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

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**Abstract:** Rice blast caused by *Pyricularia oryzae* is a major rice disease worldwide. Despite the detailed knowledge on major resistance genes available to date, little is known about how these genes interact with quantitative blast resistance loci and with the genetic background. Knowledge on these interactions is crucial for assessing the usefulness of introgressed resistance loci in breeding germplasm. Our goal was to identify quantitative trait loci (QTL) for blast resistance in rice breeding populations and to describe how they interact among each other and with the genetic background. To that end, resistance to blast was mapped by genome-wide association study (GWAS) in two advanced rice breeding subpopulations, one made of 305 *indica* type inbred lines, and the other of 245 *tropical japonica* inbred lines. The interactions and main effects of blast resistance loci were assessed in a multilocus model. Well known, major effect blast resistance gene clusters were detected in both *tropical japonica* (*Pii/Pi3/Pi5*) and *indica* (*Piz/Pi2/Pi9*) subpopulations with the GWAS scan 1. When these major effect loci were included as fixed cofactors in subsequent GWAS scans 2 and 3, additional QTL and more complex genetic architectures were revealed. The multilocus model for the *tropical japonica* subpopulation showed that *Pii/Pi3/Pi5* had significant interaction with two QTL in chromosome 1 and one QTL in chromosome 8, together explaining 64% of the phenotypic variance. In the *indica* subpopulation a significant interaction among the QTL in chromosomes 6 and 4 and the genetic background, together with *Piz/Pi2/Pi9* and QTL in chromosomes 1, 4 and 7, explained 35% of the phenotypic variance. Our results suggest that epistatic interactions can play a major role modulating the response mediated by major effect blast resistance loci such as *Pii/Pi3/Pi5*. Furthermore, the additive and epistatic effects of multiple QTL bring additional layers of quantitative resistance with a magnitude comparable to that of major effect loci. These findings highlight the need of genetic background-specific validation of markers for molecular assisted blast resistance breeding and provide insights for developing quantitative resistance to blast disease in rice.

**Keywords:** leaf blast; GWAS; QTL by QTL interaction; QTL by genetic background interaction; *Magnaporthe oryzae*; *Pyricularia oryzae*

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## 1. Introduction

Rice is a major staple food worldwide, feeding half of the world population [1]. One of the major threats to the rice crop is blast, a major disease caused by the fungus *Pyricularia oryzae* (PO) [2]. Genetic resistance to blast is a key objective in rice breeding programs, given its economic and environmental advantages over chemical control [3]. Current breeding methods for rice blast resistance involves phenotypic and marker assisted selection strategies [4]. The high genetic instability of PO mediated by parasexuality generates variations in fungal avirulence genes that prevent gene-for-gene interaction with rice resistance genes, leading to frequent breaks of race specific resistance [5].

More than 100 race specific blast resistance genes have been identified up to date in rice [6,7], and hundreds of quantitative trait loci (QTL) associated with blast resistance have been mapped [8]. Molecular markers linked to these genes and loci have been widely reported and used for assisted breeding of blast resistance [9–12]. Pyramiding several resistance genes is a common breeding strategy to broaden resistance spectrum and delay resistance breakdown [13–16]. This usually requires gene or QTL introgression from diverse donors to an adapted recipient, rising the issues of epistasis, namely, QTL by QTL interaction and QTL by genetic background interaction [17].

Low order QTL by QTL interaction effects are often estimated in biparental QTL studies [18], while the ability to test the interaction between QTL and genetic background is limited in biparental QTL studies [19,20], mainly due to the low number of QTL and genetic backgrounds combinations [21]. A widespread approach for identifying main effect QTL that exploits the diversity of allelic combinations from diverse genetic backgrounds is the genome-wide association study (GWAS) [22,23]. Assessment of QTL by QTL interaction in GWAS through multi loci models has been proposed [24], developed [25], and further implemented for several traits and crops [26–28], including disease resistance in rice [29]. Furthermore, the multiple genetic backgrounds and levels of relatedness present in GWAS mapping populations can be exploited to effectively investigate QTL by genetic background interactions [30]. A model that incorporates a term for QTL by genetic background interaction in GWAS analysis has been proposed [19]. GWAS has been extensively used for QTL mapping in highly diverse rice germplasm collections [31], and more recently in rice advanced breeding populations [32–34]. GWAS has been also used for successfully identifying blast resistance QTL in different types of mapping populations, such as a multi-parent advanced generation inter-cross population [35], collections of *indica* type landraces [36], a small set of temperate japonica elite breeding lines [37], and in rice diversity panels [38,39]. However, none of these studies has investigated epistatic interaction in blast resistance genetics, despite reports from biparental QTL studies of significant effects for several traits in rice [40–44], including blast resistance [9,44,45]. Furthermore, to our knowledge no statistical analysis with experimental data on QTL by genetic background interaction has been reported for blast resistance in rice up to date. In the present work, QTL main effects, QTL by genetic background, and QTL by QTL interaction effects for blast resistance in two advanced rice breeding populations were estimated using multi loci models in a GWAS framework.

