Host specificity controlled by *PWL1* and *PWL2* effector genes in the finger millet blast pathogen *Magnaporthe oryzae* in eastern Africa

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Abstract

Magnaporthe oryzae, a devastating pathogen of finger millet (*Eleusine coracana*), secretes effector molecules during infection to manipulate host immunity. This study determined the presence of avirulence effector genes *PWL1* and *PWL2* in 221 *Eleusine* blast isolates from eastern Africa. Most Ethiopian isolates carried both *PWL1* and *PWL2*. Kenyan and Ugandan isolates largely lacked both genes, and Tanzanian isolates carried either *PWL1* or lacked both. The roles of *PWL1* and *PWL2* towards pathogenicity on alternative *Chloridoid* hosts, including weeping lovegrass (*Eragrostis curvula*), were also investigated. *PWL1* and *PWL2* were cloned from Ethiopian isolate E22 and transformed separately into Ugandan isolate U34, which lacked both genes. Resulting transformants harboring either gene gained varying degrees of avirulence on *E. curvula* but remained virulent on finger millet. Strains carrying *PWL1* and/or *PWL2* infected the *Chloridoid* species *Sporobolus phyllotrichus* and *Eleusine tristachya*, indicating the absence of cognate resistance (*R)* genes for *PWL1* and *PWL2* in these species. Other *Chloridoid* grasses, however, were fully resistant, regardless of the presence of *PWL1* and/or *PWL2*, suggesting the presence of effective *R* genes against *PWL* and/or other effectors. Partial resistance in some *E. curvula* accessions to some blast isolates lacking *PWL1* and *PWL2* also indicated the presence of other *AVR*-*R* interactions. Related Chloridoid species thus harbour resistance genes that could be useful to improve finger millet for blast resistance. Conversely, loss of *AVR* genes in the fungus could expand its host range, as demonstrated by *E. curvula*'s susceptibility to finger millet blast isolates that had lost *PWL1* and *PWL2*.

Keywords: Blast disease, *Chloridoid* grasses, *Eleusine coracana*, *Eragrostis curvula,* Host resistance, *Magnaporthe oryzae*, *PWL1*, *PWL2*

INTRODUCTION

The filamentous ascomycete fungus *Magnaporthe oryzae* causes blast disease, one of the most devastating diseases affecting more than 50 grass species, including finger millet (*Eleusine coracana*). In endemic areas, complete yields can be lost, especially if blast infections occur in combination with abiotic stresses (Senthil et al., 2012). The fungus infects finger millet at all developmental stages, but infection of the panicle (head blast) and peduncle (neck blast) are the most destructive (Ramakrishnan et al., 2016; Takan et al., 2012). The most cost effective solution for small holder farmers to manage blast disease is to deploy tolerant plant varieties that carry resistance (*R*) genes against the infecting blast strain (Vleeshouwers and Oliver, 2014). However, because of their adaptability, fungal pathogens often overcome resistance in newly deployed cultivars within a few years (Orbach et al., 2000).

Individual strains of *M. oryzae* are host specific and have been classified into distinct subgroups according to their pathogenicity on a variety of plants, such as *Eleusine* pathotypes that infect finger millet, *Setaria* pathotypes on foxtail millet, *Oryza* pathotypes on rice and *Triticum* pathotypes on wheat (Kato et al., 2000). The fungus utilizes a hemi-biotrophic infection strategy to interact with the host plant. During the initial biotrophic phase, it colonizes living tissues, from which it acquires nutrients, before switching to a necrotrophic phase when it acquires nutrients from the dead cells (Park et al., 2009; Vleeshouwers and Oliver, 2014; Jones et al., 2021). To subvert the host defense mechanisms and cellular activities, *M. oryzae* secretes effector proteins that facilitate host colonization (Yoshida et al., 2016; Valent and Khang, 2010). Some effectors exhibit avirulence properties (Zhang and Xu, 2014) and are recognized by the host plant's R proteins (De Wit et al., 2009), resulting in a rapid and effective hypersensitive response (Yoshida et al., 2009).

To date, a limited number of *M. oryzae* avirulence *(AVR)* effector genes have been identified and analyzed for mutations that can affect the avirulence, including *AVR-Pita1* (Orbach et al., 2000; Khang et al., 2008), *ACE1* (Böhnert et al., 2004), *AvrPiz-t* (Li et al., 2009), *Avr-Pia* (Yoshida et al., 2009), *Avr-Pii* and *Avr-Pik/km/kp* (Yoshida et al., 2009), *Avr-CO39* (Ribot et al., 2013) and *PWL* (Kang et al., 1995; Sweigard et al., 1995). Of these, *AVR-Pita1* was shown to be linked to the subtelomeric region in a Chinese rice field isolate, O-137, indicating that loss of chromosome tips could result in gain of virulence (Orbach et al., 2000). Previous studies of *M. oryzae* have revealed that the fungus employs gene gain or loss to change its host specificity (Sone et al., 2013; Yoshida et al., 2016). This process often involves transposable elements, which can generate gene duplications, gene disruptions, recombination, mutations and the adaptive evolution of blast fungal effector genes (Chuma et al., 2011; Khang et al., 2008; Gomez, Luciano et al., 2019; Thon et al., 2006; Wang et al., 2017)

The diversified, rapidly evolving *PWL* gene family in *M. oryzae*, which determines pathogenicity in weeping lovegrass (*Eragrostis curvula*), includes four genes - *PWL1*, *PWL2*, *PWL3* and *PWL4*. *PWL1* was identified from a cross between *Eleusine* isolate WGG-FA40 and weeping lovegrass isolate K76-79 (Valent et al., 1986) and subsequently cloned (Kang et al., 1995). The second gene, *PWL2,* was identified and cloned from a cross between two laboratory strains virulent on rice, of which one strain (4224-7-8) was virulent and the other (6043) avirulent on weeping lovegrass (Sweigard et al., 1995). *PWL3* and *PWL4* were found not to confer avirulence in weeping lovegrass, although *PWL4* became functional when its expression was driven by the promoter of either *PWL1* or *PWL2* (Kang et al., 1995).

In this study, we cloned and transferred *PWL1* and *PWL2* from the Ethiopian finger millet blast (FMB) isolate E22, which is avirulent on weeping lovegrass, to the Ugandan strain U34, which lacks both *PWL1* and *PWL2*, and used the transformants as well as native isolates in infection assays to determine the role of *PWL1* and *PWL2* in pathogenicity on eight

Chloridoid species, including finger millet. In addition, PCR amplification of *PWL1* and *PWL2* in 221 *Eleusine* isolates collected across Ethiopia, Kenya, Tanzania and Uganda was used to investigate the presence of *PWL1* and *PWL2* genes in *M. oryzae* across eastern Africa.

RESULTS

Distribution of *PWL1* and *PWL2* genes across finger millet blast isolates from eastern Africa

To determine the presence in FMB strains of *PWL1* and *PWL2*, which have been shown to mediate resistance of weeping lovegrass to rice blast strains (Kang et al., 1995; Sweigard et al., 1995), a collection of 221 *Eleusine M. oryzae* isolates were investigated in this study. The 221 strains were isolated from blast-infected finger millet tissues collected between 2015 and 2017 from Ethiopia (E isolates), Kenya (K), Tanzania (T) and Uganda (U). PCR amplification with gene-specific primers (**Table 1)** and/or bioinformatic mining of resequencing data revealed that *PWL1* and *PWL2* were present either alone or in combination in 89 isolates (**Table 2)**. Thirty-six isolates contained only *PWL1*, three contained only *PWL2* and 50 had both. The remaining 132 isolates, which included all those from Uganda and the majority of Kenyan isolates lacked both *PWL1* and *PWL2* (**Table 2)**. The isolates were subsequently grouped into four groups, based on whether they contained neither *PWL1* nor *PWL2* (FMB-1), both *PWL1* and *PWL2* (FMB-2)*, PWL1* only (FMB-3) or *PWL2* only (FMB-4). In FMB-1, 76.5% of the isolates had been collected from Kenya and Uganda; in FMB-2, 90.0% originated in Ethiopia; and in FMB-3, 83.3% originated in Tanzania. Presence of *PWL2* only was found in only three isolates, all originating from Tanzania. Tanzanian isolates were mainly present in groups FMB-1 (34.5 %) and FMB-3 (51.7%) (**Table 2**).

Nucleotide sequence comparison of *PWL1* and *PWL2* with those from other *E. coracana* and rice blast isolates

We compared the level of sequence conservation between the FMB *PWL1* (444 bp) and *PWL2* (438 bp) open reading frames (ORFs) cloned from Ethiopian isolate E22 with those reported in GenBank and with resequencing data for both genes in a subset of the 221 FMB isolates we collected from eastern Africa. The *PWL1* sequence isolated from E22 (this study; GenBank acc. MT669814) was identical to those from the Japanese FMB strains reported by Asuke et al. (2019) (GenBank acc. AB480169) and Gomez et al. (2019) (GenBank acc. CP034204.1). Similarly, no variants were identified upon alignment of resequencing reads from 49 Ethiopian, 35 Tanzanian and two Kenyan strains to *PWL1* of strain E22. Ten single base substitutions, predicted to result in three amino acid substitutions, were found between *PWL1* from E22 and the orthologous sequence in a rice blast strain (GenBank acc. CP091458; region 363,360 – 363,803) **(Supplementary Figs. S1 and S2)**. Two of the amino acid changes are located in the N-terminal signal peptide, but predictions by SignalP 6.0 (Teufel et al. 2022) indicated that they do not affect cleavage. The third amino acid substitution is located near the C-terminus of PWL1.

