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1 # Epistasis and Quantitative Resistance to *Pyricularia oryzae*
2 Revealed by GWAS in Advanced Rice Breeding Populations

3

4 Juan E. Rosas[1,2¶*], Maia Escobar[3¶], Sebastián Martínez[1], Pedro Blanco[1], Fernando
5 Pérez[1], Gaston Quero[3,4], Lucía Gutiérrez[2,5] and Victoria Bonnecarrère[3].

6

7 [1]Instituto Nacional de Investigación Agropecuaria (INIA), Programa Producción de
8 Arroz, Estación Experimental INIA Treinta y Tres, Ruta 8 km 281, Treinta y Tres 33000,
9 Uruguay.

10 [2]Universidad de la República, Facultad de Agronomía, Departamento de Biometría y
11 Estadística, Garzón 780, Montevideo 12900, Uruguay.

12 [3]Instituto Nacional de Investigación Agropecuaria (INIA), Unidad de Biotecnología,
13 Estación Experimental Wilson Ferreira Aldunate, Ruta 48 km 10, Canelones 90200,
14 Uruguay.

15 [4] Universidad de la República, Facultad de Agronomía, Departamento de Biología
16 Vegetal, Garzón 780, Montevideo 12900, Uruguay.

17 [5]Department of Agronomy, College of Agricultural and Life Sciences, University of
18 Wisconsin-Madison, 1575 Linden Dr., Madison, WI 53706, USA

19 [¶]Equal contribution

20 *Corresponding author, jrosas@inia.org.uy

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21 **Abstract**

22 **Background:** Rice blast caused by *Pyricularia oryzae* is a major rice disease
23 worldwide. Despite the detailed knowledge on major resistance genes available to
24 date, little is known about how these genes interact with quantitative blast resistance
25 loci and with the genetic background. Knowledge on these interactions is crucial for
26 assessing the usefulness of introgressed resistance loci in breeding germplasm. Our
27 goal was to identify blast resistance loci in rice breeding populations and to describe
28 how they interact among each other and with the genetic background. To that end,
29 resistance to blast was mapped in two advanced rice breeding populations, one made
30 of 305 indica type inbred lines, and the other of 245 tropical japonica inbred lines. The
31 interactions and main effects of blast resistance loci were assessed in a multilocus
32 model.

33 **Results:** Well known, major effect blast resistance gene clusters were detected in
34 both tropical japonica (*Pii/Pi3*/*Pi5*) and indica (*Piz*/*Pi2*/*Pi9*) populations with the GWAS
35 scan 1. When these major effect loci were included as fixed cofactors in subsequent
36 GWAS scans 2 and 3, additional QTL and more complex genetic architectures were
37 revealed. The multilocus model for the tropical japonica population showed that
38 *Pii/Pi3*/*Pi5* had significant interaction with two QTL in chromosome 1 and one QTL in
39 chromosome 8, together explaining 64% of the phenotypic variance. In the indica
40 population a significant interaction among the QTL in chromosomes 6 and 4 and the
41 genetic background, together with *Piz*/*Pi2*/*Pi9* and QTL in chromosomes 1, 4 and 7,
42 explained 35% of the phenotypic variance.

43 **Conclusions:** Our results suggest that epistatic interactions can play a major role
44 modulating the effect of major effect blast resistance loci such as *Pii/Pi3*/*Pi5*.
45 Furthermore, the additive and epistatic effects of multiple QTL bring additional layers

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46 of quantitative resistance with a magnitude comparable to that of major effect loci.
47 These findings highlight the need of genetic background-specific validation of markers
48 for molecular assisted blast resistance breeding and provide insights for developing
49 quantitative resistance to blast disease in rice.
50

51 **Keywords**
52 Leaf blast, GWAS, QTL by QTL interaction, QTL by genetic background interaction,
53 *Magnaporthe oryzae*.
54

55 Background

56 Rice is a major staple food worldwide, feeding half of the world population (Khush
57 2005). One of the major threats to the rice crop is blast, a major disease caused by
58 the fungus *Pyricularia oryzae* (Khush and Jena 2009). Genetic resistance to blast is a
59 key objective in rice breeding programs, given its economic and environmental
60 advantages over chemical control (Ashkani et al. 2015). Current breeding methods for
61 rice blast resistance involves phenotypic and marker assisted selection strategies
62 (Miah et al. 2013). The high genetic instability of PO mediated by parasexuality
63 generates variations in fungal avirulence genes that prevent gene-for-gene interaction
64 with rice resistance genes (Jia et al. 2000; Miah et al. 2013), leading to frequent breaks
65 of race specific resistance (Liu et al. 2010).

