

1 **A Genome-Wide Association Study Identifies SNP Markers for Virulence in**
2 ***Magnaporthe oryzae* Isolates from Sub-Saharan Africa**

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29 **ABSTRACT**

30 The fungal phytopathogen *Magnaporthe oryzae* causes blast disease in cereals such as
31 rice and finger millet worldwide. In this study, we assessed genetic diversity of 160
32 isolates from nine sub-Saharan Africa (SSA) and other principal rice producing
33 countries and conducted a genome-wide association study (GWAS) to identify the
34 genomic regions associated with virulence of *M. oryzae*. GBS of isolates provided a
35 large and high-quality 617K single nucleotide polymorphism (SNP) dataset. Disease
36 ratings for each isolate was obtained by inoculating them onto differential lines and
37 locally-adapted rice cultivars. Genome-wide association studies were conducted using
38 the GBS dataset and sixteen disease rating datasets. Principal Component Analysis
39 (PCA) was used as an alternative to population structure analysis for studying population
40 stratification from genotypic data. A significant association between disease phenotype
41 and 528 SNPs was observed in six GWA analyses. Homology of sequences
42 encompassing the significant SNPs was determined to predict gene identities and
43 functions. Seventeen genes recurred in six GWA analyses, suggesting a strong
44 association with virulence. Here, the putative genes/genomic regions associated with
45 the significant SNPs are presented.

46 **INTRODUCTION**

47 Rice is of increasing importance as a staple food crop in sub-Saharan Africa (SSA),
48 particularly due to the rapidly growing urban population. (Amberwaves; Nigatu *et al.*,
49 2017 USDA, 2017). Currently, 80% of rice production in SSA takes place in eight
50 countries: Nigeria, Madagascar, Mali, Guinea, Côte d'Ivoire, Tanzania, Sierra Leone
51 and Senegal with 20% from the rest of SSA. Nigeria and Madagascar alone account for

52 a third of SSA rice production. It is estimated that rice consumption in SSA will grow
53 from the current 27-28 million tonnes to 36 million tonnes by the year 2026
54 (Amberwaves; Nigatu *et al.*, 2017 USDA, 2017). Considering the predictions of rice
55 consumption required to attain self-sufficiency, SSA will need to increase production by
56 at least 10% per year for the next 10 years, or succumb to international rice trade with
57 large imports impacting the economy of already financially fragile countries
58 (Amberwaves; Nigatu *et al.*, 2017 USDA, 2017). Because of the increase in demand,
59 several strategies are underway to increase rice production, which include increased
60 usage of fertilizers, planting high-yielding cultivars and increasing the area of rice
61 production (Balasubramanian *et al.*, 2007). In spite of efforts to increase production,
62 biotic constraints such as damage by fungal pathogens, remain a major challenge
63 towards achieving this food security goal. Recent statistics indicate that while the total
64 rice harvest has grown at an average rate of 4.2% from 2007 to 2016, there are several
65 abiotic and biotic stresses that restrict rice production, including rice blast disease
66 (USDA 2017, Mgonja *et al.*, 2016, Mutiga *et al.*, 2017). Annual crop losses due to rice
67 blast disease, caused by the fungus *Magnaporthe oryzae*, are reported to be up to 30%,
68 with regional blast disease outbreaks causing up to 80% yield losses (Nalley *et al.*,
69 2016). Another major challenge in enhancing rice production is that smallholder
70 farmers, who cannot afford the cost of fungicides, are the major growers of rice (Saito *et*
71 *al.*, 2013; Nalley *et al.*, 2016). Therefore, farmer-friendly technologies such as breeding
72 for rice blast resistance and surveillance of the fungal pathogen are urgently needed
73 (Mutiga *et al.*, 2017).

74 To breed for blast resistant rice, an understanding of the pathology and
75 molecular interactions between the fungus and its host is required. Knowledge of the
76 pathosystem is necessary and involves information of different lifestyles that fungi can
77 adopt and their co-evolution with plants, which also reflects their biological diversity and
78 worldwide distribution (Occhipinti, 2013). Owing to adaptations of both the pathogen
79 and crop plants, plant-pathogen interactions differ significantly across agro-ecological
80 systems. Building on the many studies that have begun to unravel the genetic basis of
81 plant-fungal interactions, current work is aimed at identifying the molecular signatures
82 for virulence (Burdon and Thrall, 2009). The rice blast pathosystem is a long-standing
83 co-evolutionary model system, however, the molecular interactions between *M. oryzae*
84 and rice has not been well studied using isolates from sub-Saharan Africa.

85 Hemibiotrophic fungal phytopathogens, such as *M. oryzae*, secrete effector
86 proteins that counter plant defense signals and are key determinants of pathogenesis
87 and virulence (Kamoun, 2007; Dodds *et al.*, 2009). Genomics-based effector discovery
88 has identified genomic regions encoding effectors (Gibriel *et al.*, 2016). Plants in turn,
89 exhibit recognition mechanisms that trigger production of resistance (*R*) proteins that
90 directly, or indirectly, interact with effector molecules also called avirulence (*Avr*)
91 proteins (Dodds and Rathjen, 2010; Petit-Houdénot and Fudal 2017). The activation of
92 Effector-Triggered Immunity (ETI) in plants occurs upon the recognition of a pathogen
93 *Avr* protein leading to a hypersensitive reaction (HR), which causes localized cell death,
94 hence blocking disease progression (Jones and Dangl, 2006). This knowledge of *R-Avr*
95 gene interaction is frequently utilized by scientists in breeding for superior crops that
96 possess novel dominant resistance genes that cannot be infected by resident pathogen

97 races. Genetic control of blast disease therefore involves development of resistant rice
98 cultivars that harbor major *R* genes (Petit-Houdenot and Fudal, 2017). There is however
99 a significant risk of resistance breakdown because of the selection for *Avr* genes to
100 mutate leading to loss of recognition by *R* gene products. To overcome this risk, a new
101 strategy is to pyramid multiple *R* genes into a locally adapted rice variety (Mutiga *et al.*,
102 2017; Pilet-Nayel *et al.*, 2017). More than 100 rice blast *R* genes have been identified in
103 rice to date (Sharma *et al.*, 2012) and many of these have been cloned and
104 characterized. The rice panel used in this GWAS includes lines harboring six major *R*
105 genes (*Pia*, *Pita*, *Pi9*, *Pik*, *Pizt*, *Piz-5*) that have been functionally characterized and the
106 *R* gene *Pi3* that is organized into a gene cluster with *Pii* (Wu *et al.*, 2015).

107 The advent of Next Generation Sequencing (NGS) and availability of fungal
108 genomes has led to accelerated identification of *Avr* genes (Petit-Houdenot and Fudal
109 2017). However, due to exertion of selection pressure by *R* genes, fungal pathogens
110 very frequently become virulent through evolution of *Avr* genes. In such cases, virulence
111 can be achieved by inactivation, down-regulation or complete deletion of the *Avr* gene,
112 or simply generating point mutations such as single nucleotide polymorphisms (SNPs)
113 that disable recognition (Guttman *et al.*, 2014; Jones and Dangl, 2006). Accessibility of
114 whole genomes or reduced representation of genomes has enabled genome-level
115 analysis of plant pathogens. Sequence variation is often used to identify complex traits
116 in plants, animals and microorganisms. There are several high-throughput methods that
117 combine NGS with reduced representation of genomes (Glaubitz *et al.*, 2014).
118 Genotyping-by-sequencing (GBS) is a simple, robust multiplex method that generates
119 large numbers of SNPs at very low cost (Elshire *et al.*, 2011). GBS has been mostly

120 used to study host plants (Torkamaneh *et al.*, 2017; Fernandez-Mazuecos *et al.*, 2017;
121 Hussain *et al.*, 2017) because of the emphasis on resistance, however this technique
122 can equally be applied to a pathogen. There are several combinatorial factors that can
123 complicate the characterization of virulence in plant-pathogenic fungi, including genetic
124 diversity present in natural populations, difficulty in conducting controlled crosses
125 among genotypes, complexity of inheritance, inaccessibility to genomic information and
126 the high costs associated with whole genome sequencing (Leboldus *et al.*, 2015). Using
127 low cost GBS data can circumvent some of these issues (Leboldus *et al.*, 2015), and
128 recent studies using GBS-based identification of quantitative trait loci in fungal
129 pathogens have begun to emerge (Norelli *et al.*, 2017). GBS can be used to study
130 population diversity and conduct Genome-wide Association Studies (GWAS) (Mjonga *et*
131 *al.*, 2017).

132 GWAS is a potent tool used to detect genomic regions associated with natural
133 variation in biological systems. This method is increasingly being used to study variation
134 in plants, especially to fine map resistance genes in plants. However, GBS has only
135 been sparingly used to detect genetic variants associated with pathogenicity/virulence in
136 pathogens (Sanchez-Vallet *et al.*, 2017). In the last couple of years, GWAS has been
137 utilized for successful identification of a wide range of alleles and candidate genes
138 associated with disease or pathogenicity factors/phenotypes (Plissonneau *et al.*, 2017).
139 There are more than 35 GWAS that have used SNPs as genetic markers for identifying
140 genomic regions associated with plant response to pathogen infection (Bartoli and
141 Roux, 2017) but only six of those GWAS reports on plant pathogens identified candidate
142 pathogenicity determinants/genes. This includes one study of the bacterial pathogen

143 *Pseudomonas syringae* (Monteil *et al.*, 2016) and five GWAS on fungal
144 phytopathogens; *Heterobasidion annosum* s.s., *Parastagonospora nodorum*, *Fusarium*
145 *graminearum*, *Puccinia triticina* and *Zymoseptoria tritici* (Dalman *et al.*, 2013; Gao *et al.*,
146 2016; Talas *et al.*, 2016, Wu *et al.*, 2017, Hartmann *et al.*, 2017). These studies were
147 conducted to directly identify loci in the genome of the pathogen contributing to
148 virulence.

