangladesh in 2016 before spreading to neighboring India. In these 23 Asian countries, wheat blast strains are now co-occurring with the destructive rice blast 24 pathogen raising the possibility of genetic exchange between these destructive pathogens. We 25 assessed the recently described MoT3 diagnostic assay and found that it did not distinguish between wheat and rice blast isolates from Bangladesh. The assay is based on primers 26 27 matching the WB12 sequence corresponding to a fragment of the M. oryzae MGG 02337 28 gene annotated as a short chain dehydrogenase. These primers could not reliably distinguish 29 between wheat and rice blast isolates from Bangladesh based on DNA amplification 30 experiments performed in separate laboratories in Bangladesh and in the UK. Specifically, all 31 eight rice blast isolates tested in this study produced the WB12 amplicon. In addition, 32 comparative genomics of the WB12 nucleotide sequence revealed a complex underlying 33 genetic structure with related sequences across M. oryzae strains and in both rice and wheat blast isolates. We, therefore, caution against the indiscriminate use of this assay to identify 34 35 wheat blast and encourage further development of the assay to ensure its value in diagnosis.

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### 38 INTRODUCTION

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Outbreaks caused by fungal diseases have increased in frequency and are a chronic 40 41 threat to global food security (Fisher et al. 2012). A prime case is blast, a disease caused by 42 the ascomycete fungus Magnaporthe oryzae (syn. Pyricularia oryzae), which is best known 43 as the most destructive disease of rice. However, in addition to rice, M. oryzae can also infect 44 other cereal crops such as wheat, barley, oat and millet destroying food supply that could feed 45 hundreds of millions of people (Pennisi 2010; Fisher et al. 2012; Liu et al. 2014). Increased 46 global trade, climate change, and the propensity of this pathogen to occasionally jump from 47 one grass host to another, have resulted in increased incidence of blast diseases. For example, 48 only a few decades ago blast was not known to affect wheat, a main staple crop critical to 49 ensuring global food security. But in 1985, blast disease on wheat was first reported in Paraná 50 State, Brazil (Igarashi et al. 1986). It has since spread throughout Brazil and subsequently 51 moved into neighboring South American countries where it is now a major threat to wheat 52 production (Goulart et al. 1992; Goulart et al. 2007; Kohli et al. 2011). Currently, wheat blast 53 affects as many as 3 million hectares of cultivated wheat, seriously limiting the potential for 54 wheat production in the vast grasslands region of South America.

55 More recently, in February 2016, wheat blast was detected for the first time in Asia. 56 following reports of a severe outbreak in Bangladesh (Islam et al. 2016). The outbreak was 57 particularly destructive, affecting  $\sim 16\%$  of the cultivated wheat area in Bangladesh and with 58 yield losses reaching up to 100% (Islam et al. 2016). In 2017, the pathogen spread to the 59 neighboring West Bengal region of India and hundreds of hectares were cleared by burning to 60 limit the accumulation and spread of the pathogen according to local press reports. An open 61 source population genomics project led by Islam *et al.* (2016) revealed that the Bangladeshi 62 wheat blast outbreak was caused by a South American genotype of *M. oryzae*, and therefore 63 most likely introduced from South America (OpenWheatBlast http://wheatblast.net). The 64 2016 Bangladeshi epidemic vividly illustrates the clear and present danger caused by blast 65 diseases in an era of global trade. It is especially worrisome because blast could spread further to other wheat-producing areas in South Asia, such as India and Pakistan, thus threatening 66 67 food security across South Asia.

68 Until recently, the genetic structure of *M. oryzae* remained somewhat unclear. Whole-69 genome sequence analyses of 76 isolates from 12 different grass hosts confirmed the status of 70 *M. oryzae* as a single species and revealed genetic exchanges among the different host71 specific lineages (Gladieux et al. 2018). Overall, M. oryzae lineages exhibit low levels of nucleotide polymorphisms within single-copy genes, with  $\pi$  ranging from 7.75e<sup>-04</sup> to 1.24e<sup>-03</sup> 72 in the various host-specific lineages of this species. This is one order of magnitude lower than 73 74 divergence between M. oryzae and its closest relatives Magnaporthe grisea and Magnaporthe 75 pennisetigena (Gladieux et al. 2018). Nonetheless, the different lineages of M. oryzae exhibit 76 significant genome plasticity probably due to the activity of transposable elements and 77 selection for optimal disease effector repertoires (Yoshida et al. 2016). Notably, effector 78 genes often exhibit presence/absence polymorphisms, and particular effector genes can be 79 associated with a given lineage probably contributing to the specialization of individual genotypes to certain hosts (Inoue et al. 2017). 80

Given the propensity of *M. oryzae* to jump hosts and spread to new geographic regions, 81 82 reliable and cost-effective diagnostic tools are needed to monitor this pandemic pathogen at 83 the genotype level. This is not necessarily a straightforward problem considering the 84 relatively low genetic diversity and potential for gene flow among lineages of M. orvzae. 85 Pieck et al. (2017) recently reported a polymerase chain reaction (PCR) assay diagnostic for 86 wheat blast based on a single molecular marker named MoT3. The assay is based on primers 87 matching the WB12 sequence corresponding to a fragment of the M. oryzae MGG 02337 88 gene annotated as a short chain dehydrogenase. When evaluated with 285 M. oryzae isolates 89 from various hosts, the assay produced the expected amplicon for 113 of 115 wheat blast 90 isolates from North and South America, whereas it did not yield amplicons for the four rice 91 blast isolates tested (Pieck et al. 2017). Here, we report that these primers could not reliably 92 distinguish between wheat and rice blast isolates from Bangladesh based on DNA 93 amplification experiments performed in separate laboratories in Bangladesh and in the UK. In 94 addition, comparative genomics of the WB12 nucleotide sequence revealed a complex 95 underlying genetic structure with WB12 related sequences occurring across M. oryzae strains and in both rice and wheat blast isolates. We, therefore, caution against the indiscriminate use 96 97 of this assay to identify wheat blast. Moving forward, we recommend the design of new 98 primers that unambiguously distinguish between the WB12 sequence from related sequences, 99 and to take into account the distribution of the WB12 sequence across the various lineages of 100 M. oryzae.