## 2. Materials and Methods

### 2.1. Plant Material

Two mapping populations were selected representing the genetic variability of Uruguayan rice advanced breeding germplasm at the National Institute of Agricultural Research (INIA). The *tropical japonica* subpopulation comprised 245 *tropical japonica* advanced inbred lines, and the cultivars INIA Tacuarí [46] and Parao [47]. The *indica* subpopulation had 305 advanced inbred lines, and the cultivars El Paso 144 [48] and INIA Olimar [49].

## 2.2. Phenotyping of Disease Resistance and *Pyricularia Oryzae* Isolate

For phenotyping blast resistance two separate greenhouse experiments were run, one for each mapping population. Each population was planted in 8- by 13-cell seedbeds with a completely randomized experimental design with three replicates. The experimental unit was the seedbed cell. Four seeds were sown per cell and trimmed to left one plant per cell at inoculation. Rice seedlings at 3–4 leaf-stage were inoculated spraying a suspension of  $3.0 \times 10^5$  conidia ml<sup>-1</sup>, following the standard procedure [50]. Relative humidity was maintained at >98% and temperature from 24 to 28 °C during the first two days post inoculation. The area under disease progress curve (AUDPC) was used as a continuous trait for association analysis and was calculated with the *audpc* function from the *agricolae* package [51] in R software [52], which implements Equation (1) for the trapezoidal method [53]:

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

where  $AUDPC_k$  is the total accumulated disease until time  $k$ ,  $y_i$  is the disease score rated at time  $i$ ,  $t_i$  is the time point  $i$  when a disease score was rated, and  $n$  is the total number of rating times. Disease scores were rated at 7, 14 and 21 days post inoculation on the standard 0–5 scale [50], with 0–3 considered resistant and 4–5 susceptible reactions. Adjusted phenotypic means were estimated with the mixed model in Equation (2):

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

where  $Y_{ijmn}$  is the AUDPC score;  $\mu$  is the intercept;  $\gamma_i$  is the random block effect with  $\gamma \sim N(0, \sigma_B^2)$ ;  $G_j$  is the genotypic effect modeled as  $G_j = g_k + c_l$ , where  $g_k$  is the random effect of the  $k$ th genotype line with  $g \sim N(0, \sigma_G^2)$  for estimation of genetic variances and as fixed effect for estimating adjusted means, and  $c_l$  is the fixed effect of the  $l$ th check;  $R_{m(i)}$  and  $C_{n(i)}$  are the row and column as coordinates for each experimental unit in the greenhouse modelled as random effects nested within blocks, with  $R \sim N(0, \sigma_R^2)$  and  $C \sim N(0, \sigma_C^2)$ ; and  $\varepsilon_{ijmn}$  the residual. The models were fit in R with the package *lme4* [54]. AUDPC estimates equal to or below 30 were considered resistant, between 30 and 45 intermediate, and equal to or above 45 susceptible reaction. Heritability for AUDPC was estimated following Cullis et al. [55], and its standard error was estimated with a bootstrap resampling procedure following Holland et al. [56] with the *boot* package in R [57]. *Pyricularia oryzae* strain Po 188 isolated from leaves of rice cv. Samba [58] in naturally infected commercial fields in Rio Branco, Cerro Largo, Uruguay (32°67' S, 53°32' W), was selected based on its high virulence to locally adapted *tropical japonica* and *indica* rice germplasm. The isolate was grown at 25 °C for 15 days in rice bran agar media (1:1 w/w) and exposed for 4 days to sunlight radiation to induce sporulation.

## 2.3. Genotyping

DNA was isolated from rice plant seedlings using the DNeasy kit (Qiagen, Hamburg, Germany). The 550 advanced breeding lines and cultivars were genotyped-by-sequencing (GBS) [59]. GBS libraries and sequencing were performed in the Biotechnology Resource Center at Cornell University using digestion with enzyme *ApeKI* following standard GBS protocol [59]. GBS data was analyzed separately for *indica* and *tropical japonica*. Single nucleotide polymorphisms (SNP) were called with the TASSEL version 3.0 GBS pipeline [60]. Sequences were aligned with the MSU version 7.0 of Nipponbare reference genome [61] using BWA-0.7.5a [62]. SNPs with a minor allele frequency (MAF) below 5% were removed from the datasets. A detailed description of data curation can be found elsewhere [34]. Curated genotypic data is available as Supplementary Tables S1 and S2.

## 2.4. Population Structure and Genetic Background Analyses

Population structure was determined with principal component analysis (PCA) in both *indica* and *tropical japonica* subpopulations, computing the singular value decomposition of the centered and

scaled SNP score matrix for each subpopulation independently using the *prcomp* base function in R. Pedigree groups within each subpopulation were defined based on the origin and ancestry of the parental lines.