149 Our previous research (Mutiga *et al.*, 2017) on the virulence spectrum and
150 genetic diversity of *M. oryzae* samples from SSA suggested that regional breeding
151 strategies would be required for East and West Africa based on the observed
152 associations between genetic relatedness and virulence spectrum. The objectives of the
153 current study are as follows;

- 154 1. To dissect the pathogen-host interaction for African collections of *M.*
155 *oryzae* based on inoculations on genotypes carrying known *R*-genes
- 156 2. To discover new avirulence genes, which could be useful in providing
157 insights onto how *M. oryzae* interacts with rice
- 158 3. To gain insight into pathogen-host co-evolution; through knowledge of
159 shared virulence genes; to assist the team in developing a breeding
160 strategy to achieve durable resistance to rice blast disease

161 Here we use GBS and GWAS to detect SNPs to identify loci and putative genes
162 as markers of virulence profile. To the best of our knowledge, this is the first study to
163 report GWAS of *M. oryzae* isolates from sub-Saharan Africa. Natural isolates of *M.*
164 *oryzae* tend to be female sterile (Ray *et al.*, 2016) and thus, this is also the first report of
165 GWAS of an asexually reproducing fungal pathogen.

166 **MATERIALS AND METHODS**

167 **Fungal Population used in the study**

168 The genotyped *M. oryzae* population comprise 160 isolates from nine African countries
169 ($n=128$) including Tanzania, Kenya and Uganda from East Africa, Nigeria, Burkina
170 Faso, Benin, Togo, Mali and Ghana from West Africa, and international isolates ($n=32$)
171 from seven major rice producing countries of China, India, Egypt, Philippines, South
172 Korea, Colombia and United States (Table 1). The isolates were provided by D.
173 Tharreau (Centre de Coopération Internationale en Recherche Agronomique pour le
174 Développement, CIRAD, Montpellier, France), and by collaborators in the rice blast
175 project which was supported through the Sustainable Crop Production Research for
176 International Development (SCPRID) initiative funded by Bill & Melinda Gates
177 Foundation through the Biotechnology and Biological Sciences Research Council
178 (BBSRC) and Department for International Development (DFID) of the United Kingdom
179 between 2012 and 2014.

180 **Fungal culture preparation, genomic DNA extraction, GBS library construction** 181 **and Pathogenicity assays**

182 Fungal isolates were grown in liquid PD broth and DNA was extracted using DNeasy
183 Plant Mini Kit (Qiagen GmbH). GBS library construction, MiSeq sequencing and
184 pathogenicity assays were conducted as previously described in Mutiga et al., 2017. A
185 visual disease rating scale of 0-9 was used to assess foliar blast in pathogenicity
186 assays.

187 **Rice Germplasm used in the study**

188 Sixteen rice cultivars were used in the pathotyping assays. Differential rice lines IRBLA-
189 a, IRBLTA CP1, IRBL3-CP4, IRBL9-W, IRBLK-KA, IRBLZT-T, IRBLZ5-CA(R), IRBLTA
190 CT2 (n=8), African Upland Rice NERICA2 and NERICA5, African lowland rice FKR62N,
191 African intraspecific lines TS2 and F6-36, susceptible check UZROZ275, *Pi9* donor line
192 75-1-127 and African *Oryza glaberrima* rice AR105. Nine of these cultivars had the
193 presence of known blast resistance genes *Pia*, *Pita*, *Pi3*, *Pi9*, *Pizt*, *Piz-5*, *Pita* (Table 3).
194 Rice cultivars containing the R-genes are known to be in the common background of an
195 Asian *japonica* rice cultivar called Lijiangxituanheigu (LTH). The *Pi9* donor line 75-1-127
196 was kindly provided by the Wang lab (Plant Pathology, The Ohio State University), in
197 collaboration with Liu *et al.*, 2002. IRBLs (IRRI-bred blast-resistant lines) were provided
198 by IRRI and bulked at DBNRRC, Stuttgart, Arkansas. UZROZ275 was provided by
199 Correll lab (University of Arkansas). AR105, F6-36 and NERICA lines were provided by
200 Dr. Ouédraogo (INERA-Burkina Faso, Linares 2002), NERICA lines were established by
201 Africa Rice Center utilizing crosses of *O.glaberrima* and *O.sativa*.

202 **Phylogenetic and PC analysis**

203 The high-quality filtered SNP panel consisting of 617,281 SNPs was used to conduct
204 the neighbor-joining phylogenetic cluster and principal component analyses using
205 TASSEL v5.2.1.6 (Bradbury *et al.*, 2007). The unrooted neighbor-joining tree was
206 constructed using the calculations derived from pairwise distance matrix between
207 individuals/taxa. PC analysis was performed and the values of first three components
208 were exported to R for plotting the 3D plot using P3D function (R core team 2015)

209 **Genome-wide association analysis**

210 Association between SNPs (GBS) and disease scores (pathogenicity assay) data was
211 implemented using TASSEL v5.2.3 (Bradbury et al., 2007). GWA analyses were named
212 after the cultivar of rice from which the disease scores were obtained. A Generalized
213 Linear Model (GLM) was used to execute the association analysis utilizing the least
214 squares fixed effects linear model. The p -values obtained from the analysis were
215 visualized in a Q-Q plot using the R package “qqplot” (R core team., 2013). p -value
216 cutoffs differed each analysis as they were dependent on the deviation of p -values from
217 $X=Y$ line representing true associations.

218 **Identification of putative virulence-related genes**

219 A region 500bp upstream and downstream surrounding the SNPs was extracted from
220 the respective contigs using a customized R script (R Core team., 2017). The size of the
221 sequence determined was based on the average length of a protein sequence in NCBI
222 db. The homology of the sequence surrounding the SNPs was identified using the DNA
223 database from NCBI (v 2.7.1, 23 Oct, 2017).

224 **RESULTS**

225 **Geographical representation of fungal isolates from sub-Saharan Africa and the** 226 **major rice growing areas of the world**

227 The field isolates used for both pathotyping and genotyping in this study ($n=160$) were
228 collected from blast-infected rice plants from nine countries of sub-Saharan Africa,
229 including East African countries (Kenya, Tanzania and Uganda), and West African
230 countries (Benin, Burkina Faso, Ghana, Mali, Nigeria, and Togo, as shown in Fig. 1A. In
231 addition, some isolates from major rice growing areas outside of SSA, such as China,

232 India, Egypt, Philippines, Colombia, South Korea and United States, were included as
233 comparators in the analysis. SSA isolates represent the major regions as follows: those
234 from West African countries were the highest ($n=75$, 46.9%) compared to East African
235 ($n=53$, 33.1%) and International isolates ($n=32$, 20%) (Table 1). There was variable
236 sampling within countries and Burkina Faso had the largest sampling size ($n=36$,
237 22.5%) while the least sampling size was from Ghana ($n=2$, 1.25%).

238 **GBS identifies genetic diversity among SSA field isolates**

239 Genotyping of 160 isolates of *M. oryzae* from diverse geographic regions generated a
240 617K (617,281) panel of polymorphic markers. SNPs were then used to assess diversity
241 of isolates using unrooted neighbor-joining trees based on a pair-wise distance matrix.
242 The analyses showed segregation of isolates into 8 clades and 1 independent clade X.
243 Each cluster of isolates belonged to the same country or region (Fig. 1B and C). Most of
244 the East African countries grouped into clades 1, 4, 6, and 8 and West African countries
245 grouped into clades 2, 3, 5, 7, and 8. Two isolates, one from Burkina Faso (BF0017)
246 and another from Nigeria (NG0149), segregated from the rest of the major clades as an
247 independent subclade X.

248 Isolates from the West African country of Burkina Faso clustered into 3 clades
249 (called 2, 5 and 7) although some isolates from the East African country Tanzania were
250 also present in these clades. Kenyan isolates segregated into 2 predominant clades (1
251 and 6), isolates in Clade 1 clustered with 2 international isolates (from the USA and
252 South Korea) along with a Tanzanian isolate. Seven independent Kenyan isolates
253 clustered in Clade 4 with Tanzanian, Ugandan and 2 isolates from the United States.
254 Clade 3 members were mostly from the West African country of Nigeria, which clustered

255 closely with isolates from other West African countries of Benin, Togo, Ghana and
256 Burkina Faso, an isolate from East African country of Uganda and the international
257 isolates from USA, China, Colombia and Philippines. Clade 8 had the largest number of
258 isolates at 39, and its subclades mostly showed clustering of isolates within each
259 country. Isolates from Tanzania were, for example, more similar to the international
260 isolates 70-15, CHNOS-06 and BMI-24, whereas 3 isolates from Benin sub-clustered
261 with international isolates from the USA and Egypt. Isolates from Mali and Togo were
262 similar to the single Indian isolate IN24.

263 To explore the similarity among isolates in greater detail, we conducted Principal
264 Component Analysis (PCA) and constructed a 3D scatter plot based on the
265 discriminatory power of individual SNPs and used this to identify population structure
266 patterns within West African and East African isolates, (Fig. 1C). The first three principal
267 components (PCs) explained 23.7% of the total variance observed in the dataset. The
268 scatter plot based on the first 3 PCs also corresponded to a close phylogenetic
269 relationship between the isolates wherein, they segregated into 8 groups mostly based
270 on country or region. While PC1 accounted to 10.5% of the variability, PC2 and PC3
271 accounted for 8.3% and 4.9% of the variability respectively. The clustering of isolates
272 observed in the unrooted neighbor joining tree was consistent with the PC analysis.

273 **Description of isolates chosen for GWAS analysis**

274 Representative isolates from different SSA countries were selected based on the
275 distinct clades they occupied based on phylogenetic analysis and the availability of
276 pathotyping data for GWAS analysis. A total of 78 isolates were used for the analysis,
277 which accounts to 49% of the complete dataset and 80% of SSA dataset. All of the

278 isolates from the clades 6, 7, 8 and “X” were used for GWAS, whereas for clades
279 1,2,3,4 and 5, 85%, 25%, 80%, 82.3% and 38.8% of the isolates were analyzed as
280 shown in Table 2. International isolates were not included in the GWA analysis.

281 **GWAS identifies genomic markers of virulence**

282 Principal Component Analysis (PCA) has been used as an alternative to population
283 structure analysis for studying population stratification from genotypic data (Patterson et
284 al., 2006). In this study, we used PCA scores from the genotypes as covariates in the
285 GWAS analyses. GWA analyses were conducted to identify genomic locations/putative
286 genetic loci associated with the ability to cause rice blast disease in a given host
287 cultivar. A Generalized Linear Model (GLM) was used to perform GWA using least
288 squares fixed effects linear analysis using SNPs derived from GBS and maximum score
289 from the disease rating observed. From the 16 GWA analyses conducted, six showed
290 association with disease and 10 of them showed no association (Table 3). Most p -
291 values observed in the scatter plot with association to disease were similar to the
292 expected diagonal in the QQ-plot, demonstrating the appropriateness of the GLM Model
293 for GWAS used in this analysis (see Fig. 2).