Two synonymous and one non-synonymous single base substitution were present between *PWL2* from finger millet blast strain E22 (GenBank acc. MT669815) and a rice blast strain (GenBank acc. U26313.1; Sweigard et al. 1995) **(Supplementary Figs. S3 and S4)**. The amino acid substitution is located in the signal peptide and, as for PWL1, SignalP 6.0 predictions do not indicate an effect on cleavage. It should be noted that the amino acid substitution does not correspond to the aspartic acid to asparagine mutation that rendered *PWL2* in the rice blast fungus non-functional as an avirulence gene (Sweigard et al. 1995). No variants were observed for PWL2 in resequencing data from a set of 45 Ethiopian and eight Tanzanian strains isolated from finger millet.

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146 ***PWL1* and *PWL2* from *Eleusine* isolates confer avirulence on weeping lovegrass**

147 Using whole plant spray inoculations, we tested the pathogenicity of select FMB-1 and FMB-
148 2 isolates on weeping lovegrass (*E. curvula* PI 197425) and found that virulence was correlated
149 with the absence of the *PWL1* and *PWL2* genes. Two Ugandan isolates, U34 and U44 (*pwl1-*
150 */pwl2-*; FMB-1 group in **Table 2**), showed severe infections on weeping lovegrass, causing the
151 infected leaves to completely shrivel with merged lesions (**Figs. 1 and 2**). This was in stark
152 contrast to the response of PI 197425 to two Ethiopian isolates, E2-GFP and E22
153 (*PWL1+/PWL2+*; FMB-2 group in **Table 2**), which caused barely visible lesions or some
154 uniform dark brown pinpoint lesions without visible centers (typical avirulent lesions; Valent
155 et al., 1991) with occasional isolated lesions with distinct tan centers surrounded by a darker
156 brown margin.

157

158 To demonstrate that the *PWL1* and *PWL2* genes in FMB isolates indeed confer
159 avirulence on weeping lovegrass, we first cloned *PWL2* from the avirulent isolate E22 and
160 introduced the cloned gene into the virulent isolate U34. The resulting U34 transformants (n=5)
161 caused typical avirulent lesions with occasional small lesions, exhibiting gain of avirulence in
162 weeping lovegrass (**Fig. 1; Supplementary Table S1**). Similarly, we transformed U34 with
163 *PWL1* cloned from E22. The resulting transformants (n=3) also showed gain of avirulence in
164 weeping lovegrass, but there was some variation among transformants; avirulent lesions were
165 more evident in the transformant CKF4183 than the other two transformants (**Fig. 1;**
166 **Supplementary Table S1**). While no native isolates containing solely *PWL1* (group FMB-3)
167 or *PWL2* (group FMB-4) were tested, the expectation is these strains would yield infection
168 results similar to those obtained with the *PWL1* and *PWL2* transformants.

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170 ***PWL1* and *PWL2*-independent avirulence on weeping lovegrass**

171 To further explore the nature of avirulence of FMB isolates on weeping lovegrass, we tested
172 the response of different *E. curvula* accessions to pathogens with different effector repertoires.
173 As expected, E2-GFP (*PWL1+/PWL2+*) was avirulent on *E. curvula* cv. Ermelo (Oklahoma,
174 US), whereas U44 (*pwl1-/pwl2-*) was virulent on this cultivar (**Fig. 2**). This correlation between
175 presence of the *PWL1* and *PWL2* genes and avirulence on *E. curvula* cv. Ermelo was consistent
176 with the results obtained with the Kenyan *E. curvula* accession PI 197425 (**Fig. 1**). Intriguingly,
177 the Ugandan isolate U27, lacking both *PWL1* and *PWL2*, caused mostly avirulent lesions on *E.*
178 *curvula* cv. Ermelo (**Fig. 2**). The lack of successful colonization of U27 and E2-GFP on *E.*
179 *curvula* cv. Ermelo was not due to a lack of pathogenicity because both strains were highly
180 virulent on the finger millet cultivar AAUFM-44 (**Fig. 2**). These results suggest that U27
181 possesses an *AVR* gene(s), other than *PWL1* and *PWL2*, which is recognized by a yet-to-be-
182 characterized resistance gene(s) in *E. curvula* cv. Ermelo.

183 Occurrence of additional *AVR-R* gene combinations controlling the disease response in
184 *E. curvula* to FMB isolates was further supported by the variation found within *E. curvula*
185 germplasm for the resistance response to FMB isolate U34 (*pwl1-/pwl2-*). An *E. curvula*
186 accession from South Africa, PI 295694, displayed fewer symptoms when inoculated with U34
187 compared to *E. curvula* cv. Ermelo (**Fig. 3**). All three *E. curvula* accessions tested in this study
188 were resistant to E22 (*PWL1+/PWL2+*) (**Figs. 1 and 3**).

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190 ***PWL1* and *PWL2* do not confer avirulence in *Sporobolus phyllotrichus* and *Eleusine***
191 ***tristachya***

192 To test if *PWL1* and *PWL2* can confer an avirulent response against *M. oryzae* in *E. curvula*-
193 related plant species, we inoculated another *Chloridoid* grass, *Sporobolus phyllotrichus*, with
194 FMB isolates as well as *PWL1* and *PWL2* transformants. Our whole plant spray inoculation

assays showed that both E22 (*PWL1+/PWL2+*) and U34 (*pwl1-/pwl2-*) had high infection levels on *S. phyllotrichus* (acc. PI 226098) and that similar high infection levels were observed with U34 transformants carrying either *PWL1* or *PWL2* (**Fig. 1**). This indicates that, unlike *E. curvula* (PI 197425), *S. phyllotrichus* (PI 226098) does not recognize *PWL1* or *PWL2.*

Infection of five other *Chloridoid* species, two of which belong to the genus *Eleusine*, with either U34 or U40, both of which lack *PWL1* and *PWL2*, showed avirulence on *Eragrostis tef* (U40), *Calamovilfa longifolia* (U34)*, Dactyloctenium giganteum* (U34) and *Eleusine floccifolia* (U34), and virulence on *Eleusine tristachya* (U34)(**Fig. 4)**. The same pathogenecity response was observed when these lines were inoculated with E2-GFP (*PWL1+/PWL2+*), demonstrating that *PWL1* and *PWL2* are not recognized by the *E. tristachya* accession analyzed. While we cannot derive from the infection results whether *R* genes that recognize the *PWL* genes are present in the four resistant Chloridoid species, we can conclude that the strains lacking the PWL effectors carry other effectors for which *R* genes are present in the finger millet relatives.

DISCUSSION

Distribution of *PWL1* and *PWL2* in finger millet blast isolates from eastern Africa

The present study found that the 221 *Eleusine* blast isolates from eastern Africa grouped into four classes, differentiated by the absence or presence of *PWL1* and/or *PWL2* genes. Isolates from Kenya and Uganda predominantly lacked both *PWL1* and *PWL2* (FMB-1), while the majority of Ethiopian isolates carried both genes (FMB-2) (**Table 2**). Around 50% of Tanzanian lines belonged to FMB-1, while the other half carried only *PWL1* (FMB-3) (**Table 2**). The lack of *PWL1* in Ugandan FMB isolates was also observed by Asuke et al. (2019), who classified *Eleusine* blast isolates collected from Japan, Nepal, India, and Uganda into two

groups: EC-I isolates that did not contain *PWL1* and were infectious on both weeping lovegrass and finger millet, and EC-II isolates that contained *PWL1* and were virulent on finger millet but avirulent on weeping lovegrass (Asuke et al., 2019). Similarly to what was observed by Asuke et al. (2019), our study shows that the FMB-1 isolates U34 and U44 (*pwl1-/pwl2-*) were virulent on both finger millet AAUFM-44 and *E. curvula* cv. Ermelo. The FMB-2 isolates E2-GFP and E22 (*PWL1+/PWL2+*), on the other hand, were avirulent on the same *E. curvula* cultivar, while they were highly virulent on finger millet. *E. curvula* originates in southern Africa and then moved northwards to eastern Africa. While the species is present in Ethiopia, our data suggest that *E. curvula* may be an alternative host to finger millet for *Eleusine* isolates in Kenya, Uganda and some regions of Tanzania, but not in Ethiopia.

We hypothesize that the presence or absence of *PWL1* and/or *PWL2* is brought about by the prevailing environmental conditions and/or co-evolution with the host, and that the ancestral FMB lineage carried both *PWL1* and *PWL2*. The latter is suggested by the fact that Asian (Asuke et al. 2019), Ethiopian and about half of the Tanzanian blast isolates analyzed carried *PWL1* either by itself or in combination with *PWL2*, and that gene loss leading to a broader host range is a more likely scenario than gene gain which would reduce the host range. Subsequently, an adaptation strategy to survive on alternative hosts such as *E. curvula* in the absence of finger millet may have been adopted through the loss of *PWL1* and *PWL2*. Our results also show that gene loss is more likely than inactivation through the accumulation of point mutations; no variants were identified for *PWL1* or *PWL2* across the more than 50 strains tested.