### 2.5. GWAS Scans

Three GWAS scans were performed for each subpopulation, fitting the linear mixed model in Equation (3) for each SNP in the genotypic matrix at a time.

$$y = X\beta + Zu + e \quad (3)$$

where  $y$  is a vector of adjusted phenotypic means;  $\beta$  is a vector of fixed effects (single SNP for GWAS scan 1, or single SNP and SNPs selected as covariates from the previous scans for GWAS scans 2 and 3);  $u$  is a vector of random genotypic effects with  $u \sim N(0, G\sigma_G^2)$ ;  $e$  is a vector of residual effects with  $e \sim N(0, I\sigma_e^2)$ ;  $X$  and  $Z$  are incidence matrices that relate  $y$  to  $\beta$  and to  $u$ , respectively;  $G$  is the realized additive genotypic relationship matrix [63];  $\sigma_G^2$  is the genetic variance;  $I$  is an identity matrix; and  $\sigma_e^2$  is the residual variance. QTL of major effect were found in the GWAS scan 1 in both subpopulations co-localizing with well-known and physically mapped blast resistance genes (*Pii/Pi3/Pi5* locus in *tropical japonica*, and *Piz/Pi2/Pi9* locus in *indica*). To explore additional QTL that may have been hidden by these major effect QTL, GWAS scans 2 and 3 were performed using selected SNP from QTL of previous GWAS scans as cofactors. GWAS scans were run with the GWAS function from the *rrBLUP* R package [64]. Significant thresholds for GWAS scans were adjusted by the effective number of independent tests [65].

### 2.6. QTL Identification

The significant SNP of each chromosome were clustered by their physical positions using the *hclust* and *cutree* base R functions with an  $h$  parameter of a quarter of the maximum height of the tree. Clusters with three or more significant SNP at less than 1Mb from each other were considered a QTL. The 1Mb window was defined based on the average linkage disequilibrium (LD) decay in each chromosome for both *indica* and *tropical japonica* subpopulations [34]. The SNP with the highest  $-\log_{10}(p)$  within each QTL was selected and represented the QTL in all further analyses. The same criteria were used for GWAS scans 1, 2, and 3.

### 2.7. LD Blocks

LD in the surrounding region and within each QTL found in GWAS scans 1 and 2 was computed as the pairwise  $R^2$  between all SNP in the region. Limits between LD blocks were graphically assessed with the R package *LDheatmap* [66].

### 2.8. Multilocus Models, Epistasis, and Candidate Locus Search

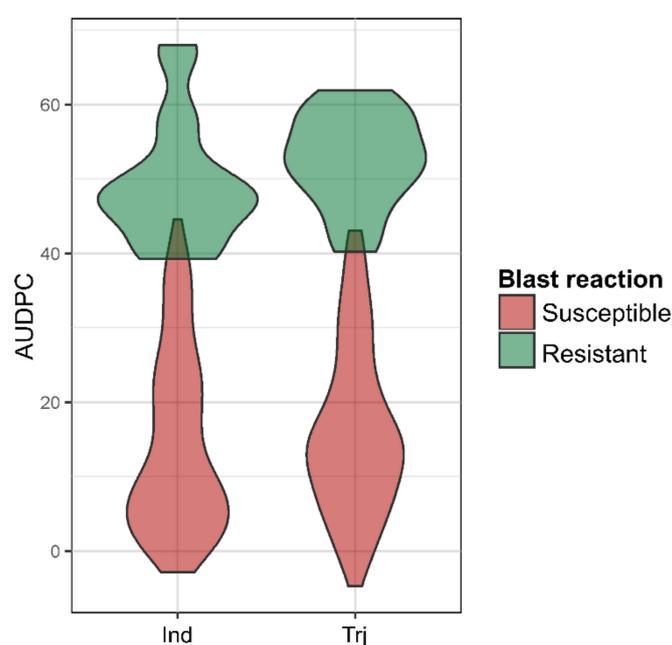
Two separate multilocus models were fit, one to estimate QTL main effects and QTL by genetic background interaction effects, and another one to estimate QTL main effects and QTL by QTL interaction effects. An initial full model considering all the identified QTL and all second level interactions was fit, progressively removing non-significant effects ( $\alpha = 0.01$ ) with a backwards elimination procedure. To evaluate epistasis, multilocus models following Equation (3) were used. For QTL by genetic background interaction, the fixed effects in the  $\beta$  vector were the QTL main effects; and the QTL by group interaction effect (for *tropical japonica*) or QTL by proportion of allele sharing with INIA Olimar interaction effect (for *indica*). For QTL by QTL interaction, the fixed effects in the  $\beta$  vector were the QTL main effects, and the QTL by QTL interaction effects. The random effects in the  $u$  vector were the genotypes for all the models, with  $u \sim N(0, G\sigma_G^2)$ , where  $G$  is the realized additive genotypic relationship matrix and  $\sigma_G^2$  is the genetic variance. These multilocus models were fitted with the *relmatLmer* function from *lme4qtl* R package [67] and least-squared means for QTL and genetic background effects were estimated with the *emmeans* R package [68]. Searches for loci

reportedly associated with blast resistance that co-localized with the QTL that had a significant effect in the multilocus model were performed in literature and public databases [69,70].