66 More than 100 race specific blast resistance genes have been identified up to
67 date in rice (Ashkani et al. 2014; Tanweer et al. 2015), and hundreds of quantitative
68 trait loci (QTL) associated with blast resistance have been mapped (Ballini et al. 2008).
69 Molecular markers linked to these genes and loci have been widely reported and used
70 for assisted breeding of blast resistance (Wang et al. 1994; Koide et al. 2009; Tacconi

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71 et al. 2010; Miah et al. 2013). Pyramiding several resistance genes is a common
72 breeding strategy to broaden resistance spectrum and delay resistance breakdown
73 (Hittalmani et al. 2000; Tabien et al. 2000; Koide et al. 2010; Fukuoka et al. 2015).
74 This usually requires gene or QTL introgression from diverse donors to an adapted
75 recipient, rising the issues of epistasis, namely QTL by QTL interaction and QTL by
76 genetic background interaction (Collard and Mackill 2008).

77 Low order QTL by QTL interaction effects are often estimated in biparental QTL
78 studies (Holland 2007), while the ability to test the interaction between QTL and
79 genetic background is limited in biparental QTL studies (Jannink 2007; Bernardo 2008),
80 mainly due to the low number of QTL and genetic backgrounds combinations (Jannink
81 2008). A widespread approach for identifying main effect QTL that exploits the
82 diversity of allelic combinations from diverse genetic backgrounds is the genome-wide
83 association study (GWAS, Thornsberry et al. 2001; Arbelbide and Bernardo 2006).
84 Assessment of QTL by QTL interaction in GWAS is possible through multi loci models
85 such as those proposed by von Zitzewitz et al. (2011), developed by Segura et al.
86 (2012), and further implemented for several traits and crops (Lipka et al. 2013;
87 Locatelli et al. 2013; Gutierrez et al. 2015) including disease resistance in rice (Rosas
88 et al. 2018). Furthermore, the multiple genetic backgrounds and levels of relatedness
89 present in GWAS mapping populations can be exploited to effectively investigate QTL
90 by genetic background interactions (Hamblin et al. 2011). A model that incorporates a
91 term for QTL by genetic background interaction in GWAS analysis has been proposed
92 by Jannink (2007). GWAS has been extensively used for QTL mapping in highly
93 diverse rice germplasm collections, reviewed by Zhang et al. (2016a), and more
94 recently in rice advanced breeding populations (Begum et al. 2015; Liang et al. 2016;
95 Quero et al. 2018). GWAS has been also used for successfully identifying blast

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96 resistance QTL in different types of mapping populations, such as a multi-parent
97 advanced generation inter-cross population (Bandillo et al. 2013), collections of indica
98 type landraces (Wang et al. 2014; Chen et al. 2017), a small set of temperate japonica
99 elite breeding lines (Shinada et al. 2015), and in rice diversity panels (Kang et al. 2016;
100 Raboin et al. 2016). However, none of these studies has investigated epistatic
101 interaction in blast resistance genetics, despite reports from biparental QTL studies of
102 significant effects for several traits in rice (Li et al. 1997; Lin et al. 2000; Xing et al.
103 2002; Fan et al. 2005; Wang et al. 2012b), including blast resistance (Wang et al. 1994;
104 Li et al. 2007; Urso et al. 2016; Inoue et al. 2017). Furthermore, to our knowledge no
105 statistical analysis with experimental data on QTL by genetic background interaction
106 has been reported for blast resistance in rice up to date. In the present work, QTL
107 main effects, QTL by genetic background, and QTL by QTL interaction effects for blast
108 resistance in two advanced rice breeding populations were estimated using multi loci
109 models in a GWAS framework.
110

111 **Results**

112 **Phenotyping and population structure**

113 Pairwise correlations between adjusted phenotypic means of disease evaluations at
114 14, 21, and 28 days post inoculation, and of the area under the disease progress curve
115 (AUDPC) were high (>0.98, P <0.001). Distributions of disease scores in the tropical
116 japonica and indica populations are shown in Figure 1. Resistant genotypes (i.e. rated
117 3 or below in the three evaluation dates) had adjusted phenotypic means of AUDPC
118 from -2.8 to 44.5 in indica and from -4.7 to 43.0 in tropical japonica. Genotypes rated
119 as susceptible (at least one score above 3) had adjusted phenotypic means of AUDPC
120 ranging from 39.3 to 68.0 in indica and from 40.2 to 61.9 in tropical japonica (Figure

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121 1). Heritability for AUDPC was 0.56 (0.07 SE) in the tropical japonica population
122 experiment and 0.26 (0.10 SE) in the indica population experiment.