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# 103 MATERIALS AND METHODS

105 **MoT3** assay. To confirm that MoT3 primers amplify sequences from wheat blast isolates, we 106 (in Sophien Kamoun's UK Lab) used genomic DNA extracted from a *M. oryzae* wheat blast 107 isolate from Bangladesh (BTJP4-1) and one from Brazilian wheat blast (BR32). To test the 108 specificity of MoT3 primers, we also included genomic DNA from three *M. oryzae* rice blast 109 isolates from Bangladesh (RB9c, RB11a, and RB13-1c) and one from Japan (INA168). In M. 110 Tofazzal Islam's Lab in Bangladesh, genomic DNA was independently extracted from four 111 Bangladeshi wheat blast isolates (BTMP 13-1, BTMP 13-2, BTJP 4-5 and BTJP 4-6) and four rice blast isolates (RB-3b, RB-9d, RB-11b and RB-11d) and one isolate of Colletotrichum sp. 112 113 All remaining procedures for PCR and gel electrophoresis were similar in both labs and are 114 described below. In these experiments, we used PoT2 transposon primers as a positive control 115 for the presence of *M. oryzae* genomic DNA.

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PCR assays with MoT3 primers (MoT3F 5'-GTCGTCATCAACGTGACCAG-3' and MoT3R 117 118 5'-ACTTGACCCAAGCCTCGAAT-3') (Pieck et al. 2017), elongation factor-1a primers 119 (forward 5'-CTYGGTGTTAGGCAGCTCA-3' 5'and reverse 120 GAAMTTGCAGGCRATGTGGG-3'), and – in the Bangladesh lab – PoT2 (forward 5'-CGTCACACGTTCTTCAACC-3' and reverse 5'-CGTTTCACGCTTCTCCG-3') (Harmon et 121 122 al. 2003; Castroagudin et al. 2016) were performed following the methods described in Pieck 123 et al. (2017). Briefly, 50 µl PCR reactions containing 1x DreamTag Green buffer (Thermo-124 Fisher Scientific, Waltham, MA), 0.2 mM dNTPs, 200 nM each primer, 100 ng template 125 genomic DNA, and one unit of DreamTaq polymerase (Thermo-Fisher) were set up and 126 amplification was performed using a C1000 Thermal Cycler (Bio-Rad, Hercules, CA) with 127 the following program: initial denaturation at 94 °C for 60 s; 30 cycles of 94 °C for 30 s, 62 °C for 30 s, 72 °C for 90 s; and extension at 72 °C for 120 s. 128

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PCR products (10 µl each) were run on 1.5% agarose gel and stained with ethidium bromide.
The rest of the PCR products were purified with a PCR purification Kit (Qiagen, Hilden,
Germany) and used for sequencing from both strands by GATC Biotech (Konstanz,
Germany). DNA sequence contigs were assembled from sequencing chromatograms using
Sequencer (Genecodes, Ann Arbor, MI) for each amplicon and exported in fasta format for
further analysis.

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137 Sequences from each amplicon contig were aligned together with genomic regions from
138 several *M. oryzae* isolates corresponding to the target sequence WB12 described in Pieck *et*

*al.* (2017). These regions were identified by BLASTN in the genomic sequences of *M. oryzae*isolates from rice (FR13, GUY11, PH14-rn, TH16), *Eleusine indica* (CD156), *Setaria italica*(US71) and wheat (BR32), as well as one *M. grisea* isolate (BR29) from *Digitalis sanguinalis*[downloaded from <u>http://genome.jouy.inra.fr/gemo/</u> (Chiapello et al. 2015)]. Primer
sequences were removed from the alignment and a gene tree was constructed using
ClustalW2 neighbor joining algorithm and bootstrapped 1000 times.

WB12 sequence similarity analyses. We used BLASTN from BLAST suite version 2.2.26
(Altschul et al. 1997) to search for the WB12 sequence (Pieck et al. 2017) in the genome
assemblies of 67 *M. oryzae* isolates, originating from various hosts as and deposited in
Genbank.

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### 152 RESULTS AND DISCUSSION

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154 MoT3 PCR assays yield similar sized bands with *M. oryzae* wheat and rice blast isolates. 155 To evaluate the MoT3 assay, we first performed PCR using MoT3 primers in the laboratory 156 of MTI in Bangladesh. We used as template genomic DNA of *M. oryzae* isolates collected from wheat (BTMP 13-1, BTMP 13-2, BTJP 4-5, and BTJP4-6) and rice plants (RB-3b, RB-157 158 9d, RB-11b, and RB-11d) that showed blast disease symptoms during the 2016 and 2017 159 epidemics in Bangladesh. We found that both primer pairs amplified amplicons of the 160 expected size (361 and 687 bp for MoT3 and PoT2, respectively) from all genomic DNA 161 from *M. oryzae* isolates (Fig. 1A).