### 3. Results

#### 3.1. Phenotyping

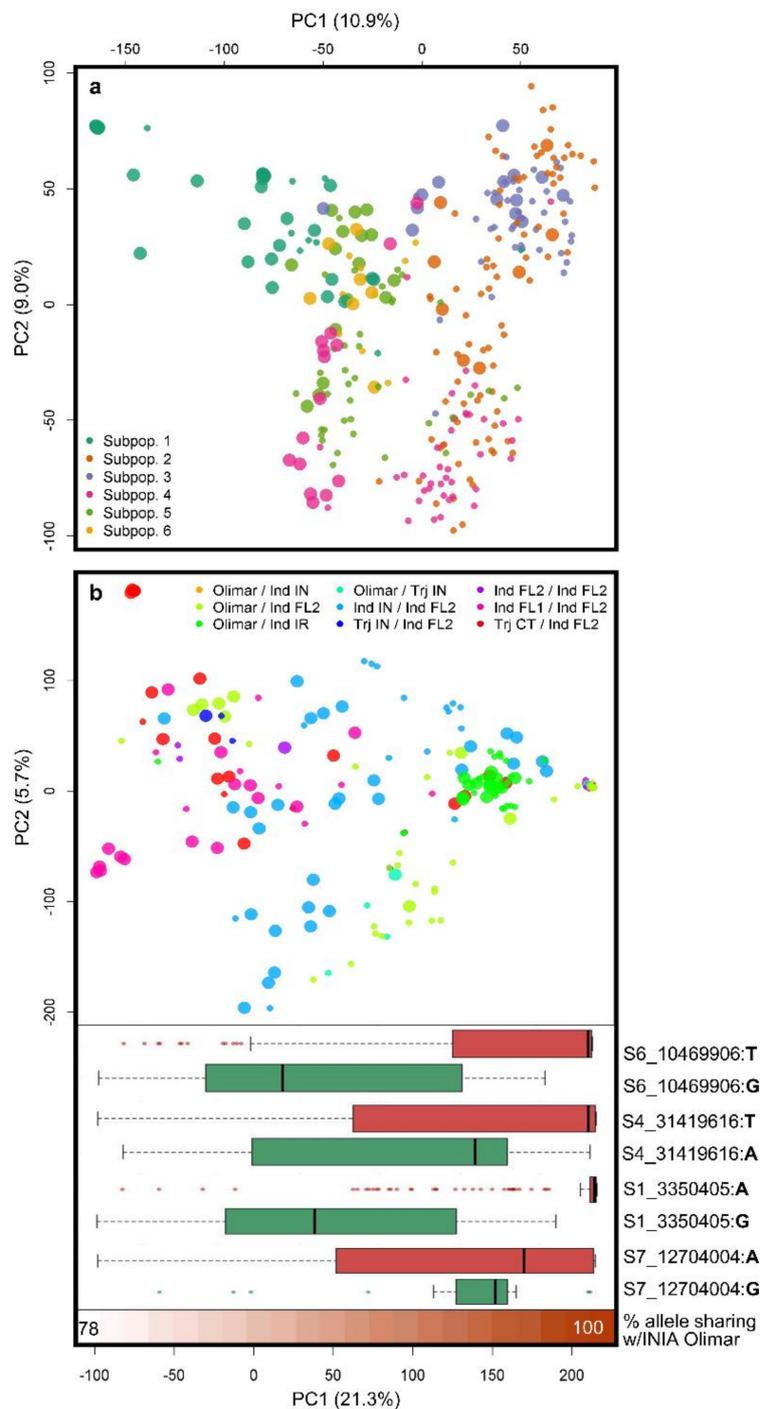
Pairwise correlations between adjusted phenotypic means of disease evaluations at 14, 21, and 28 days post inoculation, and of the area under the disease progress curve (AUDPC) were high ( $>0.98$ ,  $p < 0.001$ ). Distributions of disease scores in the *tropical japonica* and *indica* subpopulations are shown in Figure 1. Resistant genotypes (i.e., rated 3 or below in the three evaluation dates) had adjusted phenotypic means of AUDPC from  $-2.8$  to  $44.5$  in *indica* and from  $-4.7$  to  $43.0$  in *tropical japonica*. Genotypes rated as susceptible (at least one score above 3) had adjusted phenotypic means of AUDPC ranging from  $39.3$  to  $68.0$  in *indica* and from  $40.2$  to  $61.9$  in *tropical japonica* (Figure 1 and Supplementary Tables S3 and S4). Heritability for AUDPC was  $0.56$  ( $0.07$  SE) in the *tropical japonica* subpopulation experiment and  $0.26$  ( $0.10$  SE) in the *indica* subpopulation experiment.