123 For the tropical japonica population, six subpopulations were defined based on
124 pedigree (Figure 2a). Subpopulations 1 and 5 had indica type lines in their pedigree
125 record, derived from CIAT-IRRI dwarf germplasm nurseries tested in Uruguay in the
126 80's. However, locally developed tropical japonica lines were predominant in the
127 pedigree of both subpopulations. Subpopulations 1 and 3 shared the same local
128 tropical japonica lines as parents. Subpopulations 2, 4, 5 and 6 were composed by
129 locally developed lines in whose pedigrees predominated (not exclusively) the US
130 tropical japonica cultivars Cypress (Linscombe et al. 1993), Gulfmont (Bollich et al.
131 1990)(Bollich et al. 1990), Newbonnet (Johnston et al. 1984)(Johnston et al. 1984),
132 and Lemont (Bollich et al. 1985)(Bollich et al. 1985), respectively. Pedigree groups
133 were clustered in the PCA plot (Figure 2a). PC1 explained 10.9% of the genotypic
134 variance and separated subpopulations 1 and 5 from 2 and 3, and PC2 explained 9.0%
135 of the genotypic variance and separated subpopulation 4 from 1 and 3.

136 For the indica population, nine subpopulation groups were defined based on
137 the origins of parents (Figure 2b, upper section). INIA Olimar (Blanco et al. 2004), an
138 indica cultivar with high yield and quality, was one of the parents in four out of nine of
139 those groups (65% of the indica population). A clear gradient of allele sharing with
140 INIA Olimar ranged from 78 to 99% and was found to correspond with PC1 (r = 0.98,
141 P<0.001), the latter explaining 21.3% of the genetic variance (Figure 2b, upper and
142 lower sections). PC2 explained 5.7% of the genetic variance. Furthermore, lines
143 derived from crosses between INIA local germplasm, including INIA Olimar, clustered
144 on the left side of PC1 and at the center of PC2, indicating lower genetic variability
145 and more similarity to INIA Olimar. Lines derived from more diverse germplasm

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146 sources (FLAR and CIAT, including tropical japonica parents) are found on the right
147 side of PC1, corresponding with higher levels of diverse genomic introgressions and
148 more genetic distance from INIA Olimar.
149

150 **QTL found in GWAS scans 1, 2 and 3, and colocalizing reported blast resistance**
151 **loci**

152 Results of GWAS scans 1, 2 and 3 are displayed together in Figure 3, and by scan in
153 Additional file 1: Fig. S1. In the tropical japonica population (Figure 3a, outer circle),
154 GWAS scan 1 identified a single QTL on chromosome 9, with SNP S9_9786203
155 having the highest $-\log_{10}(P$ value) (Additional file 1: Fig. S1). LD block analysis in the
156 surrounding region of S9_9786203 (Figure 4a) defined an LD block in chromosome 9
157 from 9.64 to 9.79 Mb. This LD block had an average pairwise R_2 of 0.96 (Figure 4a,
158 lower section) and precisely colocalized with the reported physical position for the
159 *Pi5/Pi3/Pii* locus from 9.63 to 9.80 Mb (Figure 4a, Lee et al. 2009). GWAS scan 2,
160 using S9_9786203 as fixed cofactor to remove the effect of the *Pi5/Pi3/Pii* locus,
161 showed two additional QTL located in chromosomes 1 (S1_37612210) and 8
162 (S8_14597990, Figure 3a outer circle and Additional file 1: Fig. S1). LD blocks for
163 these QTL ranged from 37.5 to 37.9 Mb in chromosome 1 and from 12.8 to 14.6 Mb
164 in chromosome 8. With GWAS scan 3, using S9_9786203, S1_37612210 and
165 S8_14597990 as fixed cofactors, two additional QTL were found: one in chromosome
166 1 (S1_1631976, located in an LD block from 1.6 to 2.1 Mb), and another in
167 chromosome 10 (S10_17378459, located in an LD block from 17.3 to 17.6 Mb,
168 Additional file 1: Fig. S1).

169 In the indica population (Figure 3a, inner circle), GWAS scan 1 identified a
170 major effect QTL in chromosome 6 located in an LD block from 9.8 to 12.5 Mb with

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171 SNP S6_10469906 having the highest $-\log_{10}(P \text{ value})$ (Figure 4b and Additional file 1:
172 Fig. S1). Within this region the averaged pairwise R_2 was 0.44, while linkage with other
173 surrounding LD blocks was weak, with an averaged pairwise R_2 between LD blocks
174 ranging from 0.14 to 0.35 (Figure 4b, lower section). The QTL with S6_10469906
175 colocalized with the blast resistance gene cluster *Piz/Pi2/Pi5* (Wang et al., 2012a,
176 Figure 4b, upper section). Subsequent GWAS scan 2 using S6_10469906 as fixed
177 cofactor identified QTL S4_31419616 in an LD block from 31.4 to 32.7 Mb in
178 chromosome 4 (Additional file 1: Fig. S1). GWAS scan 3 with S6_10469906 and
179 S4_31419616 as fixed cofactors identified QTL S1_3350405 in an LD block from 3.2
180 to 3.4 Mb in chromosome 1, and S7_12704004 in an LD block from 12.7 to 14.8 Mb
181 in chromosome 7 (Figure 3a inner circle and Additional file 1: Fig. S1).
182 Allelic distribution of QTL was unbalanced across subpopulations. In the
183 tropical japonica population, allele S9_9786203:C was the major allele with an overall
184 frequency of 0.70 (Figure 2a). Its frequencies within subpopulations ranged from 0.22
185 in subpopulation 1, to 0.91 in subpopulation 2. In the indica population, allele
186 frequencies of QTL SNPs varied across PC1 (Figure 2b). The indica cultivar INIA
187 Olimar alleles predominated in pedigree groups originated from crosses that included
188 that cultivar and were rare or minor in pedigree groups with other ancestries (Figure
189 2b).
190
191 **QTL by QTL interaction**
192 All the main effects of QTL found in the GWAS scans for tropical japonica were
193 significant in the multi locus model analysis ($P<0.001$). The multi locus model provided
194 evidence for two significant triple interactions, one involving S9_9786203,
195 S1_37612210, and S1_1631976, and another with S9_9786203, S1_37612210, and