162 We independently repeated the MoT3 assays in The Sainsbury Laboratory, UK, with 163 wheat blast isolate BTJP 4-1 and three rice blast isolates (RB9c, RB11a, and RB13-1c) from 164 Bangladesh, along with the reference wheat isolate BR32 from Brazil and rice isolate INA168 from Japan (Fig. 1B). We used elongation factor (EF) primers as positive control. Both primer 165 166 pairs produced amplicons of the expected size (361 and 722 bp for MoT3 and EF, 167 respectively) with all tested isolates (Fig. 1B, Suppl. Fig. 1). Further experiments performed 168 in consultation with Mark Farman's lab at the University of Kentucky in the U.S. revealed 169 that the annealing temperature, and possibly the brand and age of the thermocycler, influences 170 MoT3 amplifications from rice blast isolates (Suppl. Fig. 1).

172 MoT3 PCR amplifications yield diverse sequences. To further explore the PCR assays 173 shown in Fig. 1 and determine their identity, we sequenced the amplicons (Suppl. Fig. S2). 174 We then constructed a multiple sequence alignment using the amplicon nucleotide sequences 175 (excluding the primer sequences) along with genomic sequences from WB12-like regions of 176 several *M. oryzae* isolates identified by BLASTN search of genome sequences available from 177 the Magnaporthe GEMO Database website (http://genome.jouy.inra.fr/gemo/) (Chiapello et 178 al. 2015) (Fig. 2A). Amplicon sequences from *M. oryzae* from Bangladesh rice blast isolates 179 (RB9c, RB11a, and RB13-1c) showed that they bear different WB12 sequences despite the 180 fact that MoT3 primers were able to produce amplicons with expected size from these isolates 181 (Fig. 2A). The Bangladesh wheat blast isolate shows high sequence identity to the WB12 182 sequence itself, whereas the Bangladeshi rice isolates have a high degree of similarity with 183 genome sequences of reference rice isolates (FR13, GUY11, PH14-rn, and TH16.) and the 184 one isolate from *Eleusine* (CD156). The WB12-like sequence of *M. oryzae* wheat isolate 185 BR32 from Brazil was more similar to those from rice isolates than to the wheat isolates 186 indicating that the distribution of the different WB12 sequences is not strictly correlated with 187 host or origin (Fig. 2B). We compared the sequence chromatograms corresponding to Fig. 3 188 and found that MoT3 reverse primer sequences were recovered in amplicons from various M. 189 orvzae samples including Bangladeshi wheat and rice blast isolates. We conclude that the 190 reverse primer showed mis-priming despite the single mismatch at the 3' extension end (based 191 on FR13 sequence, see below), allowing WB12-like sequences to be amplified. 192

193 Comparative genomics analyses reveal that WB12-paralogous sequences can serve as 194 templates for MoT3 primers. To confirm the amplicon sequencing analyses and assess the 195 diversity and distribution of the WB12 sequence within populations of *M. oryzae*, we 196 searched the genome sequences of 67 M. oryzae isolates from various host plants available in 197 Genbank using BLAST v.2.2.26. We found significant hits with >98% identity across at least 198 400 bp in the majority of isolates from the wheat-infecting lineage, including *Bromus* isolate 199 P29, which was previously grouped as a member of a wheat-infecting lineage (Suppl. Table 200 1). However, two wheat isolates, notably BR32, and a *Bromus* isolate P28 did not yield a 201 BLAST match to WB12 (Suppl. Table 1). We conclude that the WB12 sequence is not 202 present in all wheat-infecting isolates. 203

BLASTN searches also produced sequences related to WB12 (we refer to as WB12-like) in several contigs of the assembled genomes of the 67 *M. oryzae* isolates. The first set of WB12-

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like sequence had 70 to 98% identity to the WB12 sequence over a 400 bp matched region,
and was present in 65 isolates except in US71 and GrF52 from *Setaria* spp. The second set of
WB12-like sequences had 60 to 98% identity over 100 to 400 bp of the matching region, and
was only found in US71 and GrF52. We conclude that the WB12 region is specific to a subset
of wheat-infecting isolates of *M. oryzae* and missing in other lineages (Suppl. Table 1). In
contrast, the WB12-like sequences are present in almost all wheat and non-wheat isolates
(Suppl. Table 1).

We checked the degree to which the MoT3 primers could match the WB12-like sequences. MoT3 primer sequences can be aligned with high identity to the WB12-like sequence. For example, MoT3 primer sequences can be aligned to WB12-like sequence of *M. oryzae* wheat isolate BR32 (Fig. 3).

219 Although there were a few mismatches between primers and BR32 genome sequence, these 220 sequence differences apparently do not interfere with PCR amplification under the conditions 221 we described in the methods. Our findings lead us to caution against the indiscriminate use of 222 the MoT3 assay to identify wheat blast. The MoT3 primers can under certain amplification conditions yield positive amplicons with the great majority of M. orvzae isolates independent 223 224 of their host of origin. Moving forward, we recommend the design of new primers, ideally 225 nested within the current MoT3 primers, that unambiguously distinguish between the WB12 226 sequences from different isolates. We also advise to take into account the uneven distribution 227 of the WB12 sequence across the various lineages of M. oryzae. Identifying additional 228 diagnostic loci for wheat blast that could be used in conjunction with the MoT3 primers 229 would also provide improved reliability and robustness to the assay.