**Figure 1.** Distribution of the area under disease progress curve (AUDPC) in the *indica* (Ind) and *tropical japonica* (Trj) subpopulations according to their blast reaction rated at 7, 14, and 21 days post inoculation, with the rates equal to or below 3 as resistant, and at least one rate higher than 3 as susceptible reaction.

#### 3.2. Population Structure

For the *tropical japonica* subpopulation, six groups were defined based on pedigree (Figure 2a). Groups 1 and 5 had *indica* type lines in their pedigree record, derived from dwarf germplasm nurseries developed by the International Rice Research Institute (IRRI) and the International Center for Tropical Agriculture (CIAT) and tested in Uruguay in the 1980s. However, locally developed *tropical japonica* lines were predominant in the pedigree of both groups. Groups 1 and 3 shared the same local *tropical japonica* lines as parents. Groups 2, 4, 5 and 6 were composed by locally developed lines in whose pedigrees predominated (not exclusively) the US *tropical japonica* cultivars Cypress [71], Gulfmont [72], Newbonnet [73], and Lemont [74], respectively. Pedigree groups were clustered in the PCA plot (Figure 2a). PC1 explained 10.9% of the genotypic variance and separated groups 1 and 5 from 2 and 3, and PC2 explained 9.0% of the genotypic variance and separated group 4 from 1 and 3.



**Figure 2.** Principal component (PC) showing population structure of the two mapping populations used in this study. **(a)** PC analysis of 28850 SNP from 245 *tropical japonica* rice advanced inbred lines. Pedigree based groups are color coded and point sizes represent the allele of S9\_9786203 (small = S9\_9786203:T, big = S9\_9786203:C). **(b)** Upper section: PC analysis of 49589 SNP from 305 advanced *indica* rice inbred lines. Parents' origins are color coded, and point sizes represent the allele of S6\_10469906 (small = T, big = G). Origins of parents of pedigree groups: Olimar = susceptible *indica* cultivar INIA Olimar (Uruguay); Ind IN = *indica* type inbred lines from the National Institute of Agricultural Research (INIA, Uruguay); Ind FL1 = old *indica* type inbred lines from the Latin American Fund for Irrigated Rice (FLAR)

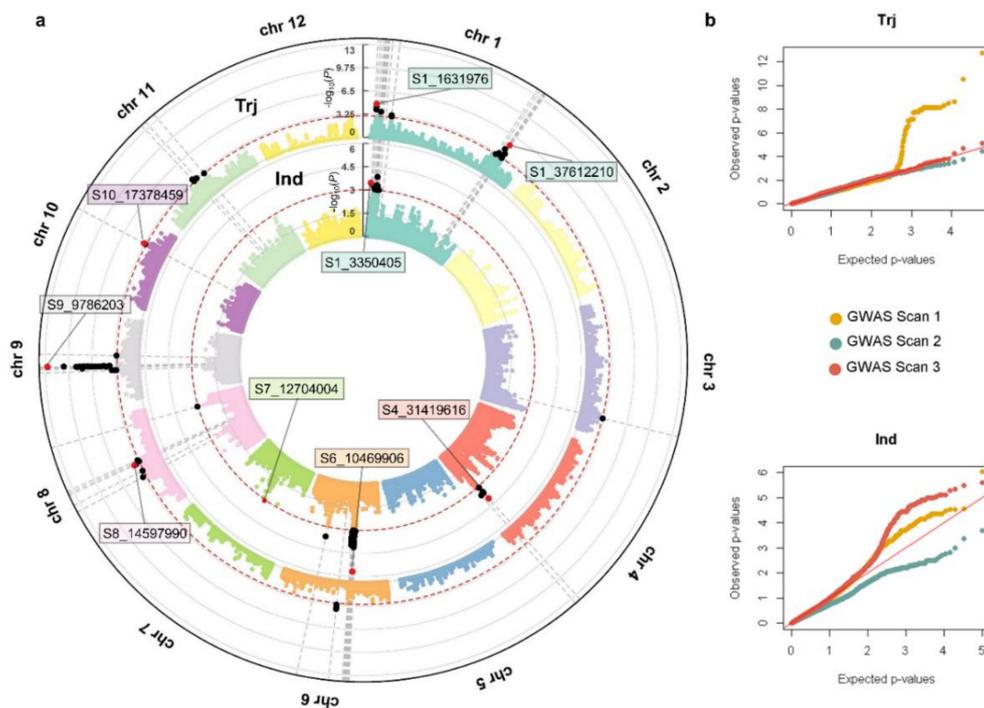
diverse germplasm (Latin America); Ind FL2 = new *indica* type inbred lines from diverse FLAR germplasm (Latin America); Ind IR = *indica* type inbred lines from the Rio Grande do Sul Rice Institute (IRGA, Brazil); Trj IN = *tropical japonica* inbred lines from INIA (Uruguay); Trj CT = *tropical japonica* inbred lines from diverse germplasm developed at the International Center for Tropical Agriculture (Latin America and Asia). Middle section: Distribution of alleles for blast resistance QTL across PC1, with red box for the susceptible allele and green box for the resistance allele. Lower section: Gradient of mean allele sharing between genotypes and the *indica* cultivar INIA Olimar across PC1.