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196 S8_14597990 (P<0.001, Figure 5a). All genotypes with allele S1_1631976:C and
197 allele S1_37612210:T were susceptible regardless the alleles of S9_9786203.
198 Analogously, all genotypes with the allelic combination S8_14597990:A and
199 S1_37612210:T had intermediate resistance regardless the alleles of S9_9786203.
200 In the indica population the multi locus model analysis had significant main
201 effects for QTL S6_10469906, S1_3350405, S4_31419616, and S7_12704004
202 (P<0.001, Figure 3, intermediate circle). A significant interaction between
203 S6_10469906 and S4_31419616 (P<0.001) was found, determined by the effect of
204 S4_31419616 when the susceptible allele S6_10469906:T was fixed (Figure 5c).
205
206 **QTL by genetic background interaction**
207 Significant QTL by subpopulation interaction in the tropical japonica population was
208 found for S9_9786203 (*P*<0.001, Figure 5b), while there was not significant interaction
209 between other QTL and the genetic background. Allele S9_9786203:C was the
210 resistance allele across all subpopulations. Allele S9_9786203:T was associated with
211 susceptibility in all but subpopulation 2, where it was significantly associated with
212 resistance.
213 In the indica population, a triple interaction involving S6_10469906,
214 S4_31419616, and the genetic background modeled as the proportion of allele sharing
215 with the susceptible indica cultivar INIA Olimar was found to be significant (*P*=0.004,
216 Figure 5c). The magnitude of the allele substitution effect for S6_10469906 varied
217 proportionally with the percentage of allele sharing with INIA Olimar, and across allelic
218 combinations of S4_31419616. All genotypes with low allele sharing with INIA Olimar
219 and carrying the resistance allele S4_31419616:T were resistant regardless the
220 S6_10469906. Genotypes with the susceptible allele S6_10469906:T still had

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221 intermediate levels of AUDPC when combined whether with the resistance allele
222 S4_31419616:T, or with low proportion of allele sharing with INIA Olimar. The highest
223 AUDPC and magnitude of allele substitution effect for S6_10469906 were associated
224 with the susceptible allele S4_31419616:A and high proportion of allele sharing with
225 INIA Olimar.
226
227 **Discussion**
228 A single major effect QTL was found in each population, S9_9786203 in tropical
229 japonica and S6_10469906 in indica (Figure 3). These major effect QTL were in strong
230 LD and corresponded with well-known blast resistance loci: the *Pii/Pi3/Pi5* gene
231 cluster (Lee et al. 2009) in tropical japonica; and the *Piz/Pi2/Pi9* gene family (Wang et
232 al. 2012a) in indica. The *Pii/Pi3/Pi5* and *Piz/Pi2/Pi9* regions harbor clusters of genes
233 with nucleotide binding site-leucine-rich repeat (NBS-LRR) sequences (Liu et al. 2002;
234 Jeon et al. 2003), a gene class commonly associated to complete resistance
235 (DeYoung and Innes 2006).
236 Other QTL in regions previously reported containing blast resistance loci were
237 identified in this study. In the tropical japonica population, QTL S1_37612210 was
238 detected where the blast resistance locus OsPdk1 was localized at 37.85 Mb (Matsui
239 et al. 2010). Also, in the proximity to S8_14597990 a QTL linked to a marker at 16.5
240 Mb in chromosome 8 was reported, which had an additive effect together with gene
241 Pi13 in chromosome in a biparental mapping population (Ebitani et al. 2011). As for
242 QTL in the indica population, QTL S4_31419616 was found in an LD in chromosome
243 4 that contains a cluster of blast resistance loci. Several blast resistance loci were
244 mapped in this cluster, such as QTL-614 at 32.24 Mb, associated with complete blast
245 resistance in greenhouse (Wang et al. 1994), and the *Pi63* gene at 31.9 Mb, coding a