Multiple mechanisms of host resistance to finger millet blast exist in *Chloridoid* grasses

Previous segregation analyses showed that *PWL1* and *PWL2* determined avirulence of rice-adapted *M. oryzae* on *E. curvula* (Kang et al., 1995; Sweigard et al., 1995). Using native and

transformed finger millet-adapted blast strains, we demonstrated that the mechanism of host resistance to finger millet blast in weeping lovegrass is also governed by *PWL1* and *PWL2*, and likely the same for finger millet and rice blast. The variation seen in infection levels for different FMB-1 strains – *E. curvula* accession combinations also suggests that other genes are present in weeping lovegrass that can convey at least partial resistance to finger millet blast isolates that lack *PWL1* and *PWL2* (**Figs. 2 and 3**).

However, *PWL1* and *PWL2* do not control pathogenicity on *S. phyllotrichus*, another *Chloridoid* grass, at least not on the accession tested (PI 226098), as *PWL1* and *PWL2* transformants, as well as the parental strain U34 were highly infectious on *S. phyllotrichus* acc. PI 226098. This suggests that *S. phyllotrichus* lacks the genes that recognize the PWL1 and PWL2 effector proteins. Testing of five additional *Chloridoid* accessions, *E. tristachya, E. floccifolia*, *C. longifolia, D. giganteum*, and *E. tef* with two finger millet blast strains, one lacking *PWL1* and *PWL2,* and one containing both genes, showed the same pathogenicity response, either resistance or susceptibility, to both strains. This demonstrates that the resistance genes effective to PWL1 and PWL2 are absent from the susceptible *E tristachya* accession tested. In the four Chloridoid species that display resistant interactions, it is unknown whether *PWL1* and *PWL2* play a role in the resistance. Regardless, *R* genes, whether recognizing *PWL* effectors or other effectors in the finger millet blast strains, must be present in those Chloridoid grasses.

Importantly, our study demonstrates that despite being considered host-specific, *M. oryzae* strains likely have alternative hosts. Finger millet blast isolates are able to infect both *E. tristachya* and *S. phyllotrichus*. *E. tristachya* is native to South America while *S. phyllotrichus* is native to eastern Africa. On the other hand, resistance to finger millet-adapted *M. oryzae* strains was observed in the *Chloridoid* species *E. floccifolia*, also native to eastern Africa, *C. longifolia*, native to North America, *D. giganteum*, which is found from Kenya to

South Africa and in Madagascar, and *E. tef*, a cereal widely grown in Ethiopia. Based on the limited number of species analyzed, there does not appear to be a correlation between the presence of resistance in related Chloridoids to finger millet-adapted *M. oryzae* strains and sympatry of the species tested with finger millet. Similarly, no obvious link was discerned between the taxonomic relatedness of the species (Peterson et al., 2010) and their resistance, or lack thereof, to finger millet blast isolates **(Supplementary Fig. S5)**.

The resistance we observed in several of the Chloridoid species against finger millet infecting *M. oryzae* isolates was complete, in contrast to the resistance typically seen in finger millet accessions (Takan et al., 2012). In a recent study in *Arabidopsis,* a few susceptible transgressive segregants were found in progeny derived from intercrossing 19 parents that were resistant to white rust caused by *Albugo candida* for which *Arabidopsis* is considered a non-host (Cevik et al. 2019). Further analysis of the segregants led to the identification of resistance genes, some of which also conferred resistance to *A. candida* when introduced into susceptible lines of the host species *Brassica napus* and *B. juncea* (Cevik et al., 2019). This indicated that the resistance to *A. candida* in *Arabidopsis* was caused by effector-triggered immunity. Similarly, the resistance of wheat to ryegrass and oat-infecting *M. oryzae* isolates was caused by the incompatible interaction of the ryegrass blast AVR effectors PWT3 and PWT4 with the wheat resistance proteins RWT3 and RWT4. Widespread cultivation of *rwt3* wheat cultivars led to pathogenicity of ryegrass blast on wheat (Inoue et al. 2017). Our study identified the presence of at least two types of resistance in the finger millet blast – *Chloridoid* host system. In *E. curvula*, resistance is present against the cognate effectors *PWL1* and *PWL2*. In other *Chloridoid* species such as *E. floccifolia*, *C. longifolia, D. giganteum*, and *E. tef,* resistance to finger millet adapted *M. oryzae* is expressed in the absence of *PWL1* and *PWL2*, and hence is targeted to as yet unknown *AVR* effectors that are present in FMB. Screening germplasm collections or intercrossed populations for susceptible genotypes will provide a way forward

to genetic mapping and isolation of the genes underlying the resistance to FMB in other Chloridoids. While these genes will provide novel sources of potentially durable resistance in finger millet, knowing the basis of the resistance seen in finger millet relatives will also be important for related crop species that have overlapping cultivation areas with finger millet. *Eragrostis tef* (teff), for example, grows in sympatry with finger millet in Ethiopia, but is resistant to finger millet blast. However, teff could become vulnerable to finger millet blast if varieties were to be bred and widely cultivated that lacked the gene(s) conferring the resistance to finger millet-adapted blast strains, or if the finger millet blast fungus lost the corresponding *AVR* genes.

CONCLUSION

This study confirmed that, as in rice, *PWL1,* either alone or in combination with *PWL2,* modulates the finger millet blast fungus' virulence on *E. curvula*. Analysis of the prevalence of *PWL1* and *PWL2* across eastern Africa further showed that *Eleusine M. oryzae* isolates formed four groups based on the presence/absence of *PWL1* and/or *PWL2*. FMB-1 isolates (lacking both genes) originated almost exclusively in Kenya and Uganda. The majority of Ethiopian isolates carried both *PWL1* and *PWL2* (FMB-2) while the Tanzanian lines were divided between FMB-1, FMB-3 (*PWL1* only) and FMB-4 (*PWL2* only). This suggests that finger millet blast may use *E. curvula* as an alternative host in Kenya and Uganda but not in Ethiopia. *S. phyllotrichus*, also native to eastern Africa, may also be used as an alternative host as the accession we tested had intermediate to high susceptibility depending on the blast isolates tested, although the resistance level was independent of the presence of *PWL1* or *PWL2*. Our study also identified resistance in species other than *E. curvula* that was not, or at least not solely, based on interaction of host resistance genes with *PWL1* and/or *PWL2*. Elucidating the mechanisms of resistance to FMB in finger millet relatives will broaden the

320 portfolio of resistance genes that could be introduced into finger millet to fight blast disease.
321 Conversely, it would allow breeders to ensure that resistance is retained in other Chloridoid
322 crops that are grown in sympatry with finger millet such as *E. tef* in Ethiopia.
323
324 **MATERIALS AND METHODS**
325
326 **Fungal strains and plant materials**
327 Plant accessions used for infection assays are described in **Table 3**. The *Eleusine M. oryzae*
328 isolates used in infection assays are described in **Table 4.** This included four isolates from
329 Uganda (U27, U34, U40 and U44), eight transformants of U34, Ethiopian isolate E22 and one
330 laboratory strain (E2-GFP). The latter was generated by transforming Ethiopian isolate E2 with
331 pBV126 carrying an enhanced green fluorescent protein (GFP) under control of the *M. oryzae*
332 ribosomal protein 27 promoter (Khang et al., 2010). *Eleusine* isolate U34 was used as the
333 recipient for *PWL1* and *PWL2* by genetic transformation. Fungal transformants were generated
334 using *Agrobacterium tumefaciens*-mediated transformation (Khang et al., 2006). *M. oryzae*
335 isolates and transformants were stored dehydrated and frozen at -20°C to maintain full
336 pathogenicity and were cultured on oatmeal agar (OMA) plates at 24°C under continuous light
337 (Valent et al., 1991). In addition to the fungal and plant materials described above, DNA
338 samples and/or resequencing reads covering *PWL1* and *PWL2* from 221 *Eleusine* isolates
339 collected from Ethiopia, Kenya, Tanzania and Uganda between 2015 and 2017, were made
340 available to this study for *PWL1* and *PWL2* distribution analysis.
341
342 **Cloning of *PWL1* and *PWL2*, and their transformation in FMB strain U34**
343 *PWL1* and *PWL2* were individually amplified from genomic DNA of *Eleusine* isolate E22 with
344 the primers listed in **Table 1** (E22_PWL1_F and E22_PWL1_R for *PWL1* amplification;

E22_PWL2_F and E22_PWL2_R for *PWL2* amplification). The 1.13 kb *PWL1* fragment consisted of 444 bp of coding sequence (CDS), and 459 bp and 230 bp of upstream and downstream sequence from the start and stop codons respectively. The 1.37 kb *PWL2* fragment consisted of 438 bp of CDS, and 725 bp and 207 bp of upstream and downstream sequence respectively. PCR was performed in 25 µL containing 25 ng of genomic DNA, 1x Q5 reaction buffer, 200 µM dNTPs, 0.5 µM forward primer, 0.5 µM reverse primer and 0.02 U/µl Q5 High-Fidelity DNA Polymerase (NEB, USA). Reaction conditions were 30 cycles of denaturation at 98°C for 10 seconds, annealing at 66°C for 30 seconds and extension at 72°C for 2 minutes. The resulting PCR products of *PWL1* and *PWL2* were individually cloned into the pMiniT PCR vector (NEB, USA) to generate plasmids pHM1 and pHM2, respectively. *PWL1* and *PWL2* genes in these plasmids were verified by Sanger sequencing (GENEWIZ, USA). Using *Bam*HI and *Xho*I restrictions enzymes, the *PWL1* insert in pHM1 and the *PWL2* insert in pHM2 were excised and subsequently cloned into the *Bam*HI and *Sal*I sites of pBV1 (Mullins et al., 2001) to generate plasmids pCK2104 and pCK2106, respectively. pCK2104 (carrying *PWL1*) and pCK2106 (carrying *PWL2*) were separately transformed into *A. tumefaciens* EHA105 competent cells, and then transformed into *Eleusine M. oryzae* isolate U34 as described by Khang et al. (2006).