294 Based on the Q-Q plot analysis of each GWA analysis, independent observed –
295 $\log P$ cutoffs were then used to obtain SNPs associated with the ability to cause disease.
296 A cutoff of 3.5 was used in the analyses of IRBLTA, IRBL3 and NERICA5, which yielded
297 95, 173 and 64 associated markers, respectively. A cutoff of 4.5 was used for the
298 analyses of IRBLA-a and IRBL9-W, which yielded 135 and 54 markers, respectively,
299 while a cutoff of 4.0 was used for IRBLK-KA which generated 7 associated markers (Fig
300 3). A total of 528 markers were obtained from the 6 GWA analyses. However, due to the

301 absence of a completely annotated Guy11 genome, a *de novo* assembly of Guy11
302 contigs was utilized (Source: Darren Soanes, University of Exeter) as the reference
303 genome in the alignment process during GBS analysis. This did not provide
304 chromosome locations and SNP positions on the genome, but did provide SNP
305 locations in the *de novo* contigs. We then used the contig information, coupled with the
306 SNP marker location, to identify the genomic location of markers compared to the
307 published 70-15 *M. oryzae* genome (Dean et al., 2005).

308 **GWAS guides prediction of virulence-associated genetic loci**

309 A nucleotide sequence similarity search using NCBI BLASTn database (Altschul et al.,
310 1990) was conducted with the sequences encompassing the identified SNPs, (500bp
311 upstream and downstream based on average length of protein sequence in NCBI db;
312 325 amino acids long) associated by GWAS with the ability to cause disease on a given
313 host. Their homologies were categorized into four different types, which are SNP
314 markers that are located in (a) predicted genes (PG), (b) hypothetical proteins (HP), (c)
315 Other (OT); those present in non-coding genomic sequences, such as repeat regions,
316 (d) no similarity (NS) (Table S1-S6).

317 The highest number of SNPs associated with blast disease were observed in the
318 GWA analysis involving rice line IRBL3-CP4. This yielded 173 markers, out of which a
319 majority of the markers ($n=71$, 41%) were predicted to be hypothetical proteins,
320 uncharacterized ORFs whose structural genes exist but where corresponding
321 translation products are so far unidentified. Fifty two of the markers (30%) were
322 identified as predicted genes with known functions, and 11 genes (6.35%) reported in
323 other uncharacterized genomic regions or repeat regions. The sequence location of 18

324 markers (10.4%) showed no similarity to any sequence present in the NCBI Nucleotide
325 database (Fig 4).

326 The NERICA5 GWA analysis had the highest percentage of predicted gene
327 similarity (34.9%), while the IRBLK-KA had the lowest at 14.2%. Most of the SNP
328 markers were located in hypothetical protein-encoding genes across all the studies, with
329 an average of 58.4% (+/-4.05) showing this association. Some markers from IRBL3-
330 CP4, IRBLA-a, IRBLTA-CP1 and IRBL-W analyses had similarities with fosmid clone
331 genomic regions or repeat regions and this was predominant in 3 analyses (IRBL3-CP4,
332 IRBLA-a, IRBLTA-CP1) accounting to only 7% of the markers. In some cases, the DNA
333 sequence in which some of the markers were present were dissimilar to sequences in
334 the *nt* database and this accounted to 11.8% in IRBL3-CP4, 7.4% in IRBLA-a, 4.4% in
335 IRBLTA-CP1, 12.6% in NERICA5 and 14.2% in IRBLK-KA (Fig. 4). Overall, sequence
336 similarity to hypothetical protein-encoding genes was higher compared to predicted
337 genes.

338 **Consistent SNPs associated with virulence of *M. oryzae***

339 A closer look at predicted genes across different GWA analyses indicated the
340 recurrence of specific genes. For example, a AGC/RSK protein kinase (*MGG_07012*), a
341 class of protein previously implicated in regulating signaling events that coordinate
342 growth and morphogenesis in fungi (Lee *et al.*, 2016) and a rRNA processing protein
343 FCF2 (*MGG_00473*) involved in ribosome biogenesis (Rempola *et al.*, 2006), were
344 reported in five of six GWA analyses conducted (Table 4).

345 IRBLTA CP1, IRBLA-a, IRBL9-W and IRBL3 CP4 showed recurrence of 11
346 genes. (1) Rhamnogalacturonan lyase (*MGG_06041*) (2) Secreted glucosidase
347 (*MGG_10475*) (3) MFS (Major facilitator Superfamily) transporter (*MGG_10131*), (4)
348 Integral membrane protein (*MGG_04629*) (5) Multidrug resistance protein fnx1
349 (*MGG_07444*) (6) Nitrite reductase (*MGG_00634*) (7) Alpha/beta hydrolase
350 (*MGG_019030*) (8) Agglutinin Isolectin 1 (*MGG_15185*) (9) Elongation Factor 3
351 (*MGG_14971*) (10) Lovastatin nonaketide synthase (*MGG_05589*) (11) Dynamin
352 GTPase (*MGG_04289*) (Table 3). Three genes were identified in IRBLTA CP1 (*Pita*),
353 IRBLA-a (*Pia*) and IRBL3-CP4 (*Pi3*), they are (1) ubiquitin C-terminal hydrolase
354 (*MGG_05996*) (2) Stromal membrane-associated protein 1 (*MGG_04954*) and (3) the
355 Developmental regulator flbA (*MGG_14517*). DNA-repair protein rad13 (*MGG_00155*)
356 was reported in the three analyses of IRBLTA CP1 (*Pita*), IRBLA-a (*Pia*) and IRBL-9W
357 (*Pi9*) (Table 4).

358 **Multiple SNPs in Genes/Genomic regions associated with virulence**

359 Multiple SNPs in a region/gene were observed across 5 analyses (IRBLTA CP1, IRBL3
360 CP4, IRBLA-a, IRBLA 9-W and NERICA5). These SNPs were found to be located in
361 hypothetical protein-encoding genes, genes with predicted products and other regions
362 in the genome (Table 5). IRBLTA CP1 had the highest number of genes ($n=12$),
363 showing multiple SNPs, while NERICA5 had the lowest ($n=1$). The IRBL3 CP4 had the
364 second highest number ($n=9$) and the analyses of IRBLA-a and IRBL9-W had similar
365 number of genes with multiple SNPs ($n=3$). SNPs varied in number from a maximum of
366 nine to a minimum of two SNPs per region/gene. Seven genes/regions harboring
367 multiple SNPs were commonly present in one or more of the GWA analyses.

368 *MGG_16548* was identified in IRBLTA CP1, IRBL3 CP4, IRBL 9-W and NERICA5 while
369 *MGG_07814* and *MGG_10232* were identified in IRBLTA CP1, IRBL3 CP4 and IRBL 9-
370 W. Y34 repeat region of *M. oryzae* with accession JQ929669 was identified in IRBLTA
371 CP1 and IRBL3 CP4, the fosmid SK2054 genomic sequence was found in IRBLTA CP1
372 and IRBLA-a, *MGG_02369* was identified in IRBL3 CP4 and IRBLA-a and *MGG_17298*
373 was present in three of the GWA analyses of IRBLTA CP1, IRBL3 CP4 and IRBLA-a.

374 **DISCUSSION**

375 This study utilizes SNPs derived from high-throughput genotyping using a GBS
376 approach to characterize isolates of *M. oryzae* from Africa based on genetic diversity,
377 and to then identify genomic regions associated with virulence on rice. While in-depth
378 phylogenetic analysis of a sub-set of the isolates used in this study has been reported
379 previously (Mutiga *et al.*, 2017), we report here the results of GWAS to define putative
380 genes associated with virulence in isolates from SSA. The study involves rice lines with
381 and without known blast resistance genes. The use of monogenic lines with known blast
382 resistance genes was aimed at validating the known effector/avirulence genes, while
383 the inclusion of the other lines, is expected to provide insight into the potential
384 resistance genes and/or more insights in the pathosystem. This is the first study to
385 identify genomic regions associated with virulence of *M. oryzae* isolates from sub-
386 Saharan Africa based on GWAS. It is expected that these findings will boost knowledge
387 of molecular Magnaporthe-rice pathosystem in sub-Saharan Africa, and hence enhance
388 the breeding for durable blast resistance.

389 In this study, *M. oryzae* isolates were sampled from nine SSA countries, which
390 were categorized into East and West Africa (Table 1). Rice cultivars commonly grown in

391 this region were utilized in the pathogenicity assays to study virulence (Table 3). Efforts
392 were focused on a better understanding of the Magnaporthe-rice pathosystem by
393 studying population structure of the pathogen and conducting GWAS to identify novel
394 markers associated with pathogenicity. Previously, a robust characterization of *M.*
395 *oryzae* isolates from SSA was conducted using a combination of phylogeny of GBS-
396 derived SNPs and pathogenicity assays using rice differential lines harboring known
397 blast resistance genes (Mutiga *et al.*, 2017). GBS data phylogenetics revealed genetic
398 relatedness of the pathogen collection from different West and East African regions. A
399 comprehensive analysis of the genetic relatedness of 78 *M. oryzae* isolates from SSA
400 showed a clear segregation of East and West African isolates compared to isolates from
401 outside Africa (Mutiga *et al.*, 2017). The relationship between SNPs and virulence of the
402 isolates was studied based on disease scores using standard least square regression
403 and an evidence of association between genetic diversity and virulence of the isolates
404 was identified. Analysis of the association between genetic relatedness and virulence
405 showed that 77% of isolates in the three clades with highest mean disease score were
406 from West African region known to have had a longer history of rice cultivation
407 compared to East Africa. The emergence of avirulent and highly virulent strains may
408 have resulted from continued rice production, breeding diverse genotypes and trade,
409 resulting in a continuing modification in virulence spectrum of the isolates. It was
410 suggested that the observed differences in virulence amongst the isolates clustered
411 within independent clades conclude that some of these SNPs can lead us to the
412 identification of genomic regions associated with virulence or avirulence. However,
413 since that study used relatively few isolates, in this study the sampling size was

414 increased to obtain a more holistic view of the population structure, augment the
415 statistical power, as well as obtain meaningful associations by conducting GWAS
416 analysis to identify novel virulence factors.