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289	SUPLEMENTARY MATERIAL
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291	Table S1. WB12 region - BLASTN hits (>98% identity and > 400 bp lengths of match) of
292	WB12 sequence to genomes of Magnaporthe oryzae lineages.
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295	Figure Legends
296	
297	Figure 1. Amplification of the MoT3 regions from rice and wheat infecting Magnaporthe
298	oryzae isolates using MoT3 primers. (A) Amplification performed in the Bangladesh
299	laboratory using MoT3 primers and using PoT2 primers as a positive control for amplification
300	of PoT2 region. (B) Amplification performed in the UK laboratory using MoT3 primers and
301	elongation factor (EF) primers. EF serves as a positive control for amplification.
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303	Figure 2. MoT3 amplicon sequences of Magnaporthe oryzae. (A) Multiple sequence
304	alignment of WB12 sequence (boxed in blue), MoT3 amplicon sequences (indicated by red
305	dots), and WB12-like genomic regions of several isolates. Sequence conservation for each site
306	is indicated by box-shading the nucleotide with either black (>50%) or gray ("conservative

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mutation"). (B) A gene tree based on sequences of WB12 region in a selection of M. oryzae

isolates. Red dots = Amplicon sequences; Blue dots = Bangladeshi isolates; the rest of the
sequences were extracted from the genomic sequences.

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Figure 3. Alignment of second WB12 region in BR32 against WB12 isolate of *Magnaporthe oryzae* with MoT3 primers highlighted: forward primer in cyan; reverse primer in magenta.

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314 Figure S1. Amplification of the MoT3 regions from rice and wheat infecting Magnaporthe 315 oryzae isolates using MoT3 primers sourced in the UK (top halves of the gels) and the MoT3 316 primers sent from the U.S. (bottom halves). To determine whether the quality of the primers 317 used in the UK were comparable to those used in Pieck et al (2017), the PCR assay was 318 repeated using both UK and U.S. primer sets sent to the UK by Dr. Mark Farman's laboratory 319 at the University of Kentucky. We performed PCR as described in the main text except we 320 used a newly acquired thermocycler of the same model as described. (A) PCR performed on 321 this new thermocycler with 62 °C annealing temperature produced a bright band of expected 322 size only in the wheat isolate BTJP4-1 lane for both U.S. and UK primer sets, with an 323 exception of a faint band present in the rice strain RB9c for the UK primer set. However, (B) 324 PCR performed with 57 °C annealing temperature produced the band of expected size in both 325 rice and wheat infecting *M. orvzae* isolates for both primer sets. This demonstrates that under 326 certain conditions, PCR using the MoT3 primers may be able to amplify an expected-sized 327 band from both rice and wheat infecting *M. oryzae* isolates.

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**Figure S2.** Comparison of sequence chromatograms to show MoT3 reverse primer sequences were recovered in MoT3 amplicons from *Magnaporthe oryzae* including wheat and rice blast fungal isolates from Bangladesh. This indicates that MoT3 primers had mis-primed to the template that contained closely related sequences. MoT3 reverse primer sequences are highlighted in yellow. Polymorphic sites that are different from WB12 sequence are shown in orange circles.



Figure 1. Amplification of the MoT3 regions from rice and wheat infecting Magnaporthe oryzae isolates using MoT3 primers. (A) Amplification performed in the Bangladesh laboratory using MoT3 primers and using PoT2 primers as a positive control for amplification of PoT2 region. (B) Amplification performed in the UK laboratory using MoT3 primers and elongation factor (EF) primers. EF serves as a positive control for amplification. GeneRuler Plus (Thermo-Fisher) DNA ladder was used as standard markers.

107x157mm (300 x 300 DPI)



Figure 2. MoT3 amplicon sequences of Magnaporthe oryzae. (A) Multiple sequence alignment of WB12 sequence (boxed in blue), MoT3 amplicon sequences (indicated by red dots), and WB12-like genomic regions of several isolates. Sequence conservation for each site is indicated by box-shading the nucleotide with either black (>50%) or gray ("conservative mutation"). (B) A gene tree based on sequences of WB12 region in a selection of M. oryzae isolates. Red dots = Amplicon sequences; Blue dots = Bangladeshi isolates; the rest of the sequences were extracted from the genomic sequences.

157x99mm (300 x 300 DPI)

# Alignment between WB12 sequence and BR32\_scaffold00014

Query= WB12 Subject= BR32\_scaffold00014

Score = 370 bits (410) Expect = 1e-103 Identities = 391/513 (76%) Gaps = 25/513 (5%) Strand=Plus/Plus