Infection assays

For infection assays, spores from each fungal culture were harvested in 0.25% sterilized gelatin solution, and the spore concentrations were adjusted to $1.0x10^4$ or $1.0x10^5$ spores per mL. Five to 15 seeds of each accession (**Table 3)**, depending on the germination rate, were sown in soil in labeled pots and placed in a growth chamber at 28°C and 80% relative humidity. Inoculations were performed on 14 days old seedlings placed inside plastic bags. Each bagged seedling was sprayed with 5 mL of inoculum using an artist's air brush with compressed air at 20 psi. Bags

were then sealed to maintain humidity to support infection at room temperature for 24 hours, after which the bags were removed, and the plants were transferred to a growth chamber. Plants sprayed with the gelatin suspension without spores were used as negative controls. Seven days post inoculation, leaves were harvested, scored and scanned. Severity of infection was rated according to six progressive grades from 0 to 5 with 0 = no visible symptoms, 1 = pinpoint spots, 2 = small lesions (<1.5 mm), 3 = intermediate sized lesions (<3 mm), 4 = large lesions typical of blast infection, and 5 = complete shriveling of leaf blades **(Supplementary Figure S6)**. Raw data generated from more than three leaves in one or two independent inoculations were averaged (**Supplementary Table S1**).

Presence of and SNP variation in *PWL1* and *PWL2* across FMB isolates

PCR primers used to determine the presence or absence of the two *PWL* genes (*PWL1*_CDS_F, *PWL1*_CDS_R, PWL2_CDS_F and PWL2_CDS_R) are listed in **Table 1**. These primers were designed to amplify the *PWL1* ORF of 444 bp and *PWL2* ORF of 438 bp, respectively. Reaction conditions were 35 cycles of denaturation at 94°C for 30 seconds, annealing at 62°C for 30 seconds and extension at 72°C for 5 minutes. A 3 µL sample from each PCR reaction was run on a 1% (w/v) agarose gel.

Illumina resequencing reads for 208 FMB isolates were aligned against the *PWL1* and *PWL2* gene sequences cloned from isolate E22 with Bowtie2 (Langmead and Salzberg 2012) using the parameters *--maxin 900 --no-discordant --no-mixed*. *PWL1* and *PWL2* were considered present if ≥90% of their coding region was covered by Illumina reads to a depth ≥2x.

Accession numbers

394 The sequence data from this study can be obtained from GenBank/EMBL databases under the
395 following accession numbers: MT669814 for *PWL1* and MT669815 for *PWL2*.
396
397 **Acknowledgements:**
398 The research was supported by BMGF-PEARL grant OPP1131765 “Unraveling the molecular
399 genetics of finger millet blast disease and the existing resistance for the development of high-
400 yielding resilient varieties” and NSF-BREAD ABRDC award IOS-1543901 “Development of
401 Essential Genetic and Genomic Resources for Finger Millet”.
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404

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Tables:

Table 1. PCR primers used in this study.

Primer	**Sequence (5' → 3')**
E22_PWL1_F	CTTAGTGGACCCTTTGTCCG
E22_PWL1_R	GGAAACTAGCGAGCGTGGTTAG
E22_PWL2_F	CCTTATCACGTGAGGTGGAG
E22_PWL2_R	CCAAACAAGCTTCGAGGC
PWL1_CDS_F	ATGAAATTCAACAAAACTATCC
PWL1_CDS_R	TTACATAATATGGCAGCCC
PWL2_CDS_F	ATGAAATGCAACAACATCATCCTCCC
PWL2_CDS_R	ACATAATATTGCAGCCCTCTTCTCGC

545 **Table 2.** Distribution of *PWL1 and PWL2* in *M. oryzae* isolated from *Eleusine coracana*

Group	Gene combinations	Number of isolates (n=221)	Origins	Isolate names
FMB-1	Lacking both *PWL1* and *PWL2*	132 (59.7%)	**Ethiopia** (11; 8.3%)	E1 E3 E8 E18 E30 E32 E43 E46 E55 E61 E62
			Kenya (43; 32.6%)	K1 K2 K3 K4 K5 K6 K7 K8 K9 K10 K11 K12 K13 K14 K15 K16 K17 K18 K19 K20 K21 K22 K23 K24 K26 K27 K28 K29 K30 K32 K33 K34 K35 K36 K37 K38 K39 K40 K41 K42 K43 K44 K45
			Tanzania (20; 15.2%)	T2 T9 T11 T14 T17 T19 T20 T23 T24 T26 T28 T34 T35 T39 T40 T46 T49 T53 T54 T58
			Uganda (58; 43.9%)	U1 U2 U3 U4 U5 U6 U7 U8 U9 U10 U11 U12 U13 U14 U15 U16 U17 U18 U19 U20 U21 U22 U23 U24 U25 U26 U27 U28 U29 U30 U31 U32 U33 U34 U35 U36 U37 U38 U39 U40 U41 U42 U43 U44 U45 U46 U47 U48 U49 U50 U51 U52 U53 U54 U55 U56 U57 U58
FMB-2	Carrying both *PWL1* and *PWL2*	50 (22.6%)	**Ethiopia** (45; 90.0%)	E2 E5 E6 E12 E13 E14 E15 E16 E17 E19 E20 E21 E22 E23 E24 E25 E26 E27 E29 E33 E34 E35 E36 E37 E38 E39 E40 E41 E42 E44 E45

				E47 E48 E49 E50 E51 E52 E53 E54 E56 E57 E58 E59 E60 E63
			Tanzania (5; 10.0%)	T1 T3 T27 T37 T57
FMB-3	*PWL1* only	36 (16.3%)	**Ethiopia** (4; 11.1%)	E4 E9 E28 E31
			Kenya (2; 5.6%)	K25 K31
			Tanzania (30; 83.3%)	T4 T5 T6 T7 T8 T10 T12 T13 T16 T18 T21 T22 T29 T30 T31 T32 T33 T36 T38 T41 T43 T44 T45 T47 T48 T50 T51 T52 T55 T56
FMB-4	*PWL2* only	3 (1.4%)	**Tanzania** (3; 100.0%)	T15 T25 T42

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549 **Table 3.** Plant accessions used for infection assays

Species	**Accession**	**Origin**
Calamovilfa longifolia	PI 477995	USA
Dactyloctenium giganteum	PI 364504	South Africa
Eleusine coracana subsp. *coracana*	AAUFM-44	Ethiopia
Eleusine floccifolia	PI 196853	Ethiopia
Eleusine tristachia	PI 309950	Brazil
Eragrostis curvula	Ermelo	USA
Eragrostis curvula	PI 197425	Kenya
Eragrostis curvula	PI 295694	South Africa
Eragrostis tef	Tsedey (DZ-Cr-37)	Ethiopia
Sporobolus phyllotrichus	PI 226098	Kenya

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553 **Table 4.** *M. oryzae* isolates from finger millet used in infection assays.

Strain	**Origin**	***PWL1***[1]	***PWL2***[1]
E2-GFP	Ethiopian isolate E2 transformed with enhanced GFP	+	+
E22	Ethiopia	+	+
U27	Uganda	−	−
U34	Uganda	−	−
U40	Uganda	−	−
U44	Uganda	−	−
CKF4183	U34 transformed with PWL1	+	−
CKF4184	U34 transformed with PWL1	+	−
CKF4185	U34 transformed with PWL1	+	−
CKF4186	U34 transformed with PWL2	−	+
CKF4187	U34 transformed with PWL2	−	+
CKF4188	U34 transformed with PWL2	−	+
CKF4192	U34 transformed with PWL2	−	+
CKF4193	U34 transformed with PWL2	−	+

554 [1] Negative and positive symbols indicate absence and presence, respectively, of either *PWL1*
555 or *PWL2*

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558

559 **Figure captions**

560

561 Fig 1. Pathogenicity of finger millet blast strains on *Eragrostis curvula* and *Sporobolus*
562 *phyllotrichus*. The latter was used as a susceptible control. Leaves of *Eragrostis curvula* (PI
563 197425) and *Sporobolus phyllotrichus* (PI 226098) at seven days after spray inoculation with
564 a panel of *M. oryzae* strains; E22 and U34 are field isolates, and CKF4183 and CKF4188 are
565 transformants of U34 with *PWL1* and *PWL2*, respectively, cloned from E22. The presence or
566 absence of *PWL1* and *PWL2* is indicated by plus (+) or minus (-), respectively. Asterisks
567 indicate typical virulent lesions (straw colored and shriveled leaf with merged lesions).
568 Arrowheads indicate some avirulent lesions, and arrows indicate some isolated lesions with
569 distinct tan centers surrounded by a darker brown margin. Consistent infection results were
570 observed from more than eight leaves in two independent inoculations. Note that *M.*
571 *oryzae* transformants, carrying either *PWL1* (CKF4183) or *PWL2* (CKF4188), gained
572 avirulence on *E. curvula*, compared to the recipient strain U34, while maintaining virulence
573 on *S. phyllotrichus*. Bars = 0.5 cm.