417 A GBS-SNP phylogeny of 160 isolates including 32 international isolates was
418 conducted highlighting the clustering of isolates into eight unique clades (Fig 1).
419 Segregation of West African and East African isolates into specific clades was clearly
420 observed, which indicated the prevalence of higher variability and disease occurrence.
421 Interestingly, no isolates from a single country were monophyletic, suggesting
422 independent events of separate pathogen introductions in each country. This result is
423 supported by the fact that *M. oryzae* is a globally dispersed trade pathogen (Tharreau *et*
424 *al.*, 2009), and there is no clear country-specific geographic pattern of samples due to
425 international trade and germplasm movement. Three isolates from Tanzania were, for
426 example, closely related to most of the Burkina Faso isolates, suggesting that either of
427 them could be the main source of disease outbreak within the region. Similarly, Burkina
428 Faso isolates belonging to clades 2 and 5 are closely related to Kenyan isolates from
429 clade 1 and 6. Although clade 8 was the largest-clade containing the majority of isolates
430 ($n=39$), 53.8% of those are international strains, from the U.S.A., China, Egypt and
431 India. Moreover, 23% were from the East African country of Tanzania that were related
432 to isolates from West African countries of Togo (7.7%), Mali (7.7%) and Ghana (7.7%).
433 The presence of admixture of isolates such as this, is indicative of global rice trade.
434 With the exception of one isolate from the East African country of Uganda, clade 3
435 predominantly consists of isolates from the West African countries of Nigeria, Burkina
436 Faso, Benin, Togo, Mali, Ghana with its origins from China, Philippines, Colombia and

437 the U.S.A. Principal Component analysis was largely consistent with the phylogenetic
438 analysis (Fig 1B and C).

439 To our knowledge, there have been only two other reports apart from this (Mutiga
440 *et al.*, 2017) of a fungal study using GBS for population structure and genetic diversity
441 analysis (Milgroom *et al.*, 2014, Rafiei *et al.*, 2018). Both of those studies focused on the
442 ascomycete fungus *Verticillium dahliae* which reproduces mitotically and the population
443 structure of which is highly clonal. GBS was performed on 141 *V. dahliae* isolates
444 collected from diverse geographical and host origins that yielded 26,748 SNPs. The
445 authors identified a large number of candidate SNPs distinct to lineages that can be
446 used in the development of diagnostic markers, providing a strong suggestion that GBS
447 can be used as a potential genotyping method for the analysis of clonally propagating
448 fungi (Milgroom *et al.*, 2014). The current study yielded 617,281 filtered, high-quality
449 SNPs that constitute a substantial SNP dataset in which the SNPs are evenly
450 distributed throughout the genome, providing a more complete assessment of
451 population structure, which to our knowledge is the first report of such a magnitude of
452 SNPs in such a field study. The large panel of SNP markers used in this study will
453 provide precise discrimination among geographical regions and enhance our
454 association analysis of the Magnaporthe-Rice pathosystem.

455 The rice cultivar panel used in this study includes a diverse panel of rice lines
456 (Table 3) including IRRI-bred blast-resistant lines (IRBLs) with known single target *R*
457 genes, African rice germplasm (upland, lowland and intraspecific lines), a susceptible
458 check and *Oryza glaberrima*, the African rice species that is one parent in the
459 interspecific NERICA lines. This diverse panel was selected ensuring adequate

460 sampling of isolates across different ecosystems that also covers screening of
461 germplasm with known *R* genes.

462 A Generalized Linear Model was used in GWA analysis that utilizes a fixed
463 effects linear model for association between segregating sites and phenotypes. It
464 accounted for population structure using covariates, which indicates a degree of
465 membership in underlying populations. Principal component (PC) scores obtained from
466 PC analysis of genotypes were used as covariates, because there is no kinship in *M.*
467 *oryzae* populations. Of the 16 GWA analyses, six showed association with disease in
468 SSA, including differential rice lines that harbored the dominant *R* genes; *Pia*, *Pita*, *Pi3*,
469 *Pi9* and *Pik* and the African interspecific upland rice variety NERICA5. Varying cutoffs
470 for observed p-values were utilized to maximize number of markers associated with
471 disease in each study (Fig 2). We obtained a significant number of markers ($n=528$)
472 from the six studies (Fig 3), whose location on the genome was identified and NCBI
473 BLASTn analysis confirmed the homology of sequences (500bp upstream and
474 downstream) surrounding each SNP. The majority of those markers ($n=269$) were
475 located in genes encoding hypothetical proteins (Fig 4) with uncharacterized ORFs the
476 structural genes of which can be predicted, but where their analogous translation
477 products remain unidentified. It has been reported that *M. oryzae* has more than 12,000
478 protein-encoding genes and 65% of them are not yet annotated (Li *et al.*, 2018). Thus,
479 it is not surprising that a majority of the identified markers returned hypothetical protein
480 hits. The second highest number of markers ($n=161$) were located in genes with
481 predicted products and it can be noted that there are several genes that were
482 repeatedly identified across 5 of 6 GWA studies ($n=16$) (Table 4). These are

483 *MGG_07012*, *MGG_06041*, *MGG_10475*, *MGG_10131*, *MGG_07444*, *MGG_00634*,
484 *MGG_01903*, *MGG_15185*, *MGG_05589*, *MGG_04289*, *MGG_00473*, *MGG_04629*,
485 *MGG_14971*, *MGG_05996*, *MGG_04954* and *MGG_14517*. Some of these genes have
486 reported to be associated with disease in Magnaporthe ($n=3$), other fungal systems
487 ($n=7$) and unreported ($n=6$).

488 Out of the 16 genes that are considered as encoding putative virulence factors in
489 this study, one of them, *MGG_07012*, a AGC/RSK kinase was consistently found across
490 5 of the 6 GWA analyses, including the African rice cultivar NERICA5, which suggests
491 that this gene plays an important role in virulence of *M. oryzae* isolates from SSA. AGC
492 kinases have been reported to be a common pathogenic protein kinase in fungal
493 pathogens of humans, such as *Cryptococcus neoformans*, *Candida albicans* and
494 *Aspergillus fumigatus* (Lee *et al.*, 2016). The AGC kinase subfamily contains 60
495 members including RSK. In humans these kinases have been shown to mediate
496 important cellular functions and their mutation and/or dysregulation can cause human
497 diseases (Pearce *et al.*, 2010). Furthermore, 9 genes were repeatedly found in four
498 analyses [IRBLTA CP1 (*Pita*), IRBLA-a (*Pia*), IRBL 9-W (*Pi9*), IRBL3-CP4 (*Pi3*)], all of
499 which have been previously reported to be involved in virulence of *M. oryzae* or other
500 fungal systems. The 9 respective genes identified in SSA *M. oryzae* isolates are: (1)
501 *MGG_06041*, a Rhamnogalactouronan lyase (Quoc and Chau, 2017) that recognizes
502 and cleaves the α - 1,4 glycosidic bonds in the backbone of rhamnogalactouronan-I, a
503 major component of the plant cell wall polysaccharide, pectin. (2) the MFS transporter
504 *MGG_10131*, transporters such as these have recently been shown to be important for
505 production of mycotoxins such as deoxynivalenol (DON) in *Fusarium graminearum*

506 (Wang *et al.*, 2018). DON plays a key role in infection of host plants (Wang *et al.*, 2018).
507 (3) the Nitrite reductase (NR)-encoding gene *MGG_00634*. NR catalyzes the formation
508 of Nitric oxide (NO) which can have implications in virulence of fungal pathogens by
509 regulation of mycotoxin biosynthesis, as reported in *Aspergillus nidulans* (Baidya *et al.*,
510 2011). The requirement of NO for successful colonization of host by *M. oryzae* has been
511 reported suggesting a critical role in appressorium formation (Samalova *et al.*, 2013). (4)
512 Alpha beta-hydrolases similar to *MGG_01903*, identified in this study, were shown to be
513 induced during infection in *S. sclerotiorum*, where some of them are involved in lipid
514 degradation, as esterases, or lipases, and others act as hydrolytic enzymes in general
515 (Seifbarghi *et al.*, 2017). (5) Secreted glucosidase *MGG_10475* was identified in four of
516 the six GWA analyses. Glucosidases are plant cell-wall degrading enzymes shown to
517 play a central role in fungal pathogenesis, as reported in various other fungi and
518 oomycetes (Choi *et al.*, 2013). (6) *MGG_07444*, a multidrug resistance protein (MDR).
519 The MDR transporters CgTpo1_1 and CgTpo1_2 have been shown to play a role in
520 virulence of *Candida glabrata* (Santos *et al.*, 2016) (7) *MGG_15185*, Agglutinin isolectin
521 1 was found in four of the six analyses. Lectins mediate the attachment and binding of
522 bacteria and viruses to their hosts. Similarly, it has been reported that fungal lectins can
523 participate in the early stages of infection in humans (Houser *et al.*, 2013). (8)
524 *MGG_05589*, a Lovastatin nonaketide synthase, which synthesizes Lovastatin, a
525 polyketide metabolite in *Aspergillus terreus*. Polyketides are major fungal secondary
526 metabolites with varied biological activities, including being implicated in pathogenicity
527 in plants (Collemare *et al.*, 2008). (9) *MGG_04289*, Dynamin GTPase, such as
528 *MoDnm1* interact with partner proteins in the cytoskeleton and play important roles in

529 appressorium function and pathogenicity in *M. oryzae* (Zhong *et al.*, 2016). Interestingly,
530 none of these nine genes was found in the GWA analysis of the African cultivar
531 NERICA5.

532 Six other genes (Table 4) were identified in the current study that have been
533 either indirectly implicated in pathogenicity, or have not yet been fully functionally
534 characterized to be involved in pathogenesis. (1) *MGG_04629*, an integral membrane
535 protein was found across 4 of the 5 GWA analyses (2) *MGG_14971*, an EF3 elongation
536 factor was predicted in four of the six analyses. EF3's are necessary for growth and
537 development of an organism and thus essential for causing disease in the pathogenic
538 fungus *C. albicans* (Perfect, 1996) (3) *MGG_00473*, rRNA-processing protein FCF2 has
539 been identified in five of the six GWA analyses (4) Ubiquitin carboxyl-terminal hydrolase
540 *MGG_05996*, a ubiquitin pathway gene that was found in three of the six analyses (5)
541 *MGG_04954*, a stromal membrane associated protein was identified in 3 of 5 analyses
542 (6) *MGG_14517*, the developmental regulator *flbA* was found in 3 of the 5 analyses.