Query	5	CAGTTCGACGTCAATTTTTGGGGCCCCATGGAGCTGACCAGACATGCCGTCAGGATTATG	64
Sbjct	247737	CAGTTCGACGTCAACTTCTGGGGCCCCATGGAGCTGACCAGGCATGCCGTCAGGGTCATG	247796
Query	65	CGCGAGGAGAACCCAAAGAACGGGGGTGCTCCCGGCGGC <mark>GTCGTCATCAACGTGACCAG</mark> C	124
Sbjct	247797	CGCGAGGAGAACCCAAAGAACGGTCCCATCGGCGGCGTCATCATCAACGTCACCAGC	247853
Query	125	GCGGTTGGCTTTCTGGCCATACCCGGCAGCCCCTTTTATTGCGCATCCAAGTTTGCTATG	184
Sbjct	247854	TTCGTTGGCTTCGTGTCGATACCGGGCTGCCCCTTTTACTGCGCATCCAAGTTTGCTCTG	247913
Query	185	GAGGGATTTACAGAGACCTTTGCCAAGGATGTATGCCCTGACTGGAATAGTAAGTTTGTT	244
Sbjct	247914	GAGGGTTTCACCGAGACATTTGCTAAAGAGGTGCACCCGGACTGGAACAGTAGGTTTTAC	247973
Query	245	GTTACAACATCACGAATCGAATGGAGACAAAAACTACAAGAAA	287
Sbjct	247974	TCTAGCACTTCCTTTGCCGTGATGATTCGAATGGGAGCGAAAGAAA	248033
Query	288	ATTGAAGTACAGCTTTCTAACGGTTTGCAATTGCACAAAACAACCCAGTTCACTTTTGCA	347
Sbjct	248034	AGGATTACAGCTTTCTAACGGTATG-ACTTTTACAAAACCACAGTTCACTTTTGCC	248088
Query	348	TCGCCGAGCCCGGCGGTGTCGCTACGGAATTCATAAACAACGTCTCATTTGGACCCCACC	407
Sbjct	248089	TCGCCGAGCCCGGCGTGTCGCTACGGCATTCGCGGACAACAGAAAATTTGGGGTCCCTC	248148
Query	408	ACCCGGCATACGAAGCCCCCAACACGCCGGCGCGGGCCAGGCCTGGGTCAAGTCCG	467
Sbjct	248149	ATCCGGCATACCAGGCCCCGGCACGCCGACGCGAGAGTTCGAGGCTTGGGCCCAGAATC	248208
Query	468	CATCCACCACGAGTTTCTCGTCGGCCGAAGGGA 500	
Sbjct	248209	TATCCGACATGGGCTTCTCGACGGCAGAGGGGA 248241	

MoT3 Primers (positive strands):

Forward: 5' – GTCGTCATCAACGTGACCAG – 3' Reverse: 3' – ATTCGAGGCTTGGGTCAAGT – 5'



Figure S1. Amplification of the MoT3 regions from rice and wheat infecting *Magnaporthe oryzae* isolates using MoT3 primers sourced in the UK (top halves of the gels) and the MoT3 primers sent from the US (bottom halves). To determine whether the quality of the primers used in the UK were comparable to those used in Pieck et al (2017), PCR assay was repeated using both UK and US primer sets sent to the UK by Dr. Mark Farman's laboratory at the University of Kentucky in the US. We performed PCR as described in the Materials and Methods section of the main text except we used a newly acquired PCR machine of the same model as described. (A) PCR performed on this new machine with 62 °C annealing temperature produced a bright band of expected size only in the wheat isolate BTJP4-1 lane for both US and UK primer sets, with an exception of a faint band present in the rice strain RB9c lane for the UK primer set. However, (B) PCR performed with 57 °C annealing temperature produced the band of expected size in both rice and wheat infecting *M. oryzae* isolates for both primer sets. This demonstrates that under certain conditions, PCR using the MoT3 primers may be able to amplify an expected-sized band from both rice and wheat infecting *M. oryzae* isolates.



Figure S2. Comparison of sequence chromatograms to show MoT3 reverse primer sequences were recovered in MoT3 amplicons from *M. oryzae* including wheat and rice blast fungal isolates from Bangladesh. This indicates that MoT3 primers had mis-primed to the template that contained closely-related sequences. MoT3 reverse primer sequences are high-lighted in yellow. Polymorphic sites that are different from WB12 sequence are shown in orange circles.

# Table S1. WB12 region - BLASTN hits (>98% identity and > 400 bp lengths of match) of WB12 sequence to genomes of Magnaporthe oryzae lineages

Isolate	Host	Genbank	Assembled Contig ID	Identity	Length of match (bp)
		accession	genome size	(%)	
			(Mbp)		
Bm88324	Bracharia	GCA_002925385.1	41.3		
Bd8401	Bracharia	GCA_002925405.1	41.2		
P29 (Triticum lineage)	Bromus	GCA_002924915.1	43.2 PJYC0100005	100	500
P28 (Lolium lineage)	Bromus	GCA_002924945.1	43.5		
BR29	Digitaria	GEMO N	lot information		
EI9411	Eleusine	GCA_001548775.1	40.3		
PH42	Eleusine	GCA_002924865.1	43.2		
B51	Eleusine	GCA_002925415.1	43		
G22	Eleusine	GCA_002925165.1	41.4		
G17	Eleusine	GCA_002925205.1	42		
CD156	Eleusine	GEMO	N		
EI9604	Eleusine	GCA_001548785.1	41.6		
TF05-1	Festuca	GCA_002924755.1	43.5		
Pg1213-22	Festuca	GCA_002924875.1	42		
TH16	Hordeum	GEMO	Ν		
PL3-1	Lolium	GCA_002924825.1	42.4		
FH	Lolium	GCA_002925225.1	42.9		
PL2-1	Lolium	GCA_002924835.1	42.5		
LpKY97	Lolium	GCA_002924975.1	42.9		
HO	Lolium	GCA_002925105.1	42.9		
CHW	Lolium	GCA_002925285.1	40.3		
CHRF	Lolium	GCA_002925295.1	42.1		
GG11	Lolium	GCA 002925155.1	42.5		
70-15	Oryza+Digitaria	GCA_000002495.2	40.9		
Y34	Oryza	GCA_000292585.1	38.8		
P131	Oryza	GCA 000292605.1	37.9		
98-06	Oryza	GCA 000805855.1	42.3		
GUY11	Oryza	GCA 002368485.1	42.8		
ML33	Oryza	GCA 002924965.1	39.4		
IE1K	Oryza	GCA 002924985.1	39.6		
IC17	Oryza	GCA 002925025.1	39.5		
IB49	Oryza	GCA 002925045.1	39.4		
IB33	Oryza	GCA 002925065.1	40.2		
IA1	Oryza	GCA 002925085.1	39.4		
IE1K	Oryza	GCA 002924985.1	39.6		
IC17	Oryza	GCA 002925025.1	39.5		
IB49	Oryza	GCA 002925045.1	39.4		
IB33	Orvza	GCA 002925065.1	40.2		
IA1	Orvza	GCA 002925085.1	39.4		
PH14	Orvza	GEMO	N		
FR13	Orvza	GCA 002925215.1	39.8		