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246 NBS-LRR protein (Xu et al. 2014). The QTL S7_12704004 colocalized with the
247 reported Os07g0409900 locus at 12.8 Mb. This gene codifies for a calcium dependent
248 protein kinase and represses defense gene expression, negatively regulating rice blast
249 resistance (Xie et al. 2014). To our knowledge there are no previous reports for blast
250 resistance loci in the LD block regions where we detected QTL S1_1631976 and
251 S10_17378459 in tropical japonica, nor in the LD block for QTL S1_3350405 in indica.
252 In this study, we found statistical evidence of an epistatic interaction between
253 S9_9786203, a QTL colocalizing in the *Pii/Pi3/Pi5* locus, and newly found QTL for
254 blast resistance in chromosomes 1 and 8 in a tropical japonica population. In
255 particular, these interactions consisted in a loss of the expected resistance effect of
256 S9_9786203 when combined with certain alleles of the interacting QTL. The molecular
257 basis behind these interactions may correspond with regulation of expression and/or
258 activity of NBS-LRR genes by *trans* acting elements (Marone et al. 2013; Lee and
259 Yeom 2015). Similar QTL by QTL interactions have been reported for other
260 pathosystems such as resistance of pea (*Pisum sativum* L.) to *Ascochyta* blight
261 disease (Timmerman-Vaughan et al. 2016). Furthermore, several biparental blast
262 resistance QTL studies have reported digenic epistatic interactions involving rice blast
263 resistance genes: Wang et al. (1994) found epistatic interactions between *Pi5(t)* and
264 the *Pi7(t)* locus in chromosome 11; Sirithunya et al. (2002) reported significant
265 epistatic interactions between a QTL close to the *Pi5(t)* locus and a QTL in
266 chromosome 7; Wu et al. (2005) found two digenic interactions between partial blast
267 resistance QTL located in chromosomes 7 and 8; and Li et al. (2007) found from 2 to
268 7 digenic epistatic interactions among major- and minor-effect QTL for blast resistance
269 across different developmental stages of rice. Other indications of epistatic
270 interactions between blast resistance genes in rice have been derived from differences

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271 in resistance phenotypes found among different susceptible genotypes used as
272 recipients of blast resistance genes (Zhang et al. 2016b). Therefore, our findings
273 confirm previously observed blast resistance QTL by QTL interactions from biparental
274 populations, but within the more diverse genetic background offered by GWAS
275 mapping populations.

276 On the other hand, the triple interaction between the *Piz/Pi2/Pi9* linked QTL
277 S6_10469906, the QTL S4_31419616, and the genetic background, is probably not
278 attributable to epistasis, but to the additive effect of multiple small effect blast
279 resistance QTL that collinearly segregate with the genetic background in the indica
280 populations. That is, genotypes with low proportion of allele sharing with the
281 susceptible cultivar INIA Olimar may carry a higher number of favorable alleles
282 additional to S6_10469906 and S4_31419616 attributable to their more diverse
283 ancestry.

284

285 **Conclusions**

286 We found statistical evidence for a genetic background-dependent effect of the major
287 effect blast resistance locus *Pii/Pi3/Pi5* in a tropical japonica breeding population, that
288 suggest epistatic interactions. Also, the architecture of blast quantitative resistance
289 that is usually masked by major effect blast resistance genes was revealed in two rice
290 breeding populations. This means that, even among germplasm with relatively low
291 genetic diversity such as advanced lines from a breeding program, the contribution of
292 Pi genes to the enhancement of blast resistance may not be homogenous. Thus, a
293 genetic background-specific validation of this contribution should be addressed for
294 introgression of blast resistance loci in breeding germplasm.

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296 **Methods**

297

298 **Plant Material**

299 Two mapping populations were selected representing the genetic variability of
300 Uruguayan INIA's National rice advanced breeding germplasm. The tropical japonica
301 population comprised 245 tropical japonica advanced inbred lines, and the cultivars
302 INIA Tacuarí (Blanco et al. 1993) and Parao (Molina et al. 2011). The indica population
303 had 305 advanced inbred lines, and the cultivars El Paso 144 (Yan et al. 2007) and
304 INIA Olimar (Blanco et al. 2004).

305

306 **Phenotyping of disease resistance and *Pyricularia oryzae* isolate**

307 For phenotyping blast resistance two separate greenhouse experiments were run, one
308 for each mapping population. Each population was planted in 8 by 13-cell seedbeds
309 with a completely randomized experimental design with three replicates. The
310 experimental unit was the seedbed cell. Four seeds were sown per cell and trimmed
311 to left one plant per cell at inoculation. The inoculum solution was prepared following
312 Bonman et al. (1986). Briefly, conidia were harvested by scrapping the culture surface
313 with a metallic spatula, suspended in distilled water, filtered through gauze, and
314 centrifuged at 8000 rpm for 30 min. The resulting pellet was adjusted to 3 x 10^5 conidia
315 ml^{-1}, mixed with 4% gelatin, and sprayed over rice plants at 3-leaves stage. Relative
316 humidity was maintained at >98% and temperature from 24 to 28 °C during the first
317 two days post inoculation. The area under disease progress curve (AUDPC) was
318 calculated with the *audpc* function from the *agricolae* package (Mendiburu et al.
319 2015)(Mendiburu et al. 2015) in R software (R Core Team 2019), which implements
320 Equation [1] for the trapezoidal method (Simko and Piepho 2012):