For the *indica* subpopulation, nine groups were defined based on the origins of parents (Figure 2b, upper section). INIA Olimar, an *indica* cultivar with high yield and quality, was one of the parents in four out of nine of those groups (65% of the *indica* subpopulation). A clear gradient of allele sharing with INIA Olimar ranged from 78 to 99% and was found to correspond with PC1 ( $r = 0.98$ ,  $p < 0.001$ ), the latter explaining 21.3% of the genetic variance (Figure 2b, upper and lower sections). PC2 explained 5.7% of the genetic variance. Furthermore, lines derived from crosses between INIA local germplasm, including INIA Olimar, clustered on the left side of PC1 and at the center of PC2, indicating lower genetic variability and more similarity to INIA Olimar. Lines derived from more diverse germplasm sources (the Latin American Fund for Irrigated Rice, FLAR and CIAT, including *tropical japonica* parents) are found on the right side of PC1, corresponding with higher levels of diverse genomic introgressions and more genetic distance from INIA Olimar.

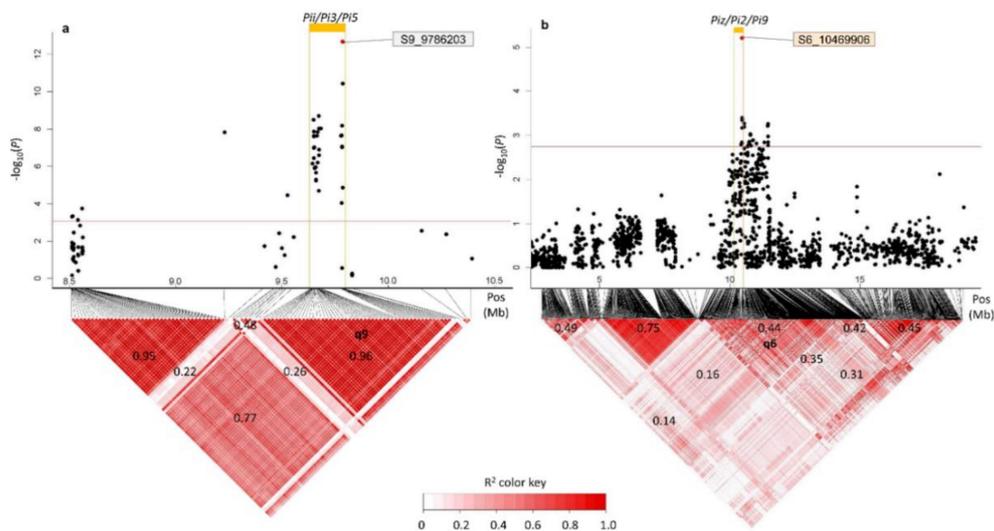
### 3.3. QTL Found in GWAS Scans 1, 2 and 3, and Colocalizing Reported Blast Resistance Loci

Results of GWAS scans 1, 2 and 3 are displayed together in Figure 3, and by scan in Supplementary Materials: Figure S1. In the *tropical japonica* subpopulation (Figure 3a, outer circle), GWAS scan 1 identified a single QTL on chromosome 9, with SNP S9\_9786203 having the highest  $-\log_{10}(p \text{ value})$  (Supplementary Materials: Figure S1). LD block analysis in the surrounding region of S9\_9786203 (Figure 4a) defined an LD block in chromosome 9 from 9.64 to 9.79 Mb. This LD block had an average pairwise  $R^2$  of 0.96 (Figure 4a, lower section) and precisely colocalized with the reported physical position for the *Pii/Pi3/Pi5* locus from 9.63 to 9.80 Mb [75] (Figure 4a). GWAS scan 2, using S9\_9786203 as fixed cofactor to remove the effect of the *Pii/Pi3/Pi5* locus, showed two additional QTL located in chromosomes 1 (S1\_37612210) and 8 (S8\_14597990, Figure 3a outer circle and Supplementary Materials: Figure S1). LD blocks for these QTL ranged from 37.5 to 37.9 Mb in chromosome 1 and from 12.8 to 14.6 Mb in chromosome 8. With GWAS scan 3, using S9\_9786203, S1\_37612210 and S8\_14597990 as fixed cofactors, two additional QTL were found: one in chromosome 1 (S1\_1631976, located in an LD block from 1.6 to 2.1 Mb), and another in chromosome 10 (S10\_17378459, located in an LD block from 17.3 to 17.6 Mb, Supplementary Materials: Figure S1).