543 This GWA study also identified multiple SNPs in predicted genes/hypothetical
544 proteins/loci/repeat regions. The number of SNPs ranged from a maximum of nine to a
545 minimum of two SNPs in any gene/genomic region (Table 5). Some of these genes
546 repeatedly occurred in multiple studies. For example, *MGG_10232* had the presence of
547 nine SNPs and was identified in three GWA analyses (IRBLTA CP1, IRBL3 CP4 and
548 IRBL 9-W).

549 This study therefore demonstrates the power of using GWAS to identify markers
550 of virulence in natural populations. This can provide insight into novel gene functions
551 associated with rice blast disease that would otherwise not be identified based on

552 conventional experimental analysis. In this way, it may be possible to identify key
553 pathogenicity loci, or genes associated with overcoming resistance in cultivars being
554 grown in SSA specifically. Further work will be necessary to test the roles of the
555 identified genes in virulence on these cultivars and then to determine how resistance is
556 conditioned in cultivars that are not susceptible.

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757 **TABLES**

758 **Table 1:** Country origins and collection sites of *M. oryzae* isolates from different parts of
 759 rice growing SSA countries and International regions (n=160). Y indicates the presence
 760 of the isolate in a clade and the asterisk represents isolates randomly chosen for GWAS
 761 (n=78).

Region/Country	No.	Isolate	Collection Site	Year	Clade 1	Clade 2	Clade 3	Clade 4	Clade 5	Clade 6	Clade 7	Clade 8	Sub-clade X	Total No.
No. of isolates					13	17	37	18	16	13	5	39	2	
East Africa														53
KENYA	1	KE0001	Mwea	2013				Y						28
	2	KE0003	Mwea	2013						Y				
	3	KE0005	Mwea	2013						Y*				
	4	KE0006	Mwea	2013						Y*				
	5	KE0007	Mwea	2013	Y									
	6	KE0008	Mwea	2013	Y									
	7	KE0009	Mwea	2013	Y									

	8	KE0010	Mwea	2013		Y			
	9	KE0015	Mwea	2013				Y*	
	10	KE0016	Mwea	2013				Y	
	11	KE0017	Mwea	2013				Y	
	12	KE0019	Mwea	2013	Y*				
	13	KE0020	Mwea	2013	Y				
	14	KE0022	Mwea	2013	Y				
	15	KE0023	Mwea	2013		Y			
	16	KE0024	Mwea	2013				Y	
	17	KE0027	Mwea	2013				Y	
	18	KE0029	Mwea	2013				Y	
	19	KE0030	Mwea	2013				Y*	
	20	KE0032	Mwea	2013				Y	
	21	KE0033	Mwea	2013	Y				
	22	KE0035	Mwea	2013	Y				
	23	KE0036	Mwea	2013	Y				
	24	KE0037	Mwea	2013		Y			
	25	KE0040	Mwea	2013	Y				
	26	KE0041	Mwea	2013		Y*			
	27	KE0210	Ahero	2014		Y*			
	28	KE0215	Mwea	2013		Y*			
TANZANIA	1	TN0001	Kyela	2013				Y*	20
	2	TN0004	Morogoro SUA-	2013		Y			
	3	TN0005	UA	2013			Y*		
	4	TN0006	Morogoro SUA-	2013			Y		
	5	TN0007	Morogoro SUA-	2013	Y				
	6	TN0008	Morogoro	2013	Y*				
	7	TN0010	Kahama	2013		Y*			
	8	TN0014	Kyela	2013				Y*	
	9	TN0016	Kyela	2013				Y*	
	10	TN0019	Kyela	2013				Y*	
	11	TN0045	Ifakara	2013		Y*			
	12	TN0050	Ifakara	2013		Y*			
	13	TN0057	Ifakara	2013		Y*			
	14	TN0065	Ifakara	2013				Y*	
	15	TN0068	Ifakara	2013				Y*	
	16	TN0070	Ifakara	2013		Y*			
	17	TN0077	Ifakara	2013				Y*	
	18	TN0078	Ifakara	2013		Y*			
	19	TN0079	Ifakara	2013				Y*	
	20	TN0098	Ifakara	2013				Y*	
UGANDA	1	UG0001	Butaleja	2013		Y*			5
	2	UG0006	Bulega	2013				Y*	
	3	UG0008	Namulonge	2013	Y*				

<i>Region/Country</i>	<i>Isolate(s)</i>	<i>Collection Site</i>	<i>Year</i>	<i>Clade 1</i>	<i>Clade 2</i>	<i>Clade 3</i>	<i>Clade 4</i>	<i>Clade 5</i>	<i>Clade 6</i>	<i>Clade 7</i>	<i>Clade 8</i>	<i>Sub-clade X</i>	<i>Total No.</i>
	4	UG0009	Butaleja	2013					Y*				
	5	UG0011	Namulonge	2013						Y			
<i>West Africa</i>													75
NIGERIA	1	NG0026	Ibadan	2009									17
	2	NG0095	Ibadan	2009									
	3	NG0103	Ibadan	2010									
	4	NG0104	Ibadan	2010									
	5	NG0110	Ibadan	2010									
	6	NG0133	Ibadan	2009									
	7	NG0134	Ibadan	2009									
	8	NG0135	Ibadan	2009									
	9	NG0149	Ibadan	2009								Y	
	10	NG0159	Ibadan	2009									
	11	NG0176	Ibadan	2009									
	12	NG0179	Ibadan	2009									
	13	NG0190	Ibadan	2009									
	14	NG0192	Ibadan	2009									
	15	NG0199	Ibadan	2009									
	16	NG0245	Abéocuta	2009									
	17	NG0253	Edozhigi	2009									
BURKINA FASO	1	BF0001	Loto	2013					Y*				36
	2	BF0002	Loto	2013					Y*				
	3	BF0003	Loto	2013		Y*							
	4	BF0004	Loto	2013		Y*							
	5	BF0005	Sinkoura	2013						Y*			
	6	BF0006	Sinkoura	2013						Y*			
	7	BF0007	Sinkoura	2013						Y*			
	8	BF0008	Niéna Dionkéle	2013					Y				
	9	BF0009	Niéna Dionkéle	2013					Y*				
	10	BF0010	Niéna Dionkéle	2013					Y				
	11	BF0012	Niéna Dionkéle	2013					Y				
	12	BF0013	Niéna Dionkéle	2013		Y							
	13	BF0014	Niéna Dionkéle	2013					Y				
	14	BF0015	Niéna Dionkéle	2013		Y							
	15	BF0016	Niéna Dionkéle	2013		Y							
	16	BF0017	Niéna Dionkéle	2013								Y*	
	17	BF0019	Niéna Dionkéle	2013		Y							
	18	BF0020	Niéna Dionkéle	2013		Y*							
	19	BF0021	Banfora	2013		Y*							
	20	BF0023	Banfora	2013					Y				

	21	BF0024	Banfora	2013											Y	
	22	BF0027	Banfora	2013			Y									
	23	BF0029	Banfora	2013											Y	
	24	BF0031	Banfora	2013		Y										
	25	BF0032	Banfora	2013		Y*										
	26	BF0035	Banfora	2013											Y*	
	27	BF0036	Banfora	2013											Y	
	28	BF0037	Banfora	2013											Y	
	29	BF0038	Banfora	2013		Y										
	30	BF0040	Banfora	2013											Y	
	31	BF0041	Banfora	2013		Y										
	32	BF0046	Banfora	2013		Y*										
	33	BF0047	Banfora	2013		Y										
	34	BF0048	Banfora	2013		Y*										
	35	BF0049	Pankatiro	2013											Y*	
	36	BF0050	Pankatiro	2013											Y	
BENIN	1	BN0013	Malanville	2011			Y*									10
	2	BN0036	Bétérou	2012			Y*									
	3	BN0040	Bétérou	2012			Y*									
	4	BN0066	Kerou Kokey-	2012			Y*									
	5	BN0125	Banikoara	2012			Y*									
	6	BN0139	Natitingou	2012			Y*									
	7	BN0152	Savalou	2012											Y*	
	8	BN0157	Savalou	2012											Y*	
	9	BN0170	Kerou	2012			Y*									
	10	BN0202	Lokossa	2010											Y*	
Region/Country		Isolate(s)	Collection Site	Year	Clade 1	Clade 2	Clade 3	Clade 4	Clade 5	Clade 6	Clade 7	Clade 8	Sub-clade X	Total No.		
West Africa																75
TOGO	1	TG0003	Assomè	2012			Y*									7
	2	TG0004	Elavayo	2012									Y*			
	3	TG0005	Assomè	2012			Y*									
	4	TG0008	Elavayo	2012									Y*			
	5	TG0011	Elavayo	2012									Y*			
	6	TG0016	Assomè	2012			Y*									
	7	TG0035	Assomè	2012			Y*									
MALI	1	ML0060	Sikasso	2009									Y*			3
	2	ML0062	Sikasso	2009									Y*			
	3	ML0066	Sikasso	2011									Y*			
GHANA	1	GH0004	Akuse AGRIC farms	2010			Y*									2
	2	GH0007	Akuse AGRIC farms	2009			Y*									
INTERNATIONAL	1	49D	USA, Arkansas	1985				Y								32
	2	6360_1	USA, Texas	2010									Y			

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774 **Table 2:** Description of isolates used in this study (a) Percentage of isolates from
775 different SSA countries randomly chosen for GWA analyses (b) Percentage of isolates
776 randomly chosen from each clade for GWA analyses.

777 (a)

Region	Country	GWAS	Total isolates	%
East Africa	Tanzania	17	20	15
	Kenya	7	28	35
	Uganda	4	5	76
West Africa	Nigeria	14	17	61
	Burkina Faso	14	36	25
	Benin	10	10	31
	Togo	7	7	80
	Mali	3	3	46
	Ghana	2	2	50
Total		78	128	

778

779 (b)

Clade	GWAS	Total isolates	%
1	17	20	85
2	7	28	25

3	4	5	80
4	14	17	82
5	14	36	39
6	10	10	100
7	7	7	100
8	3	3	100
X	2	2	100
Total	78	128	

780

781 **Table 3:** Description of rice cultivar panel used for GWAS and results of association
782 analysis.