574

575 Fig 2. Pathogenicity of finger millet blast strains on *Eragrostis curvula* and *Eleusine coracana*.
576 Leaves of *E. curvula* cv. Ermelo and *E. coracana* cv. AAUFM-44 at seven days after spray
577 inoculation with E2-GFP, U27, and U44 at a concentration of $1x10^5$ spores/mL on *E.*
578 *curvula* and $1x10^4$ spores/mL on *E. coracana*. The plus (+) and minus (-) indicate, respectively,
579 the presence or absence of *PWL1* and *PWL2*. Arrowheads indicate some of the typical avirulent
580 lesions. Asterisks indicate typical virulent symptoms with merged lesions. Consistent infection
581 results were observed from more than three leaves in two independent inoculations except for
582 U27-*E. coracana*, which was tested in one experiment. Bars = 0.5 cm.

583

584 Fig 3. *Eragrostis curvula* germplasms inoculated with U34, lacking both *PWL1* and *PWL2*, or
585 E22, carrying both genes, at a concentration of $1x10^4$ spores/mL. *Eleusine coracana* was used
586 as a susceptible control. An asterisk indicates typical virulent symptoms with merged lesions.
587 Arrowheads indicate some of the typical avirulent lesions, and arrows indicate some of the
588 small isolated lesions with distinct tan centers surrounded by a darker brown margin.
589 Consistent infection results were observed from more than three leaves in three experiments.
590 Bar = 1 cm
591
592 Fig 4. *Chloridoid* species inoculated with finger millet blast strains carrying or lacking *PWL1*
593 and *PWL2* with a concentration of $1x10^5$ spores/mL. The phylogenetic relationship between
594 the species is shown on the left hand side. Arrows indicate some of the small isolated lesions.
595 Asterisks indicate typical virulent symptoms with merged lesions. 'Not determined' indicates
596 that the plant species was not inoculated with that particular strain in this experiment.
597 Consistent infection results were observed from more than three leaves in one experiment.

Supplementary Fig. S1: Jalview alignment of the coding regions of *PWL1* from rice-infecting (RB) and finger millet-infecting (FMB) *Magnaporthe oryzae* strains.

Supplementary Fig. S2: Jalview alignment of the protein sequences of *PWL1* from rice-infecting (RB) and finger millet-infecting (FMB) *Magnaporthe oryzae* strains.

Supplementary Fig. S3: Jalview alignment of the coding regions of *PWL2* from rice-infecting (RB) and finger millet-infecting (FMB) *Magnaporthe oryzae* strains.

Supplementary Fig. S4: Jalview alignment of the protein sequences of *PWL2* from rice-infecting (RB) and finger millet-infecting (FMB) *Magnaporthe oryzae* strains.

Supplementary Fig. 5: Phylogenetic relationship between different Chloridoid species used in infection assays with finger millet-adapted *M. oryzae* strains and whether they are potential alternative hosts based on their resistance response to finger millet blast. Finger millet (*Eleusine coracana*), the species from which the *M. oryzae* strains were isolated, is indicated with an asterisk.

Supplementary Fig. 6: Leaf segments of *Eragrostis curvula* (PI 197425) showing standard infection types seven days after inoculation with indicated *M. oryzae* strains. Red bracket: leaf tissue exposed at the time of inoculation. Green bracket: new growth after inoculation. Six infection types were defined. Type 0: absence of visible lesions or evidence of infection. Type 1: small brown spots without a defined tan center (some indicated with arrowheads). Type 2: lesions with a visible tan center surrounded by a dark brown margin (some indicated with arrows). Type 3: lesions with a tan center surrounded by a dark brown margin, and some

624 merged to create larger lesions with a shared brown margin. Type 4: coexistence of straw-
625 colored and shriveled tissue (dot) towards the tip of the leaf with type 1 and type 2 lesions
626 (arrowheads and arrows, respectively) towards the edge of the inoculated leaf area. Type 5:
627 inoculated leaf tissue is completely straw-colored and shriveled compared to healthy new
628 growth. Lesions are fully merged and defined margins lost; very few isolated lesions may
629 occur at the edge of the inoculated leaf area. Note that *M. oryzae* wild-type E22, carrying
630 both *PWL1* and *PWL2*, causes Type 0 (complete avirulence), whereas another wild-type U34,
631 lacking both genes, causes Type 5 (highly virulent) on *E. curvula* (PI 197425). Also, note
632 that *M. oryzae* transformants, carrying either *PWL1* (CKF4183) or *PWL2* (CKF4188,
633 CKF4187, and CKF4193), gained a varying degree of avirulence on *E. curvula*, compared to
634 the recipient strain U34, while maintaining virulence on *S. phyllotrichus* (See Fig. 1 and
635 Supplementary Table 1).
636
637 Supplementary Table S1. Infection Summary.

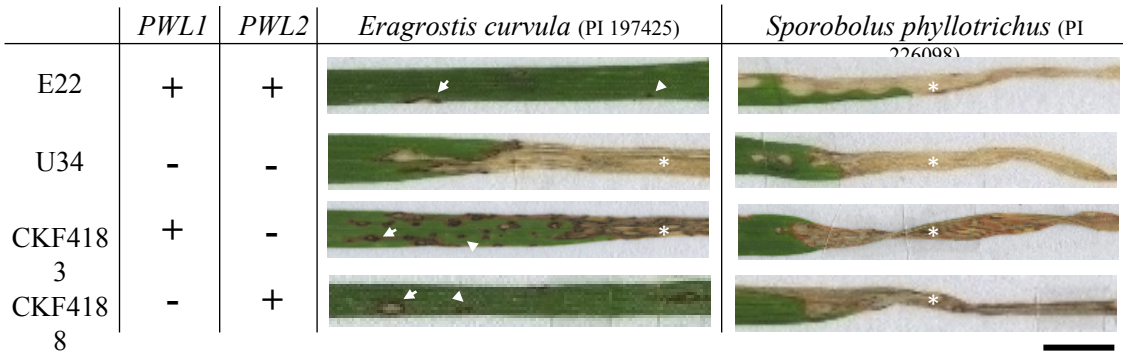


Figure 1. Pathogenicity of finger millet blast strains on *Eragrostis curvula* and *Sporobolus phyllotrichus*. The latter was used as a susceptible control. Leaves of *Eragrostis curvula* (PI 197425) and *Sporobolus phyllotrichus* (PI 226098) at seven days after spray inoculation with a panel of *M. oryzae* strains; E22 and U34 are field isolates, and CKF4183 and CKF4188 are transformants of U34 with *PWL1* and *PWL2*, respectively, cloned from E22. The presence or absence of *PWL1* and *PWL2* is indicated by plus (+) or minus (-), respectively. Asterisks indicate typical virulent lesions (straw colored and shriveled leaf with merged lesions). Arrowheads indicate some avirulent lesions, and arrows indicate some isolated lesions with distinct tan centers surrounded by a darker brown margin. Consistent infection results were observed from more than eight leaves in two independent inoculations. Note that *M. oryzae* transformants, carrying either *PWL1* (CKF4183) or *PWL2* (CKF4188), gained avirulence on *E. curvula*, compared to the recipient strain U34, while maintaining virulence on *S. phyllotrichus*. Bars = 0.5 cm.

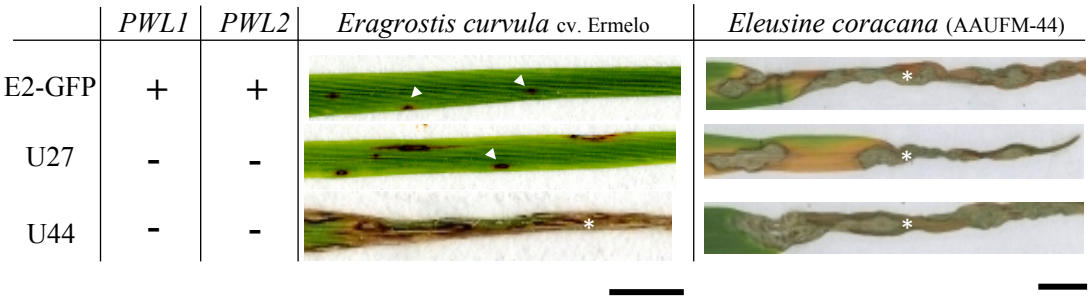


Figure 2. Pathogenicity of finger millet blast strains on *Eragrostis curvula* and *Eleusine coracana*. Leaves of *E. curvula* cv. Ermelo and *E. coracana* cv. AAUFM-44 at seven days after spray inoculation with E2-GFP, U27, and U44 at a concentration of $1x10^5$ spores/mL on *E. curvula* and $1x10^4$ spores/mL on *E. coracana*. The plus (+) and minus (-) indicate, respectively, the presence or absence of *PWL1* and *PWL2*. Arrowheads indicate some of the typical avirulent lesions. Asterisks indicate typical virulent symptoms with merged lesions. Consistent infection results were observed from more than three leaves in two independent inoculations except for U27-*E. coracana*, which was tested in one experiment. Bars = 0.5 cm.