Bracharia Isolates of Bracharia	
Bromus Isolates of Bromus	
Digitaria Isolates of Digitaria	
Eleusine Isolates of Eleusine	
Festuca Isolates of Festuca	
Hordeum Isolates of Hordeum	
Lolium Isolates of Lolium	
Oryza Isolates of Oryza	
Setaria Isolates of Setaria	
Stenotaphrur Isolates of Stenotaphrum	
Triticum Isolates of Triticum	

87-120	Oryza	GCA_003013125.1	39.1		
SV9610	Setaria	GCA_001548845.1	39.3		
SV9623	Setaria	GCA_001548855.1	39.3		
GrF52	Setaria	GCA_002925145.1	39.9		
Arcadia	Setaria	GCA_002925445.1	40.8		
US71	Setaria	GEMO	No Information		
SSFL14-3	Stenotaphrum	GCA_002924785.1	42.7		
SSFL02-1	Stenotaphrum	GCA_002924795.1	40.5		
BR32	Triticum	GEMO	No information		
P-0028	Triticum	GCA_002218475.1	42.9		
WBKY11	Triticum	GCA_002924685.1	43.3		
BdJes16-1	Triticum	GCA_001675595.1	41.3 LXOO010012	100	500
BdMeh16-1	Triticum	GCA_001675605.1	42.9 LXOP010006	100	500
BdBar16-1	Triticum	GCA_001675615.1	42 LXON010013	100	500
B71	Triticum	GCA_001675625.1	43.6 LXOQ010001	100	500
Py22.1	Triticum	GCA_002218425.1	42.4 MILZ010004	100	500
Py5020	Triticum	GCA_002218435.1	42.6 MKIG010005	100	500
B2	Triticum	GCA_002218465.1	41.7 MDUN01001	100	500
P-0029	Triticum	GCA_002218485.1	42.4 MLCC010003	100	500
WHTQ	Triticum	GCA_002924665.1	42.4 PJXP0100014	100	447
T25	Triticum	GCA_002924745.1	42.2 PJXU010002:	100	500
Р3	Triticum	GCA_002924885.1	42.6 PJYB0100009	100	500
Br130	Triticum	GCA_002925325.1	40.2 PJYW010021	98.03	507
Br7	Triticum	GCA_002925335.1	41.7 PJYY0100038	100	500
Br80	Triticum	GCA_002925345.1	42.4 PJYX0100001	100	500
B2	Triticum	GCA_002925425.1	42.4 PJZC0100032	100	500

Table S1 (continued). Set 1 of WB12-like - BLASTN hits (70-98% identity and > 400 bp lengths of match) of WB12 sequence to genomes of Magnaporthe oryzae lineages