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321 $$AUDPC_k = \sum_{i=1}^{n-1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i) \quad [1]$$

322 where $AUDPC_k$ is the total accumulated disease until time k, y_i is the disease score
323 rated at time i, t_i is the time point i when a disease score was rated, and n is the total
324 number of rating times. Disease scores data rated at 14, 21 and 28 days post
325 inoculation on a 0-5 scale, with scores of 0-3 considered resistant and of 4-5
326 susceptible reactions (Bonman et al. 1986). Adjusted phenotypic means were
327 estimated with the mixed model in Equation [2]:

328 $$Y_{ijmn} = \mu + \gamma_i + G_j + R_{m(i)} + C_{n(i)} + \varepsilon_{ijmn} \quad [2]$$

329 where Y_{ijmn} is the AUDPC score; μ is the intercept; γ_i is the random block effect with
330 $\gamma \sim N(0, \sigma_B^2)$; G_j is the genotypic effect modeled as $G_j = g_k + c_l$, where g_k is the
331 random effect of the kth genotype line with $g \sim N(0, \sigma_G^2)$ for estimation of genetic
332 variances and as fixed effect for estimating adjusted means, and c_l is the fixed effect
333 of the kth check; $R_{m(i)}$ and $C_{n(i)}$ are the row and column as coordinates for each
334 experimental unit in the greenhouse modelled as random effects nested within blocks,
335 with $R \sim N(0, \sigma_R^2)$ and $C \sim N(0, \sigma_C^2)$; and $\varepsilon_{ijmn}$is the residual. The models were fit in R
336 with the package *lme4* (Bates et al. 2005). AUDPC estimates equal to or below 30
337 were considered resistant, between 30 and 45 intermediate, and equal to or above 45
338 susceptible reaction. Heritability for AUDPC was estimated following Cullis et al.
339 (2006), and its standard error was estimated with a bootstrap resampling procedure
340 following Holland et al. (2003) with the *boot* package in R (Canty and Ripley 2015).
341 *Pyricularia oryzae* strain Po 188 isolated from leaves of temperate japonica rice cv.
342 Samba (Titone et al. 2015) in naturally infected commercial fields in Rio Branco, Cerro
343 Largo, Uruguay (32° 67'S, 53° 32'W) was selected based on its high virulence to
344 locally adapted rice germplasm. The isolate was grown at 25 °C for 15 days in rice

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345 bran agar media (1:1 w/w) and exposed for 4 days to sunlight radiation to induce
346 sporulation.
347
348 **Genotyping**
349 DNA was isolated from rice plant seedlings using the DNeasy kit (Qiagen). The 550
350 advanced breeding lines and cultivars were genotyped- by-sequencing (GBS, Elshire
351 et al. 2011). GBS libraries and sequencing were performed in the Biotechnology
352 Resource Center at Cornell University using digestion with enzyme ApeKI following
353 Elshire et al. (2011). GBS data was analyzed separately for indica and tropical
354 japonica. SNP were called with the TASSEL version 3.0 GBS pipeline. Sequences
355 were aligned with the MSU version 7.0 of Nipponbare reference genome using BWA-
356 0.7.5a. SNPs with a minor allele frequency (MAF) below 1% were removed from the
357 datasets. A detailed description of data curation can be found in Quero et al. (2018).
358
359 **Population structure and genetic background analyses**
360 Population structure was determined with principal component analysis (PCA) in both
361 indica and tropical japonica populations, computing the singular value decomposition
362 of the centered and scaled SNP score matrix for each population independently using
363 the *prcomp* base function in R. Pedigree groups within each population were defined
364 based on the origin and ancestry of the parental lines.
365
366 **GWAS scans**
367 Three GWAS scans were performed for each population, fitting the linear mixed model
368 in Equation [3] for each SNP in the genotypic matrix at a time.

369 $$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{Zu} + \boldsymbol{e} \quad [3]$$