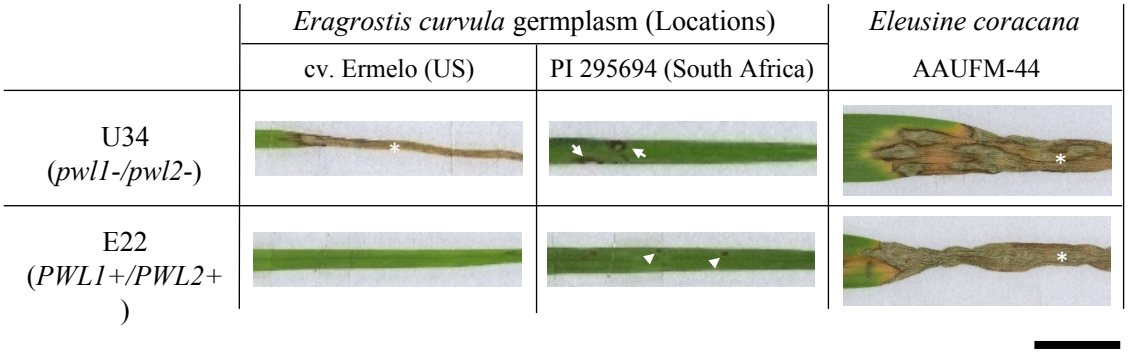


Figure 3. *Eragrostis curvula* germplasms inoculated with U34, lacking both *PWL1* and *PWL2*, or E22, carrying both genes, at a concentration of $1x10^4$ spores/mL. *Eleusine coracana* was used as a susceptible control. An asterisk indicates typical virulent symptoms with merged lesions. Arrowheads indicate some of the typical avirulent lesions, and arrows indicate some of the small isolated lesions with distinct tan centers surrounded by a darker brown margin. Consistent infection results were observed from more than three leaves in three experiments. Bar = 1 cm

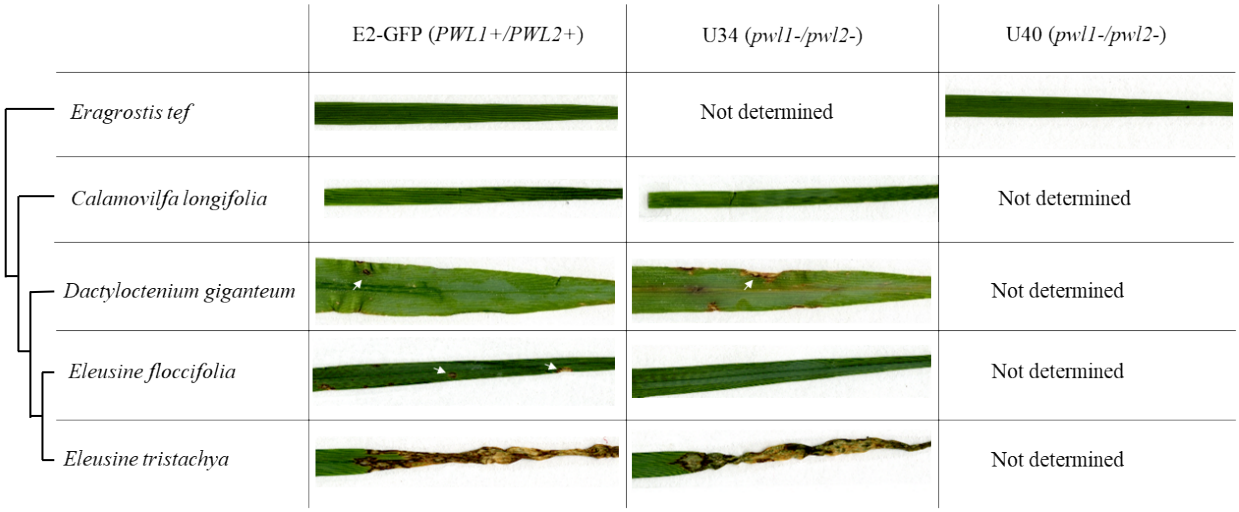
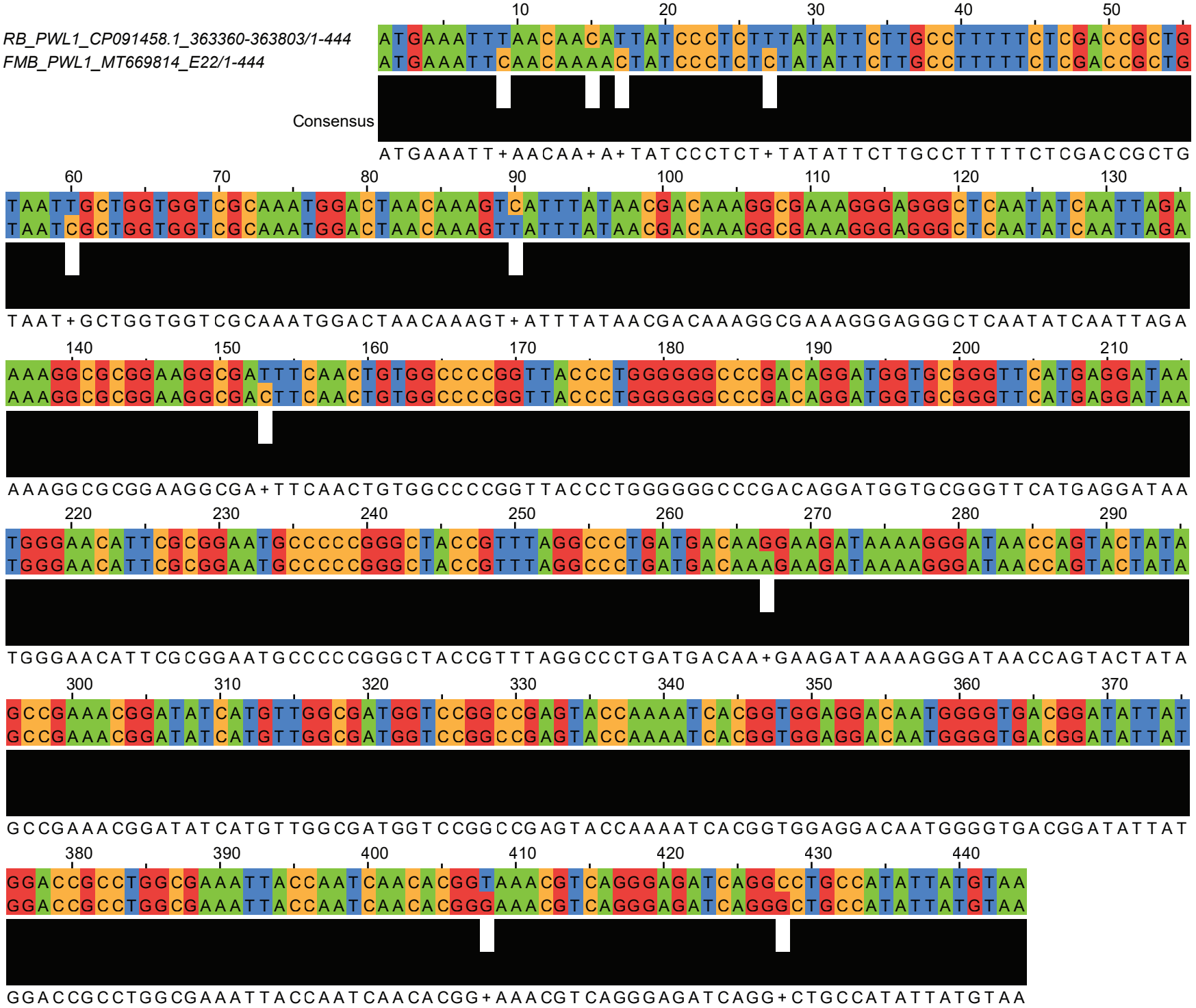
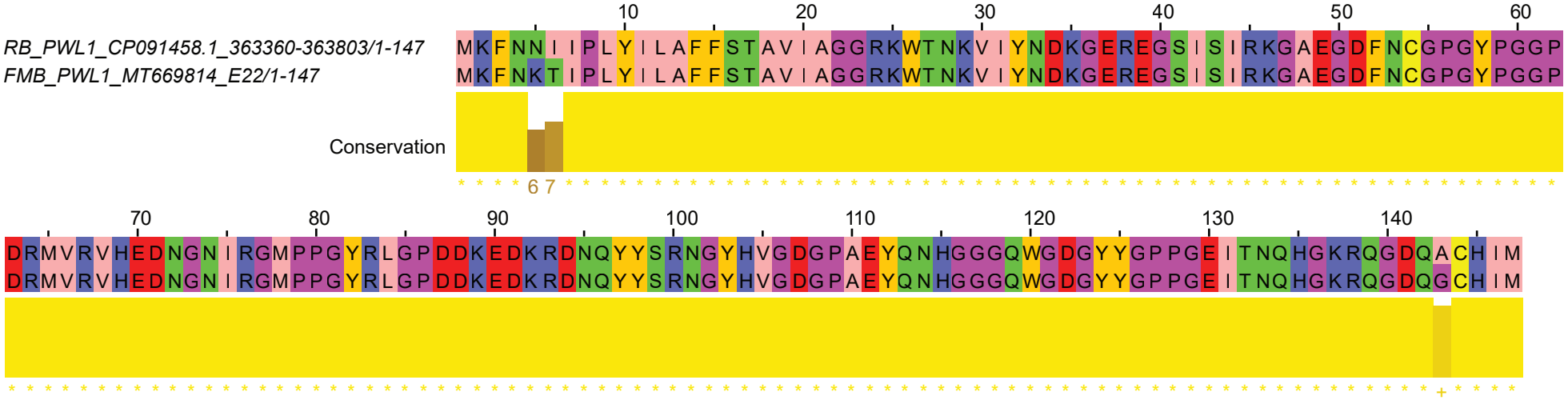