Isolate	Host	Genbank	Assembled	Contig ID	Identity	Length of match
		accession	genome size		(%)	(bp)
			(Mbp)			
Bm88324	Bracharia	GCA_002925385.1	41.3	PJYZ01000227.1	75.65	501
Bd8401	Bracharia	GCA_002925405.1	41.2	PJZA01000163.1	75.2	504
P29 (Triticum lineage)	Bromus	GCA_002924915.1	43.2	PJYC01000249.1	75.35	503
P28 (Lolium lineage)	Bromus	GCA_002924945.1	43.5	PJYD01000053.1	75.35	503
BR29	Digitaria	GEMO	no information	BR29_scaffold000	79.63	540
CD156	Eleusine	GEMO	40.3	CD156_scaffold00	( 75	504
EI9411	Eleusine	GCA_001548775.1	43.2	LOFC01001152.1	75.45	501
EI9604	Eleusine	GCA_001548785.1	43	LOFD01000071.1	75.65	501
PH42	Eleusine	GCA_002924865.1	41.4	PJXZ01000018.1	75.65	501
G22	Eleusine	GCA_002925165.1	42	PJYP01000311.1	75.65	501
G17	Eleusine	GCA_002925205.1	no information	PJYQ01000080.1	75	504
B51	Eleusine	GCA_002925415.1	41.6	PJZB01000256.1	75.65	501
TF05-1	Festuca	GCA 002924755.1	43.5	PJXT01000467.1	75.35	503
Pg1213-22	Festuca	GCA 002924875.1	42	PJYA01000371.1	75.35	503
TH16	Hordeum	GEMO	no information	TH16 scaffold000	5.35	503
PL3-1	Lolium	GCA 002924825.1	42.4	 PJXX01000001.1	75.35	503
PL2-1	Lolium	GCA 002924835.1	42.9	PJXY01000258.1	75.35	503
LpKY97	Lolium	GCA 002924975.1	42.5	PJYF01000079.1	75.35	503
НО	Lolium	GCA 002925105.1	42.9	PJYL01000256.1	75.35	503
GG11	Lolium	GCA 002925155.1	42.9	PJYO01000345.1	75.35	503
FH	Lolium	GCA_002925225.1	40.3	PJYS01000006.1	75.35	503
CHW	Lolium	GCA 002925285.1	42.1	PJYU01000383.1	75.35	503
CHRF	Lolium	GCA 002925295.1	42.5	PJYV01000418.1	75.35	503
PH14	Orvza+digitaire	GEMO	40.9	PH14-rn_scaffold0	75.35	503
Y34	Oryza	GCA 000292585.1	38.8	JH793942.1	75.2	504
P131	Oryza	GCA_000292605_1	37.9	IH794825.1	75.2	504
98-06	Oryza	GCA_000805855_1	42.3	KN711284.1	75.2	504
GUY11	Oryza	GCA_002368485.1	42.8	MOOP01000017.1	75.35	503
MI 33	Oryza	GCA_002924965_1	39.4	PIVE01000934 1	75.35	503
IF1K	Oryza	GCA_002924985.1	39.6	PIYG01000458 1	75.2	505
IF1K	Oryza	GCA_002924985.1	39.5	PIYG01000458 1	75.2	504
IC17	Oryza	GCA_0029250251	39.4	PIYH01000530 1	75.2	504
IC17	Oryza	GCA_002925025.1	40.2	PIVH01000530.1	75.2	504
IB/19	Oryza	GCA_002925045.1	39.4	PIVI01000651 1	75.2	504
IB49	Oryza	GCA_002925045.1	39.6	PIVI01000651.1	75.2	504
1822	01920	GCA_002925045.1	39.0	DIVI01000531.1	75.2	504
1833	Oryza	GCA 002925005.1	20.4	PIVI01000531.1	75.2	504
1033	Oryza	GCA_002925005.1	39.4	PJVK01000331.1	75.2	504
	01928	GCA 002923065.1	40.2	DIVK01000411.1	75.2	504
EP12	Onyza	GCA 00202523085.1	no information	DIVP01000411.1	75.2	504
87-120	01928	GCA 002013125 1		DOBK010016E6 1	75.2	504
70-15	Onyza	GCA 000003405 3	39.0	CM001221 1	75	504
11571	Setaria	GEMO	29.1	CIVIOU1231.1	/ J.33	505 No Lit
SV0610	Sotaria		39.3			
210625	Setaria	GCA_001548845.1	39.3		/5.05	505
SV9023	Setaria	GCA_00202544555.1	39.9	LUFFU1000423.1	/5.2	504
GIF52	Setaria	GCA_002925145.1	40.8	NO HI		NO HIT
Arcadia	Setaria	GCA 002925445.1	no information	PJZD01000573.1	/5.35	503

Keys	
Bracharia	Isolates of Bracharia
Bromus	Isolates of Bromus
Digitaria	Isolates of Digitaria
Eleusine	Isolates of Eleusine
Festuca	Isolates of Festuca
Hordeum	Isolates of Hordeum
Lolium	Isolates of Lolium
Oryza	Isolates of Oryza
Setaria	Isolates of Setaria
Stenotaphrum	Isolates of Stenotaphrum
Triticum	Isolates of Triticum

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SSFL14-3	Stenotaphrum	GCA_002924785.1	42.7 PJXV01000634.1	76.96	408
SSFL02-1	Stenotaphrum	GCA_002924795.1	40.5 PJXW01000073.1	76.96	408
BR32	Triticum	GEMO	no information BR32_scaffold0001	75.35	503
BdJes16-1	Triticum	GCA_001675595.1	42.9 LXOO01000451.1	75.35	503
BdMeh16-1	Triticum	GCA_001675605.1	43.3 LXOP01000434.1	75.35	503
BdBar16-1	Triticum	GCA_001675615.1	41.3 LXON01001240.1	75.35	503
B71	Triticum	GCA_001675625.1	42.9 LXOQ01000108.1	75.35	503
Py22.1	Triticum	GCA_002218425.1	42 MILZ01000218.1	75.35	503
Py5020	Triticum	GCA_002218435.1	43.6 MKIG01000337.1	75.35	503
B2	Triticum	GCA_002218465.1	42.4 MDUN01001185.1	75.35	503
P-0028	Triticum	GCA_002218475.1	42.6 MKZV01000293.1	75.35	503
P-0029	Triticum	GCA_002218485.1	41.7 MLCC01000520.1	75.35	503
WHTQ	Triticum	GCA_002924665.1	42.4 PJXP01000923.1	75.35	503
WBKY11	Triticum	GCA_002924685.1	42.4 PJXR01000009.1	75.35	503
T25	Triticum	GCA_002924745.1	42.2 PJXU01000512.1	75.35	503
Р3	Triticum	GCA_002924885.1	42.6 PJYB01000321.1	75.35	503
Br130	Triticum	GCA_002925325.1	40.2 PJYW01000240.1	75.35	503
Br7	Triticum	GCA_002925335.1	41.7 PJYY01000366.1	75.35	503
Br80	Triticum	GCA_002925345.1	42.4 PJYX01000178.1	75.35	503
B2	Triticum	GCA_002925425.1	42.4 PJZC01000243.1	75.35	503