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370 where $\boldsymbol{y}$ is a vector of adjusted phenotypic means; $\boldsymbol{\beta}$ is a vector of fixed effects (single
371 SNP for GWAS scan 1, or single SNP and SNPs selected as covariates from the
372 previous scans for GWAS scans 2 and 3); $\boldsymbol{u}$ is a vector of random genotypic effects
373 with $\boldsymbol{u} \sim \boldsymbol{N}(\boldsymbol{G}\boldsymbol{\sigma}_{\boldsymbol{G}}^{2})$; $\boldsymbol{e}$ is a vector of residual effects with $\boldsymbol{E} \sim \boldsymbol{N}(\boldsymbol{I}\boldsymbol{\sigma}_{\boldsymbol{e}}^{2})$; $\boldsymbol{X}$ and $\boldsymbol{Z}$ are incidence
374 matrices that relate $\boldsymbol{y}$ to $\boldsymbol{\beta}$ and to $\boldsymbol{u}$ respectively; $\boldsymbol{G}$ is the realized additive genotypic
375 relationship matrix (Endelman and Jannink 2012); $\boldsymbol{\sigma}_{\boldsymbol{G}}^{2}$ is the genetic variance; $\boldsymbol{I}$ is an
376 identity matrix; and $\boldsymbol{\sigma}_{\boldsymbol{e}}^{2}$ is the residual variance. QTL of major effect were found in the
377 GWAS scan 1 in both populations co-localizing with well-known and physically
378 mapped blast resistance genes (*Pii/Pi3/Pi5* locus in tropical japonica, and *Piz/Pi2/Pi9*
379 locus in indica). To explore additional QTL that may have been hidden by these major
380 effect QTL, GWAS scans 2 and 3 were performed using selected SNP from QTL of
381 previous GWAS scans as cofactors. GWAS scans were run with the *GWAS* function
382 from the *rrBLUP* R package (Endelman 2011). Significant thresholds for GWAS scans
383 were adjusted by the effective number of independent tests (Li and Ji 2005).

384 **QTL identification**

385 The significant SNP of each chromosome were clustered by their physical positions
386 using the *hclust* and *cutree* base R functions with an *h* parameter of a quarter of the
387 maximum height of the tree. Clusters with three or more significant SNP at less than
388 1Mb from each other were considered a QTL following Quero et al. (2018) and Rosas
389 et al. (2018). The SNP with the highest -$\log_{10}$(*P*) within each QTL was selected and
390 represented the QTL in all further analyses. The same criteria were used for GWAS
391 scans 1, 2, and 3.

392 **LD blocks**

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393 LD in the surrounding region and within each QTL found in GWAS scans 1 and 2 was
394 computed as the pairwise R_2 between all SNP in the region. Limits between LD blocks
395 were graphically assessed with the R package *LDheatmap* (Shin et al. 2006).

396 **Multilocus Models, Epistasis, and candidate locus search**

397 Two separate multilocus models were fit, one to estimate QTL main effects and QTL
398 by genetic background interaction effects, and another one to estimate QTL main
399 effects and QTL by QTL interaction effects. An initial full model considering all the
400 identified QTL and all second level interactions was fit, progressively removing non-
401 significant effects (α=0.01) with a backwards elimination procedure. To evaluate
402 epistasis, multilocus models following Equation [3] were used. For QTL by genetic
403 background interaction, the fixed effects in the β vector were: the QTL main effects;
404 and the QTL by subpopulation interaction effect (for tropical japonica) or QTL by
405 proportion of allele sharing with INIA Olimar interaction effect (for indica). For QTL by
406 QTL interaction, the fixed effects in the β vector were: the QTL main effects; and the
407 QTL by QTL interaction effects. The random effects in the *u* vector were the genotypes
408 for all the models, with $\boldsymbol{u} \sim N(0, G\sigma_G^2)$, where G is the realized additive genotypic
409 relationship matrix and σ_G^2 is the genetic variance. These multilocus models were fitted
410 with the *relmatLmer* function from *lme4qtl* R package (Ziyatdinov et al. 2018) and
411 least-squared means for QTL and genetic background effects were estimated with the
412 *emmeans* R package (Lenth 2018). Searches for loci reportedly associated with blast
413 resistance that co-localized with the QTL that had a significant effect in the multilocus
414 model were performed in literature and public databases (Yonemaru et al. 2010;
415 Alexandrov et al. 2015).

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416 **Abbreviations**

417 AUDPC, area under the disease progress curve; CIAT, International Center for
418 Tropical Agriculture; FLAR, Latin American Irrigated Rice Fund; GWAS, genome wide
419 association study; Ind, indica population; INIA, National Institute of Agricultural
420 Research (Uruguay); IRGA, Rice Research Institute of Rio Grande (Brazil); PO,
421 *Pyricularia oryzae*; PCA, principal component analysis; QTL, quantitative trait locus;
422 Trj, tropical japonica population.

423

424 **Consent for publication**

425 Not applicable.

426

427 **Availability of data and materials**

428 The datasets used and analyzed during the current study are available from the
429 corresponding author on reasonable request.

430

431 **Competing interest**

432 The authors have declared that no competing interests exist.

433

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440

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441 **Authors' contributions**

442 JR: designed and performed data analyses and wrote the manuscript; ME: grew rice
443 plants and inoculum, and performed blast inoculations and disease evaluation, and
444 original data analysis; SM: supervised inoculum production, blast inoculations and
445 disease evaluation; PB and FP: developed, selected and provided rice genotypes; GQ:
446 designed and supervised experiments and edited the manuscript; LG: designed and
447 supervised data analyses and edited the manuscript; VB: designed and supervised
448 the experiments and edited the manuscript. All authors read and approved the final
449 manuscript.