Rice cultivar	Description	Target R-gene	Donor Line/pedigree	Association
1. IRBLA-a	Differential Line	<i>Pia</i>	Aichi Asahi	Yes
2. IRBLTA CP1	Differential Line	<i>Pita</i>	C101PKT	Yes
3. IRBL3-CP4	Differential Line	<i>Pi3</i>	C104PKT	Yes
4. IRBL 9-W	Differential Line	<i>Pi9</i>	WHD-1S-75-1-127	Yes
5. IRBLK-KA	Differential Line	<i>Pik</i>	Kanto 51	Yes
6. NERICA5	African upland	-	WAB450-11-1-1-P31-HB	Yes
7. IRBLZT-T	Differential Line	<i>Pizt</i>	Toride-1	No
8. IRBLZ5-CA (R)	Differential Line	<i>Piz-5</i>	C101A51	No
9. IRBLTA CT2	Differential Line	<i>Pita</i>	C105TTP2L9	No
10. NERICA2	African upland	-	WAB450-11-1-P31-1-HB	No
11. FKR62N	African lowland	-	WAS122-IDSA-1-WAS-6-1	No
12. TS2	African intraspecific	-	NA	No
13. F6-36	African intraspecific	-	NA	No
14. UZROZ275	susceptible check	-	NA	No
15. 75-1-127	<i>Pi9</i> donor line	<i>Pi9</i>	NA	No
16. AR105	<i>Oryza glaberrima</i> (African)	-	NA	No

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784

785 **Table 4:** Putative virulence genes ($n=16$) that occurred in the five rice blast disease
786 associated GWA analyses.

No.	Gene	Gene ID	IRBLT A CP1 (<i>Pita</i>)	IRBL A-a (<i>Pia</i>)	IRB L 9-W (<i>Pi9</i>)	IRBL 3-CP4 (<i>Pi3</i>)	NERICA 5	Organism reported	Reference
1	AGC/RSK protein kinase	<i>MGG_07012</i>	*	*	*	*	*	<i>C. neoformans</i> , <i>C. albicans</i> , <i>A. fumigatus</i>	Lee <i>et al.</i> , 2016
2	Rhamnogalacturonan lyase	<i>MGG_06041</i>	*	*	*	*		<i>Magnaporthe oryzae</i>	Quoc and Chau, 2017
3	Secreted glucosidase	<i>MGG_10475</i>	*	*	*	*		<i>Multiple Fusarium graminearum</i>	Choi <i>et al.</i> , 2013
4	MFS transporter	<i>MGG_10131</i>	*	*	*	*			Wang <i>et al.</i> , 2018
5	Multidrug resistance protein fnx1	<i>MGG_07444</i>	*	*	*	*		<i>Candida glabrata</i>	Santos <i>et al.</i> , 2016
6	Nitrite reductase Alpha/beta	<i>MGG_00634</i>	*	*	*	*		<i>Aspergillus nidulens</i> , <i>M.oryzae</i>	Baidya <i>et al.</i> , 2011; Samalova <i>et al.</i> , 2013
7	hydrolase	<i>MGG_01903</i>	*	*	*	*		<i>Sclerotinia sclerotiorum</i>	Seifbarghi <i>et al.</i> , 2017
8	Agglutinin isolectin 1	<i>MGG_15185</i>	*	*	*	*		<i>Aspergillus fumigatus</i>	Houser <i>et al.</i> , 2013
9	Lovastatin nonaketide synthase	<i>MGG_05589</i>	*	*	*	*		<i>Aspergillus terreus</i>	Collemare <i>et al.</i> , 2008
10	Dynamin GTPase	<i>MGG_04289</i>	*	*	*	*		<i>Magnaporthe oryzae</i>	Zhong <i>et al.</i> , 2016
11	rRNA-processing protein FCF2	<i>MGG_00473</i>	*	*	*	*	*		
12	Integral membrane protein	<i>MGG_04629</i>	*	*	*	*			
13	Elongation factor 3	<i>MGG_14971</i>	*	*	*	*			
14	Ubiquitin C-terminal hydrolase	<i>MGG_05996</i>	*	*		*			
15	Stromal membrane-associated protein 1	<i>MGG_04954</i>	*	*		*			
16	Developmental regulator flbA	<i>MGG_14517</i>	*	*		*			

787

788 **Table 5:** Genes/genomic regions with multiple SNPs associated with rice blast disease.

789 Highlighted genes were identified in five of the GWA analyses.

Study	No.	Gene/Locus	Homology	No. of SNPs
IRBLTA CP1 (<i>Pita</i>)	1	<i>MGG_10232</i>	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein mRNA	9
	2	<i>MGG_07812</i>	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein partial mRNA	4
	3	<i>JQ929669</i>	<i>Magnaporthe oryzae</i> strain Y34 repeat region	4
	4	<i>MGG_16548</i>	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein partial mRNA	3
	5	<i>MGG_17298</i>	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein partial mRNA	4
	6	<i>KT599263</i>	<i>Magnaporthe oryzae</i> strain BM1-24 clone fosmid SK2054 genomic sequence	2
	7	<i>MGG_01018</i>	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein partial mRNA	2
	8	<i>MGG_02059</i>	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein mRNA	2
	9	<i>MGG_07812</i>	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein partial mRNA	2
	10	<i>MGG_04289</i>	<i>Magnaporthe oryzae</i> 70-15 dynamin GTPase partial mRNA	2
	11	<i>MGG_14358</i>	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein partial mRNA	2
	12	<i>MGG_15795</i>	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein partial mRNA	2
IRBL3 CP4 (<i>Pi3</i>)	1	<i>MGG_10232</i>	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein	8

	2	MGG_07812	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein partial mRNA	4
	3	JQ929669.1	<i>Magnaporthe oryzae</i> strain Y34 repeat region	4
	4	MGG_16548	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein	3
	5	MGG_17298	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein	4
	6	MGG_02369	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein	4
	7	MGG_00473	<i>Magnaporthe oryzae</i> 70-15 rRNA-processing protein	3
	8	MGG_03564	<i>Magnaporthe oryzae</i> 70-15 mitochondrial RNA-splicing	4
	9	MGG_17542	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein mRNA	4
IRBLA-a (<i>Pia</i>)	1	MGG_17298	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein partial mRNA	4
	2	KT599263.1	<i>Magnaporthe oryzae</i> strain BM1-24 clone fosmid SK2054 genomic sequence	3
	3	MGG_02369	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein partial mRNA	4
IRBL9-W (<i>Pi9</i>)	1	MGG_10232	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein mRNA	9
	2	MGG_07812	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein partial mRNA	3
	3	MGG_16548	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein partial mRNA	3
NERICA5	1	MGG_16548	<i>Magnaporthe oryzae</i> 70-15 hypothetical protein partial mRNA	3

790

791

792 FIGURE CAPTIONS

793 **Figure 1: Genetic Diversity of *M. oryzae* isolates from sub-Saharan Africa (SSA)**

794 (A) Geographic origin of SSA field isolates and International comparator isolates. (B)
 795 Unrooted neighbor-joining tree of *M. oryzae* isolates ($n=160$) based on pair-wise
 796 distance matrix calculated by TASSEL v5.0 (C) 3D Principal Component Analysis
 797 scatter plot showing the first three components (PC1, PC2, and PC3). Colored lines
 798 and color-filled circles represent SSA countries, black lines and filled black circles
 799 represent International isolates (Table 1).

800 **Figure 2: Quantile-Quantile plots for the 6 GWA analyses using a generalized**

801 **linear model (GLM).** The x-axis corresponds to expected values of negative logarithm
 802 of P and y-axis corresponds to observed values of negative logarithm of P . (A) Rice
 803 blast differential rice line IRBL9-W harboring the R-gene *Pi9* (B) Rice blast differential
 804 rice line IRBLK-KA harboring the R-gene *Pik* (C) Rice blast differential rice line IRBL3-
 805 CP4 harboring the R-gene *Pi3* (D) Rice blast differential rice line IRBLTA-CP1 harboring

806 the R-gene *Pita* (E) African upland rice cultivar NERICA5 (F) Rice blast differential rice
807 line IRBLA-a harboring the R-gene *Pia*. Dotted lines indicate $-\log P$ cutoff.

808 **Figure 3: Number of rice blast disease-associated SNP markers identified in**
809 **individual GWA analyses.** Fungal isolates show virulence on the respective rice
810 cultivars shown. Five cultivars carry single major blast disease resistance genes.

811 **Figure 4: Distribution of SNP marker locations in different GWA analyses as**
812 **predicted by NCBI's DNA database.** PG predicted genes; HP hypothetical protein; OT
813 other, present in genomic sequences, repeat regions on genome; NS no similarity.