### Table S1 (continued). Set 2 of WB12-like - BLASTN hits (60-98% identity and 100-400 bp lengths of match) of WB12 sequence to genomes of Magnaporthe oryzae lineages

Isolate	Host	Genbank accession	Assembled Contig ID1	Identity (%) Length of	Contig ID2	Identity	Length of
			genome size	match (bp)		(%)2	match (bp)2
			(Mbp)				
Bm88324	Bracharia	GCA_002925385.1	41.3				
Bd8401	Bracharia	GCA_002925405.1	41.2				
P29 (Triticum lineage)	Bromus	GCA_002924915.1	43.2				
P28 (Lolium lineage)	Bromus	GCA_002924945.1	43.5				
BR29	Digitaria	GEMO	o information				
EI9411	Eleusine	GCA_001548775.1	40.3				
PH42	Eleusine	GCA_002924865.1	43.2				
B51	Eleusine	GCA_002925415.1	43				
G22	Eleusine	GCA_002925165.1	41.4				
G17	Eleusine	GCA 002925205.1	42				
CD156	Eleusine	GEMO	o information				
EI9604	Eleusine	GCA 001548785.1	41.6				
TF05-1	Festuca	GCA 002924755.1	43.5				
Pg1213-22	Festuca	GCA 002924875.1	42				
TH16	Hordeum	GEMO	o information				
PL3-1	Lolium	GCA 002924825.1	42.4				
FH	Lolium	GCA 002925225.1	42.9				
PL2-1	Lolium	GCA 002924835.1	42.5				
LpKY97	Lolium	GCA 002924975.1	42.9				
но	Lolium		42.9				
CHW	Lolium	GCA 002925285.1	40.3				
CHRF	Lolium	GCA 002925295.1	42.1				
GG11	Lolium	GCA 002925155.1	42.5				
70-15	Oryza+digitai	GCA 000002495.2	40.9				
Y34	Oryza	GCA 000292585.1	38.8				
P131	Oryza	GCA 000292605.1	37.9				
98-06	, Orvza	GCA 000805855.1	42.3				
GUY11	Oryza	GCA 002368485.1	42.8				
ML33	Orvza	GCA 002924965.1	39.4				
IE1K	Orvza	GCA 002924985.1	39.6				
IC17	Orvza	GCA 002925025.1	39.5				
IB49	Oryza	GCA_002925045.1	39.4				
IB33	Orvza	GCA_002925065.1	40.2				
IA1	Orvza	GCA 002925085.1	39.4				
IF1K	Oryza	GCA_002924985_1	39.6				
IC17	Oryza	GCA_002925025.1	39.5				
IB49	Oryza	GCA_002925045_1	39.4				
IB33	Orvza	GCA 002925065.1	40.2				
IA1	Orvza	GCA_002925085_1	39.4				
PH14	Oryza	GEMO	o information				
FR13	Oryza	GCA_002925215_1	39.8				
87-120	Oryza	GCA 003013125 1	39.1				
SV9610	Setaria	GCA_001548845_1	39.3				
SV9623	Setaria	GCA_001548855 1	39.3				
GrE52	Setaria	GCA 0029251//5 1	39 9 PIVN01000512 1	83.67 241	5 PIYN01000527 1	74 57	1
Arcadia	Setaria	GCA 0029254/5 1	40.8	. 05.07 24.	///////////////////////////////////////	74.57	1
11571	Setaria	GEMO	o information US71 scaffold00	0090 (16 83 67 24	5 US71 scaffold00090 (10474-1)	76.06	1,
SSEI 14-3	Stenotanhru	GCA 002924785 1	42.7	24.		70.00	1.
	Julia		76./				

Keys	
Bracharia	Isolates of Bracharia
Bromus	Isolates of Bromus
Digitaria	Isolates of Digitaria
Eleusine	Isolates of Eleusine
Festuca	Isolates of Festuca
Hordeum	Isolates of Hordeum
Lolium	Isolates of Lolium
Oryza	Isolates of Oryza
Setaria	Isolates of Setaria
Stenotaphrun	Isolates of Stenotaphrum
Triticum	Isolates of Triticum

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SSFL02-1	Stenotaphrur	GCA_002924795.1	40.5
BR32	Triticum	GEMO	o information
P-0028	Triticum	GCA_002218475.1	42.9
WBKY11	Triticum	GCA_002924685.1	43.3
BdJes16-1	Triticum	GCA_001675595.1	41.3
BdMeh16-1	Triticum	GCA_001675605.1	42.9
BdBar16-1	Triticum	GCA_001675615.1	42
B71	Triticum	GCA_001675625.1	43.6
Py22.1	Triticum	GCA_002218425.1	42.4
Py5020	Triticum	GCA_002218435.1	42.6
B2	Triticum	GCA_002218465.1	41.7
P-0029	Triticum	GCA_002218485.1	42.4
WHTQ	Triticum	GCA_002924665.1	42.4
T25	Triticum	GCA_002924745.1	42.2
Р3	Triticum	GCA_002924885.1	42.6
Br130	Triticum	GCA_002925325.1	40.2
Br7	Triticum	GCA_002925335.1	41.7
Br80	Triticum	GCA_002925345.1	42.4
B2	Triticum	GCA_002925425.1	42.4