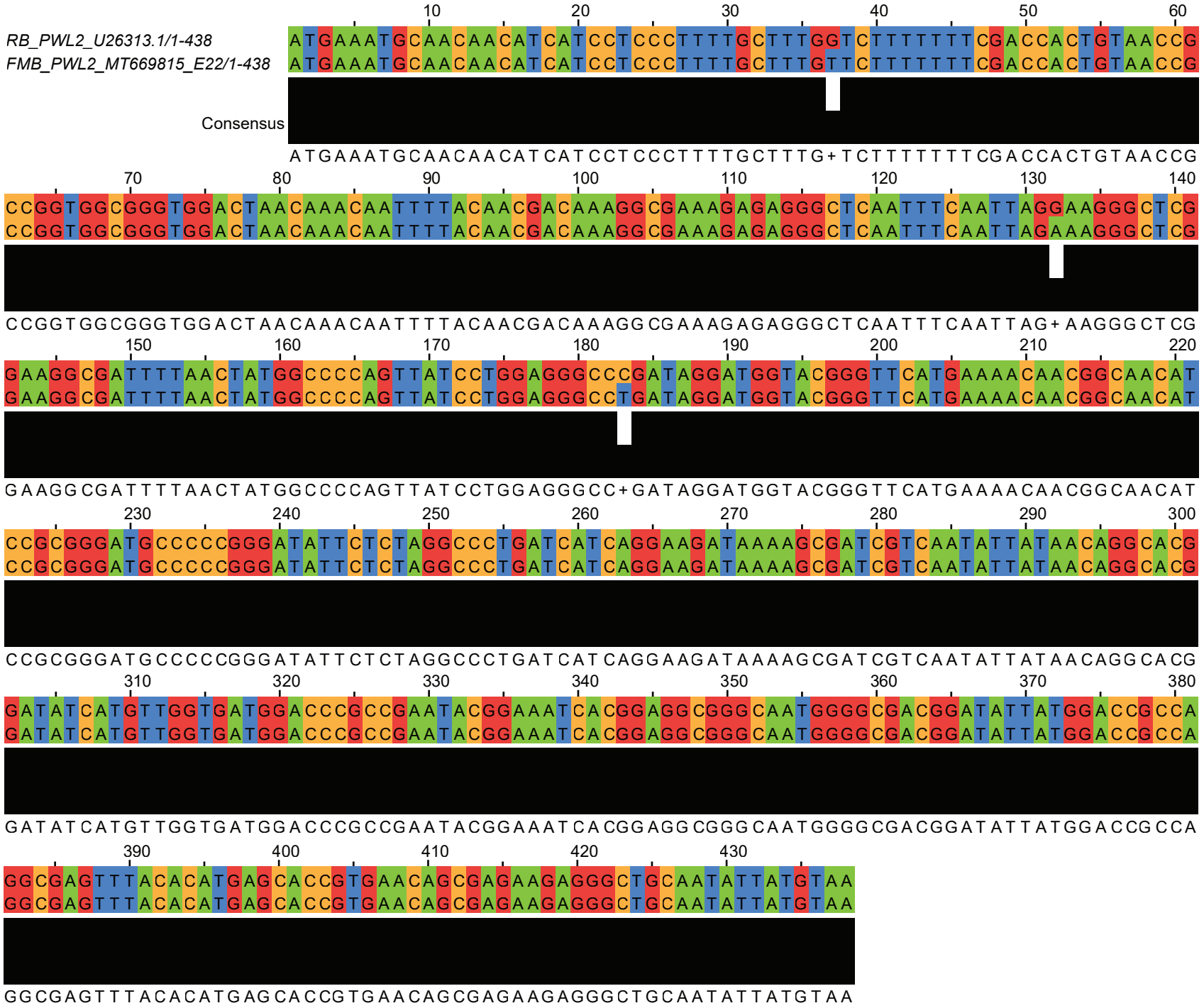
Figure 4. *Chloridoid* species inoculated with finger millet blast strains carrying or lacking *PWL1* and *PWL2* with a concentration of $1x10^5$ spores/mL. The phylogenetic relationship between the species is shown on the left hand side. Arrows indicate some of the small isolated lesions. Asterisks indicate typical virulent symptoms with merged lesions. 'Not determined' indicates that the plant species was not inoculated with that particular strain in this experiment. Consistent infection results were observed from more than three leaves in one experiment.



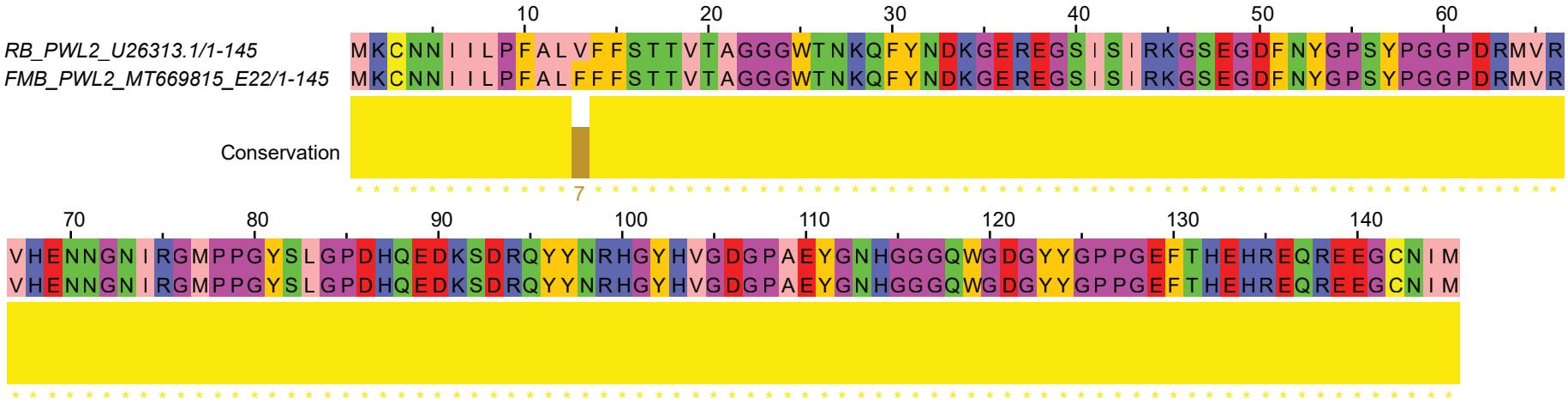
Suppl. Fig. S1. Jalview alignment of the coding regions of *PWL1* from rice-infecting (RB) and finger millet-infecting (FMB) *Magnaporthe oryzae* strains



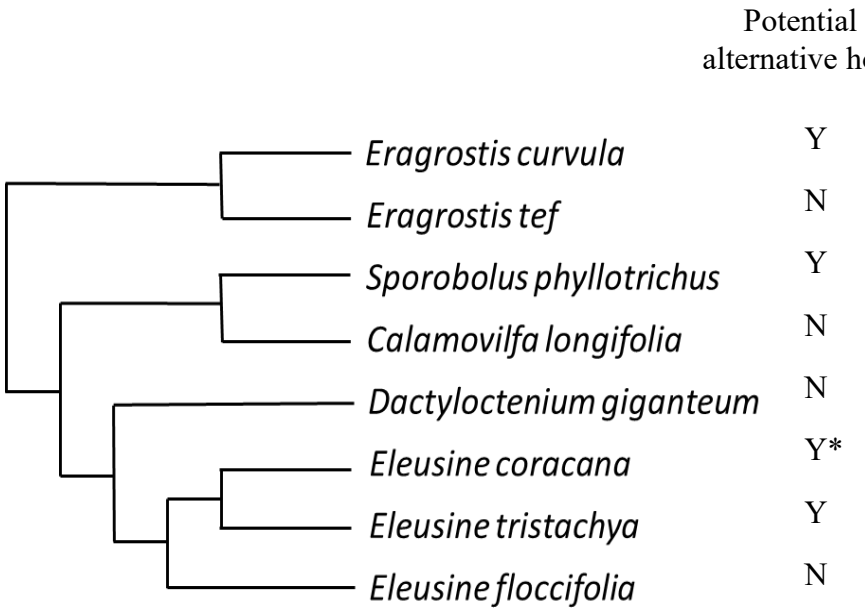
Suppl. Fig. S2. Jalview alignment of the protein sequences of *PWL1* from rice-infecting (RB) and finger millet-infecting (FMB) *Magnaporthe oryzae* strains