814 **SUPPLEMENTARY DATA CAPTIONS**

815 **Supplementary Table S1:** List of Genes/Genomic region homologs that encompass
816 the significantly associated SNPs (500bp upstream and downstream of SNP locations)
817 in the GWA analysis of NERICA5

818 **Supplementary Table S2:** List of Genes/Genomic region homologs that encompass
819 the significantly associated SNPs (500bp upstream and downstream of SNP locations)
820 in the GWA analysis of IRBLA-a

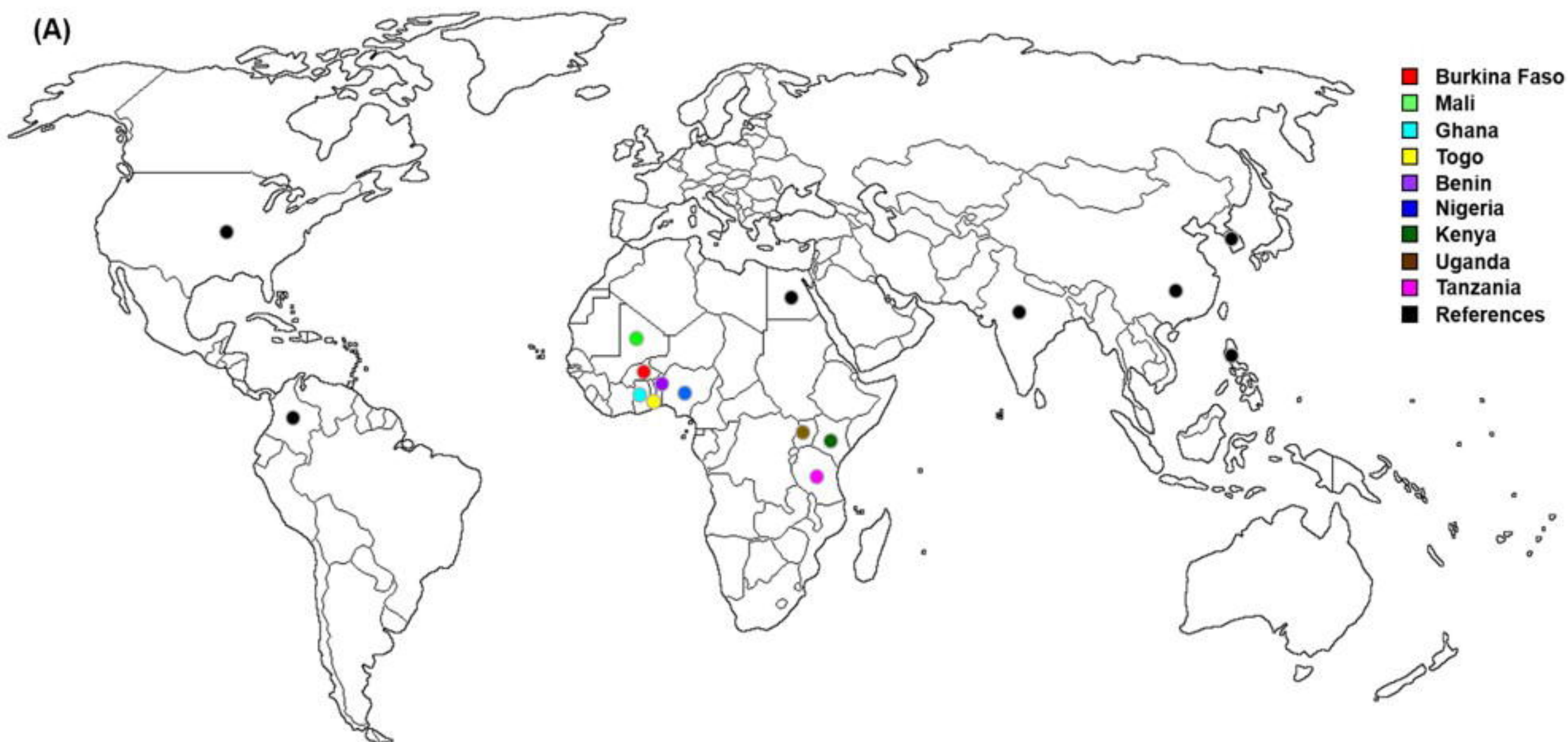
821 **Supplementary Table S3:** List of Genes/Genomic region homologs that encompass
822 the significantly associated SNPs (500bp upstream and downstream of SNP locations)
823 in the GWA analysis of IRBLTA-CP1

824 **Supplementary Table S4:** List of Genes/Genomic region homologs that encompass
825 the significantly associated SNPs (500bp upstream and downstream of SNP locations)
826 in the GWA analysis of IRBL3-CP4

827 **Supplementary Table S5:** List of Genes/Genomic region homologs that encompass
828 the significantly associated SNPs (500bp upstream and downstream of SNP locations)
829 in the GWA analysis of IRBLK-KA

830 **Supplementary Table S6:** List of Genes/Genomic region homologs that encompass
831 the significantly associated SNPs (500bp upstream and downstream of SNP locations)
832 in the GWA analysis of IRBL-9W

833



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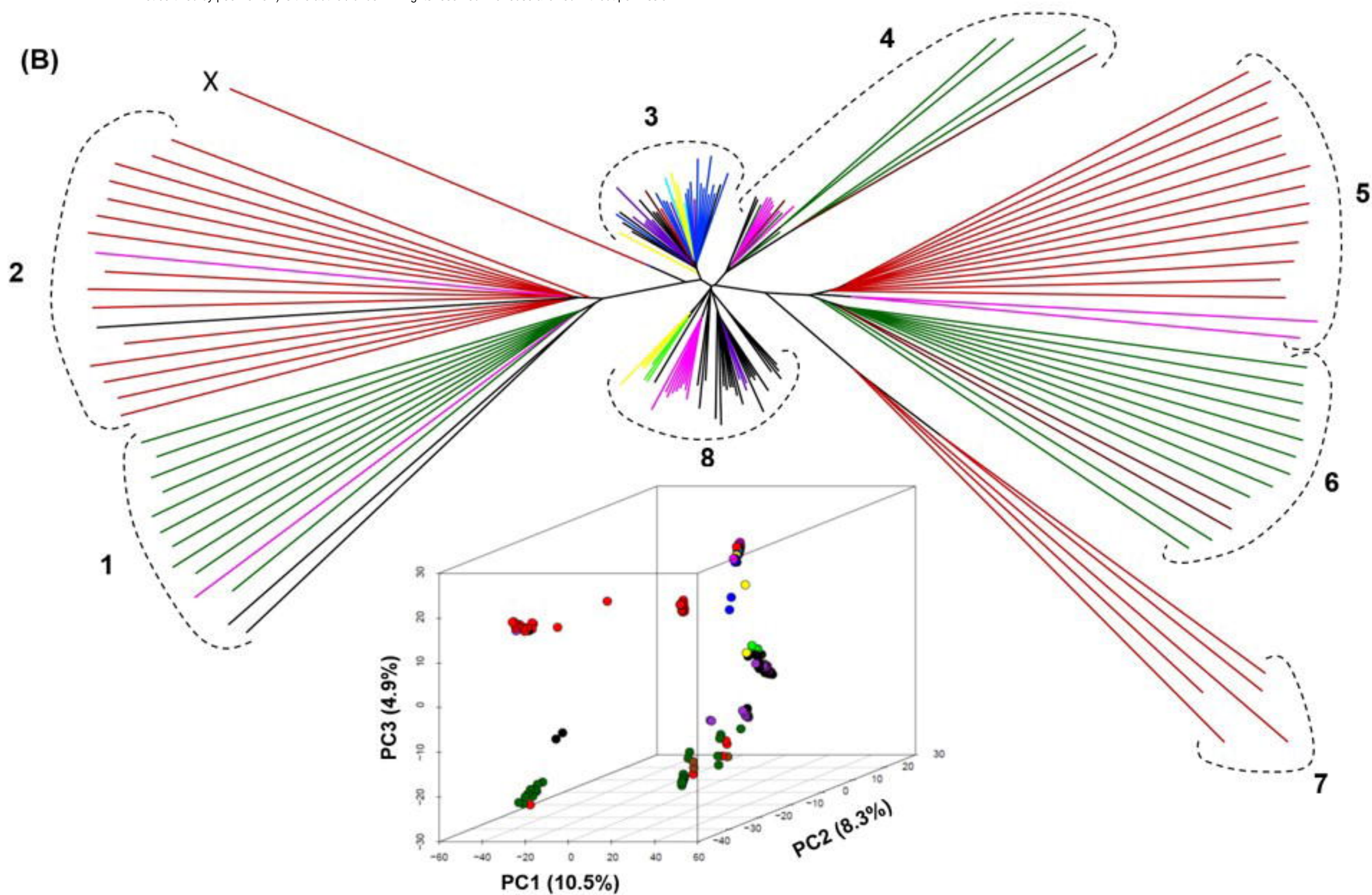


Figure 1: Genetic Diversity of *M. oryzae* isolates from sub-Saharan Africa (SSA) (A) Geographic origin of SSA field isolates and International comparator isolates. (B) Unrooted neighbor-joining tree of *M. oryzae* isolates ($n=160$) based on pair-wise distance matrix calculated by TASSEL v5.0 (C) 3D Principal Component Analysis scatter plot showing the first three components (PC1, PC2, and PC3). Colored lines and color-filled circles represent SSA countries, black lines and filled black circles represent International isolates (Table 1).