In the *indica* subpopulation (Figure 3a, inner circle), GWAS scan 1 identified a major effect QTL in chromosome 6 located in an LD block from 9.8 to 12.5 Mb with SNP S6\_10469906 having the highest  $-\log_{10}(p \text{ value})$  (Figure 4b and Additional file 1: Figure S1). Within this region the averaged pairwise  $R^2$  was 0.44, while linkage with other surrounding LD blocks was weak, with an averaged pairwise  $R^2$  between LD blocks ranging from 0.14 to 0.35 (Figure 4b, lower section). The QTL with S6\_10469906 colocalized with the blast resistance gene cluster *Piz/Pi2/Pi9* [76] (Figure 4b, upper section). Subsequent GWAS scan 2 using S6\_10469906 as fixed cofactor identified QTL S4\_31419616 in an LD block from 31.4 to 32.7 Mb in chromosome 4 (Additional file 1: Figure S1). GWAS scan 3 with S6\_10469906 and S4\_31419616 as fixed cofactors identified QTL S1\_3350405 in an LD block from 3.2 to 3.4 Mb in chromosome 1, and S7\_12704004 in an LD block from 12.7 to 14.8 Mb in chromosome 7 (Figure 3a inner circle and Additional file 1: Figure S1).



**Figure 3.** Results of GWAS scans 1, 2, and 3 for *tropical japonica* (Trj) and *indica* (Ind) subpopulations. (a) Circular Manhattan plots with scans 1, 2 and 3 overlaid for Trj (outer circle) and Ind (inner circle). Significant single nucleotide polymorphisms (SNPs) are highlighted in black, and the SNP with the highest  $-\log_{10}(p)$  within each quantitative trait loci that was significant in the multi locus model is labelled and colored in red. (b) Quantile-quantile plots of GWAS scans 1, 2 and 3 for *tropical japonica* (Trj) and *indica* (Ind) subpopulations.

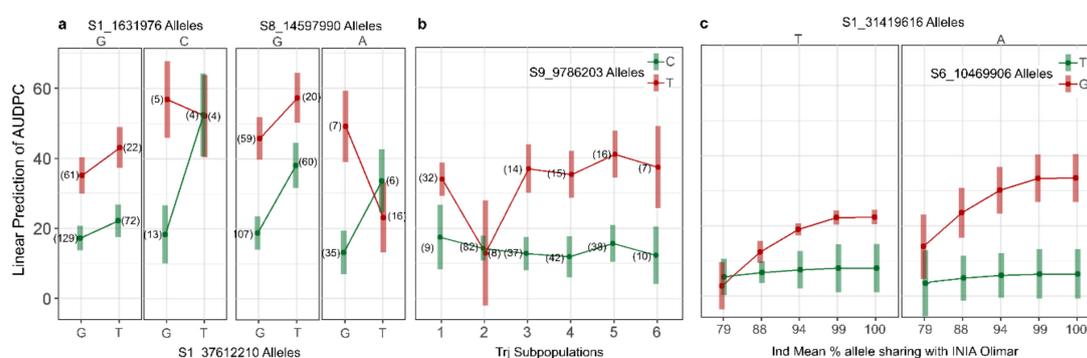


**Figure 4.** Zoomed-in views of major effect quantitative trait locus (QTL) regions with reported gene clusters. (a) chromosome 9 from 8.5 to 10.5 Mb in *tropical japonica* showing the physical position of the gene cluster *Pii/Pi3/Pi5* and the linkage disequilibrium (LD) block where this gene cluster colocalizes. (b) chromosome 6 from 3 to 19 Mb in *indica* showing the physical position of the gene cluster *Piz/Pi2/Pi9* and the q6 LD block where this gene cluster colocalizes. For both panels, the single nucleotide polymorphism with the highest  $-\log_{10}(p)$  within each QTL is labelled and colored in red. Lower triangle shows average  $R^2$  values within and between each LD block.

Allelic distribution of QTL was unbalanced across groups. In the *tropical japonica* subpopulation, allele S9\_9786203:C was the major allele with an overall frequency of 0.70 (Figure 2a). Its frequencies within groups ranged from 0.22 in group 1, to 0.91 in group 2. In the *indica* subpopulation, allele frequencies of QTL SNPs varied across PC1 (Figure 2b). The *indica* cultivar INIA Olimar alleles predominated in pedigree groups originated from crosses that included that cultivar and were rare or minor in pedigree groups with other ancestries (Figure 2b).

### 3.4. QTL by QTL Interaction

All the main effects of QTL found in the GWAS scans for *tropical japonica* were significant in the multi locus model analysis ( $p < 0.001$ ). The multi locus model provided evidence for two significant triple interactions, one involving S9\_9786203, S1\_37612210, and S1\_1631976, and another with S9\_9786203, S1\_37612210, and S8\_14597990 ( $p < 0.001$ , Figure 5a). All genotypes with allele S1\_1631976:C and allele S1\_37612210:T were susceptible regardless the alleles of S9\_9786203. Analogously, all genotypes with the allelic combination S8\_14597990:A and S1\_37612210:T had intermediate resistance regardless the alleles of S9\_9786203.