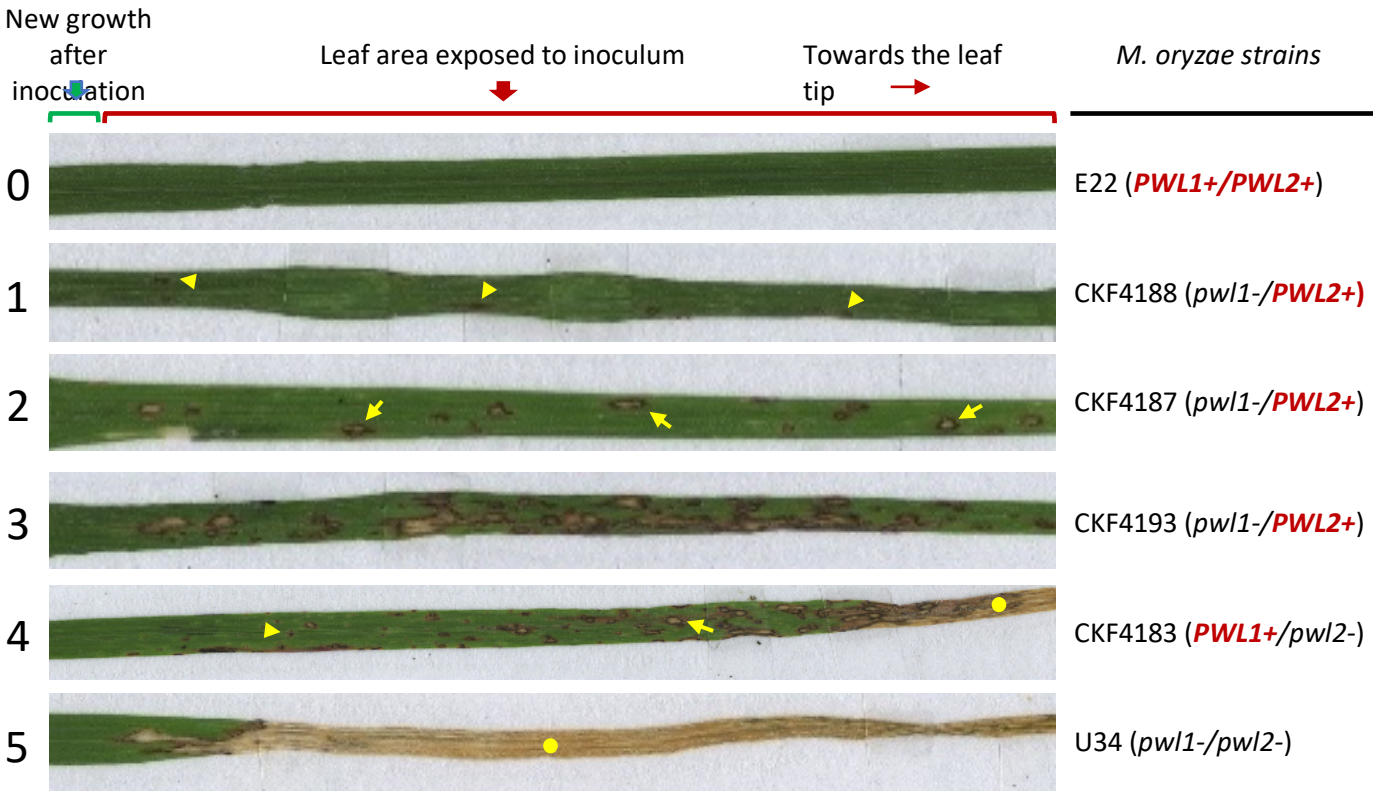
Suppl. Fig. S3. Jalview alignment of the coding regions of *PWL2* from rice-infecting (RB) and finger millet-infecting (FMB) *Magnaporthe oryzae* strains



Suppl. Fig. S4. Jalview alignment of the protein sequences of *PWL2* from rice-infecting (RB) and finger millet-infecting (FMB) *Magnaporthe oryzae* strains



Supplementary Fig. S5: Phylogenetic relationship between different Chloridoid species used in infection assays with finger millet-adapted *M. oryzae* strains and whether they are potential alternative hosts based on their resistance response to finger millet blast. Finger millet (*Eleusine coracana*), the species from which the *M. oryzae* strains were isolated, is indicated with an asterisk.



Supplementary Fig. S6: Leaf segments of *Eragrostis curvula* (PI 197425) showing standard infection types seven days after inoculation with indicated *M. oryzae* strains. Red bracket: leaf tissue exposed at the time of inoculation. Green bracket: new growth after inoculation. Six infection types were defined. Type 0: absence of visible lesions or evidence of infection. Type 1: small brown spots without a defined tan center (some indicated with arrowheads). Type 2: lesions with a visible tan center surrounded by a dark brown margin (some indicated with arrows). Type 3: lesions with a tan center surrounded by a dark brown margin, and some merged to create larger lesions with a shared brown margin. Type 4: coexistence of straw-colored and shriveled tissue (dot) towards the tip of the leaf with type 1 and type 2 lesions (arrowheads and arrows, respectively) towards the edge of the inoculated leaf area. Type 5: inoculated leaf tissue is completely straw-colored and shriveled compared to healthy new growth. Lesions are fully merged and defined margins lost; very few isolated lesions may occur at the edge of the inoculated leaf area. Note that *M. oryzae* wild-type E22, carrying both *PWL1* and *PWL2*, causes Type 0 (complete avirulence), whereas another wild-type U34, lacking both genes, causes Type 5 (highly virulent) on *E. curvula* (PI 197425). Also, note that *M. oryzae* transformants, carrying either *PWL1* (CKF4183) or *PWL2* (CKF4188, CKF4187, and CKF4193), gained a varying degree of avirulence on *E. curvula*, compared to the recipient strain U34, while maintaining virulence on *S. phyllotrichus* (See Fig. 1 and Supplementary Table 1).

Supplementary Table S1. Infection Summary

M. oryzae strains \ Plants			Finger millet	Weeping lovegrass			Chloridoid grasses					
Name	*PWL1*[a]	*PWL2*[a]	AAUFM-44	Kenya PI 197425	South Africa PI 364504	USA Ermelo	*S. phyllotrichus*	*E. tef*	*C. longifloria*	*D. giganteum*	*E. floccifolia*	*E. tristachya*
E2(GFP)	+	+	4.3 / 0.5	2.3 / 0.7	1.5 / 0.5	2.0 / 0.0	n.d.	1.0 / 0.0	1.0 / 0.0	1.0 / 0.0	2.0 / 0.0	4.7 / 0.5
E22	+	+	5.0 / 0.0	1.2 / 0.4	1.2 / 0.4	0.8 / 1.6	4.3 / 0.4	n.d.	n.d.	n.d.	n.d.	n.d.
U27	−	−	3.3 / 0.5	n.d.	n.d.	1.7 / 0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
U34	−	−	5.0 / 0.0	5.0 / 0.0	2.5 / 1.1	4.8 / 0.4	5.0 / 0.0	n.d.	1.3 / 0.5	2.0 / 0.0	1.0 / 0.0	4.3 / 0.5
U40	−	−	5.0 / 0.0	n.d.	n.d.	n.d.	n.d.	1.0 / 0.0	n.d.	n.d.	n.d.	n.d.
U44	−	−	5.0 / 0.0	n.d.	n.d.	4.0 / 1.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CKF4183	+	−	4.8 / 0.4	3.8 / 0.4	n.d.	n.d.	4.8 / 0.4	n.d.	n.d.	n.d.	n.d.	n.d.
CKF4184	+	−	n.d.	4.3 / 0.5	n.d.	n.d.	4.8 / 0.4	n.d.	n.d.	n.d.	n.d.	n.d.
CKF4185	+	−	n.d.	4.3 / 0.5	n.d.	n.d.	4.7 / 0.5	n.d.	n.d.	n.d.	n.d.	n.d.
CKF4186	−	+	n.d.	1.7 / 0.5	n.d.	n.d.	5.0 / 0.0	n.d.	n.d.	n.d.	n.d.	n.d.
CKF4187	−	+	n.d.	1.4 / 0.5	n.d.	n.d.	4.0 / 0.0	n.d.	n.d.	n.d.	n.d.	n.d.
CKF4188	−	+	4.4 / 0.5	1.8 / 0.4	n.d.	n.d.	4.5 / 0.5	n.d.	n.d.	n.d.	n.d.	n.d.
CKF4192	−	+	n.d.	2.4 / 0.8	n.d.	n.d.	4.8 / 0.4	n.d.	n.d.	n.d.	n.d.	n.d.
CKF4193	−	+	n.d.	3.7 / 0.9	n.d.	n.d.	5.0 / 0.0	n.d.	n.d.	n.d.	n.d.	n.d.

Notes

1. [a] Negative and positive symbols indicate absence and presence, respectively, of either *PWL1* or *PWL2*.
2. Infection scores are presented in the form of average / standard deviation. The standard infection types (Supplemental Figure S6) were used to determine the scores for at least two independent infection assays (more than three leaves for each assay), except for Chloridoid grasses (three leaves analyzed from one experiment). n.d. = not determined
3. Note that *M. oryzae* wild-type E22, carrying both *PWL1* and *PWL2*, is avirulent on weeping lovegrass (Kenya, PI 197425; average infection score = 1.2; see Supplementary Figure S6 for infection score key), whereas another wild-type U34, lacking both genes, is highly virulent on the same weeping lovegrass line (average infection score = 5.0). Both E22 and U34 are highly virulent on finger millet AAUFM-44 (average infection score = 5.0). Also, note that *M. oryzae* transformants, carrying either *PWL1* (CKF4183; average infection score = 3.8) or *PWL2* (CKF4186, CKF4187, and CKF4188; average infection score < 2.0), gained a varying degree of avirulence, compared to the recipient strain U34, while maintaining virulence on *S. phyllotrichus* (average infection score > 4.0) and finger millet AAUFM-44 (CKF4183 and CKF4188; average infection score > 4.4). See Fig. 1 and Supplementary Figure S6.
4. Color codes for infection scores: orange = virulent; blue = avirulent; green: gain of avirulence
5. *S. phyllotrichus = Sporobolus phyllotrichus; E. tef = Eragrostis tef; C. longifloria = Calamovilfa longifloria; D. giganteum = Dactyloctenium giganteum; E. floccifolia = Eleusine floccifolia; E. tristachya = Eleusine tristachya.*