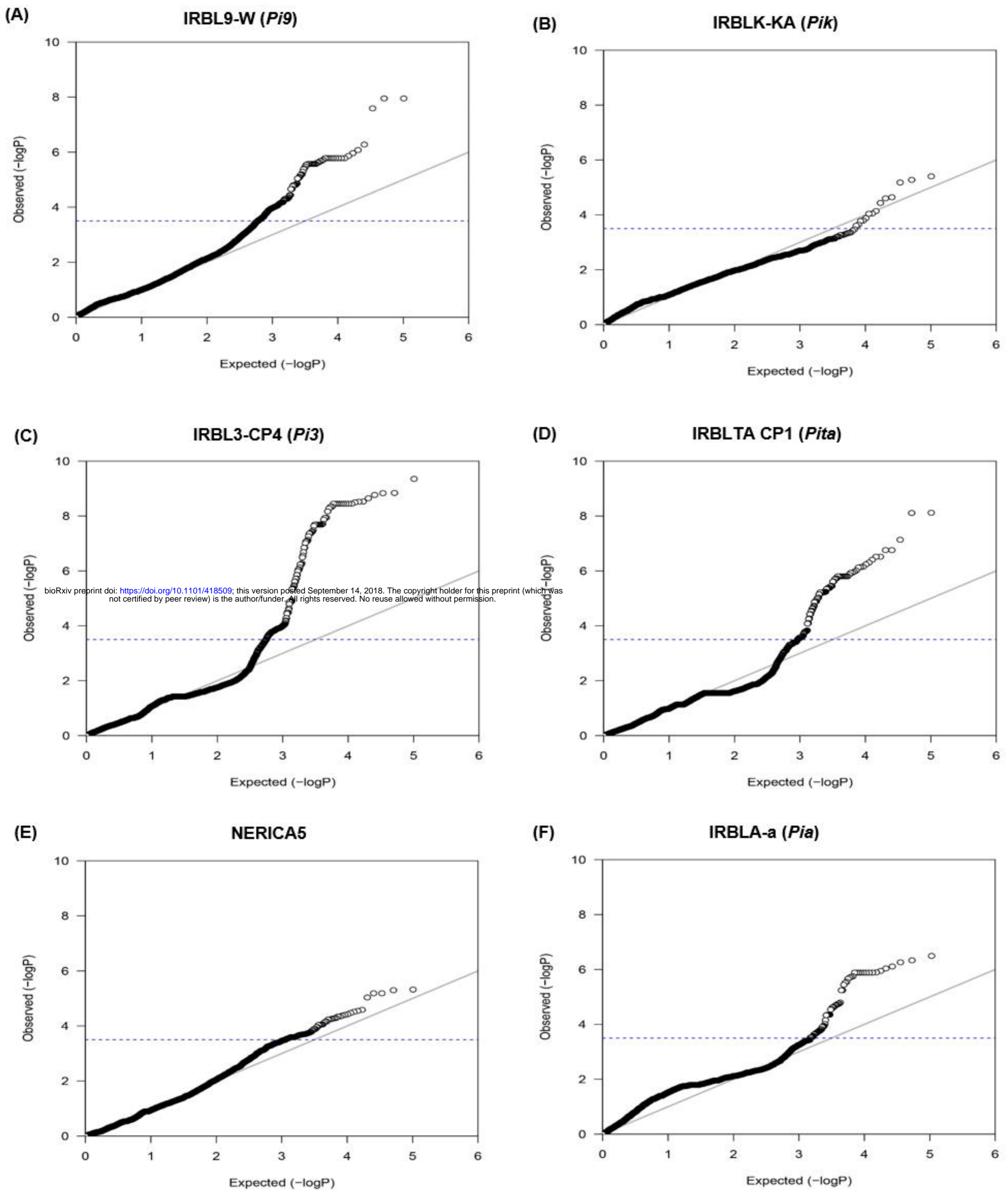


Figure 2: Quantile-Quantile plots for the 6 GWA analyses using a generalized linear model (GLM). The x-axis corresponds to expected values of negative logarithm of P and y-axis corresponds to observed values of negative logarithm of P . (A) Rice blast differential rice line IRBL9-W harboring the R-gene *Pi9* (B) Rice blast differential rice line IRBLK-KA harboring the R-gene *Pik* (C) Rice blast differential rice line IRBL3-CP4 harboring the R-gene *Pi3* (D) Rice blast differential rice line IRBLTA-CP1 harboring the R-gene *Pita* (E) African upland rice cultivar NERICA5 (F) Rice blast differential rice line IRBLA-a harboring the R-gene *Pia*. Blue dotted lines indicate $-\log P$ cutoff.

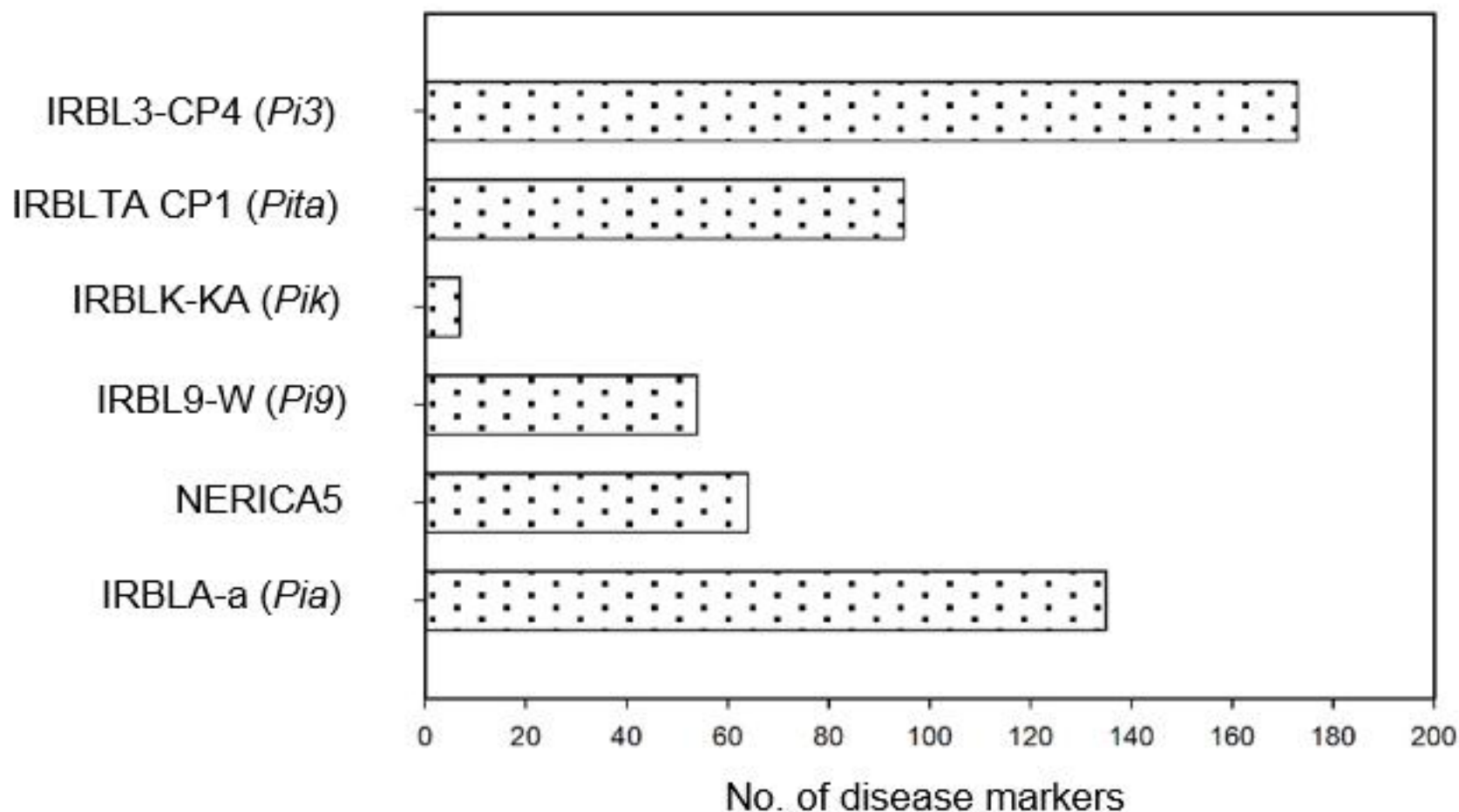


Figure 3: Number of rice blast disease-associated SNP markers identified in individual GWA analyses. Fungal isolates show virulence on the respective rice cultivars shown. Five cultivars carry single major blast disease resistance genes.

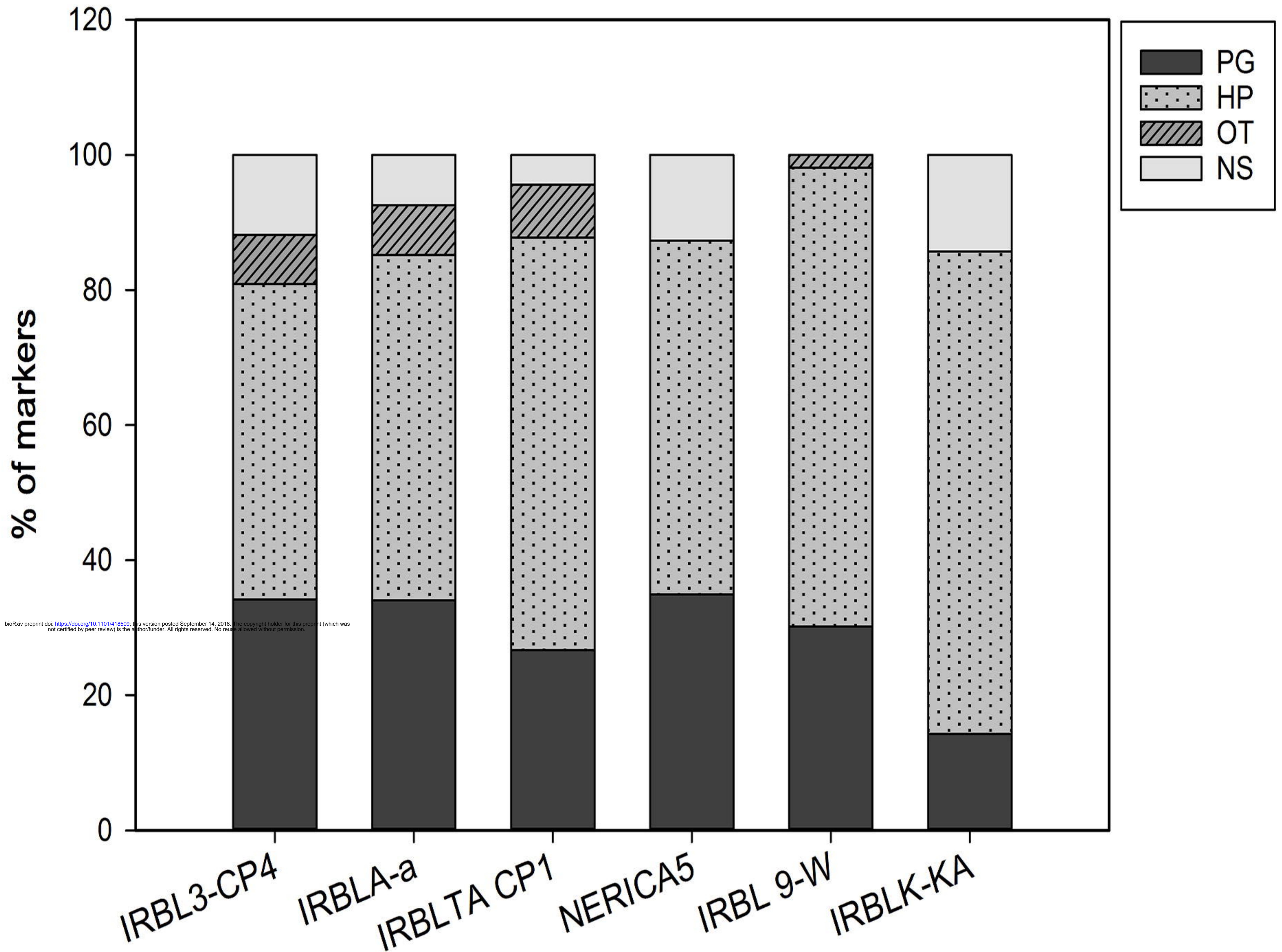


Figure 4: Distribution of SNP marker locations in different GWA analyses as predicted by NCBI's DNA database. PG predicted genes; HP hypothetical protein; OT other, present in genomic sequences, repeat regions on genome; NS no similarity.