450

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745 Figure 1. Distribution of AUDPC in the indica (Ind) and tropical japonica (Trj)
746 populations according to their blast reaction rated at 14, 21, and 28 days post
747 inoculation, with the rates at 14, 21, and 28 days post inoculation equal to or below 3
748 as resistant, and at least one rate higher than 3 as susceptible reaction.
749
750 Figure 2. Principal component (PC) showing population structure of the two mapping
751 populations used in this study. (**a**) PC analysis of 28850 SNP showing population
752 structure of 245 tropical japonica rice advanced inbred lines. Pedigree based
753 subpopulations are color coded and point sizes represent the allele of S9_9786203
754 (small = S9_9786203:T, big = S9_9786203:C). (**b**) Upper section: Principal component
755 (PC) analysis of 49589 SNP showing population structure of 305 advanced indica rice
756 inbred lines. Parents´ origins are color coded, and point sizes represent the allele of
757 S6_10469906 (small = T, big = G). Origins of parents of pedigree groups: Olimar =
758 indica cultivar INIA Olimar (Uruguay); Ind IN = indica type inbred lines from INIA
759 (Uruguay); Ind FL1 = old indica type inbred lines from FLAR diverse germplasm (Latin
760 America); Ind FL2 = new indica type inbred lines from diverse FLAR germplasm (Latin
761 America); Ind IR = indica type inbred lines from IRGA (Brazil); Trj IN = tropical japonica
762 inbred lines from INIA (Uruguay); Trj CT = tropical japonica inbred lines from diverse
763 CIAT germplasm (Latin America and Asia). Middle section: Distribution of alleles for
764 blast resistance QTL across PC1, with red box for the susceptible allele and green box
765 for the resistance allele. Lower section: Gradient of mean allele sharing between
766 genotypes and the indica cultivar INIA Olimar across PC1.
767
768 Figure 3. Results of GWAS scans 1, 2, and 3 for tropical japonica (Trj) and indica (Ind)
769 populations. (**a**) Circular Manhattan plots with scans 1, 2 and 3 overlaid for Trj (outer

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770 circle) and Ind (inner circle). Significant SNPs are highlighted in black, and the SNP
771 with the highest $-\log_{10}(P)$ within each QTL that was significant in the multi locus model
772 is labelled and colored in red. (b) Quantile-quantile plots of GWAS scans 1, 2 and 3
773 for tropical japonica (Trj) and indica (Ind) populations.
774
775 Figure 4. Zoomed-in views of major effect QTL regions with reported gene clusters. (**a**)
776 chromosome 9 from 8.5 to 10.5 Mb in tropical japonica showing the physical position
777 of the gene cluster *Pii/Pi3/Pi5* (following Lee et al. 2009) and the LD block where this
778 gene cluster colocalizes. (**b**) chromosome 6 from 3 to 19 Mb in indica showing the
779 physical position of the gene cluster *Piz/Pi2/Pi9* (following Wang et al. 2012a) and the
780 q6 LD block where this gene cluster colocalizes. For both panels, the SNP with the
781 highest $-\log_{10}(P)$ within each QTL is labelled and colored in red. Lower triangle shows
782 average R^2 values within and between each LD block.
783 Figure 5. QTL by QTL and QTL by genetic background interactions in tropical japonica
784 and indica populations. (**a**) Triple QTL interactions among tropical japonica blast
785 resistance loci S9_9786203 by S1_37612210 by S1_1631976 and S9_9786203 by
786 S1_37612210 by S8_14597990. The effect of the favorable allele S9_9786203:C (in
787 strong LD with the blast resistance gene cluster Pii/Pi3/Pi), changed from resistance
788 to susceptibility in genotypes that combine the S1_37612210:T and S1_1631976:C
789 alleles, or from resistance to intermediate resistance in genotypes combining the
790 S1_37612210:T and S8_14597990:A alleles. (**b**) QTL by genetic background
791 interaction in the tropical japonica population (Trj), with genetic background modeled
792 as pedigree-based subpopulations 1 to 6. The effect of the susceptible allele
793 S9_9786203:T was not significantly different to that of the resistance allele
794 S9_9786203:C in genotypes belonging to subpopulation 2. For (a) and (b) the number

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795 of genotypes with each allelic combinations is shown in curved brackets (**c**) QTL by
796 QTL and QTL by genetic background interactions in the indica population (Ind). As the
797 proportion of shared alleles with the susceptible indica cultivar INIA Olimar increases,
798 so does the AUDPC of genotypes with the susceptible allele S6_10469906:T. This
799 increment in susceptibility is less pronounced when the S4_31419616:T favorable
800 allele is present. Error bars show the 95% confidence intervals for the estimated
801 marginal means of AUDPC.

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