**Figure 5.** Quantitative trait locus (QTL) by QTL and QTL by genetic background interactions in *tropical japonica* and *indica* subpopulations. **(a)** Triple QTL interactions among *tropical japonica* blast resistance loci S9\_9786203 by S1\_37612210 by S1\_1631976 and S9\_9786203 by S1\_37612210 by S8\_14597990. The effect of the favorable allele S9\_9786203:C (in strong linkage disequilibrium with the blast resistance gene cluster *Pii/Pi3/Pi*), changed from resistance to susceptibility in genotypes that combine the S1\_37612210:T and S1\_1631976:C alleles, or from resistance to intermediate resistance in genotypes combining the S1\_37612210:T and S8\_14597990:A alleles. **(b)** QTL by genetic background interaction in the *tropical japonica* subpopulation (Trj), with genetic background modeled as pedigree-based groups 1 to 6. The effect of the susceptible allele S9\_9786203:T was not significantly different to that of the resistance allele S9\_9786203:C in genotypes belonging to group 2. For **(a,b)** the number of genotypes with each allelic combinations is shown in curved brackets **(c)**. QTL by QTL and QTL by genetic background interactions in the *indica* subpopulation (Ind). As the proportion of shared alleles with the susceptible *indica* cultivar INIA Olimar increases, so does the area under disease progress curve (AUDPC) of genotypes with the susceptible allele S6\_10469906:T. This increment in susceptibility is less pronounced when the S4\_31419616:T favorable allele is present. Error bars show the 95% confidence intervals for the estimated marginal means of AUDPC.

In the *indica* subpopulation the multi locus model analysis had significant main effects for QTL S6\_10469906, S1\_3350405, S4\_31419616, and S7\_12704004 ( $p < 0.001$ , Figure 3, intermediate circle). A significant interaction between S6\_10469906 and S4\_31419616 ( $p < 0.001$ ) was found, determined by the effect of S4\_31419616 when the susceptible allele S6\_10469906:T was fixed (Figure 5c).

### 3.5. QTL by Genetic Background Interaction

Significant QTL by group interaction in the *tropical japonica* subpopulation was found for S9\_9786203 ( $p < 0.001$ , Figure 5b), while there was not significant interaction between other QTL and the genetic

background. Allele S9\_9786203:C was the resistance allele across all groups. Allele S9\_9786203:T was associated with susceptibility in all but group 2, where it was significantly associated with resistance.

In the *indica* subpopulation, a triple interaction involving S6\_10469906, S4\_31419616, and the genetic background modeled as the proportion of allele sharing with the susceptible *indica* cultivar INIA Olimar was found to be significant ( $p = 0.004$ , Figure 5c). The magnitude of the allele substitution effect for S6\_10469906 varied proportionally with the percentage of allele sharing with INIA Olimar, and across allelic combinations of S4\_31419616. All genotypes with low allele sharing with INIA Olimar and carrying the resistance allele S4\_31419616:T were resistant regardless the S6\_10469906. Genotypes with the susceptible allele S6\_10469906:T still had intermediate levels of AUDPC when combined whether with the resistance allele S4\_31419616:T, or with low proportion of allele sharing with INIA Olimar. The highest AUDPC and magnitude of allele substitution effect for S6\_10469906 were associated with the susceptible allele S4\_31419616:A and high proportion of allele sharing with INIA Olimar.

#### 4. Discussion

A single major effect QTL was found in each subpopulation, S9\_9786203 in *tropical japonica* and S6\_10469906 in *indica* (Figure 3). These major effect QTL were in strong LD and corresponded with well-known blast resistance loci: the *Pii/Pi3/Pi5* gene cluster [75] in *tropical japonica*; and the *Piz/Pi2/Pi9* gene family [76] in *indica*. The *Pii/Pi3/Pi5* and *Piz/Pi2/Pi9* regions harbor clusters of genes with nucleotide binding site-leucine-rich repeat (NBS-LRR) sequences [77,78], a gene class commonly associated to complete resistance [79].

Other QTL in regions previously reported containing blast resistance loci were identified in this study. In the *tropical japonica* subpopulation, QTL S1\_37612210 was detected where the blast resistance locus OsPdk1 was localized at 37.85 Mb [80]. Furthermore, in the proximity to S8\_14597990 a QTL linked to a marker at 16.5 Mb in chromosome 8 was reported, which had an additive effect together with gene *Pi13* in chromosome 6 in a biparental mapping population [81]. As for QTL in the *indica* subpopulation, QTL S4\_31419616 was found in an LD in chromosome 4 that contains a cluster of blast resistance loci. Several blast resistance loci were mapped in this cluster, such as QTL-614 at 32.24 Mb, associated with complete blast resistance in greenhouse [9], and the NBS-LRR protein coding genes *Pi63* at 31.9 Mb [82] and *PiPR1* at 31.5 Mb [83]. The QTL S7\_12704004 colocalized with the reported Os07g0409900 locus at 12.8 Mb. This gene codifies for a calcium dependent protein kinase and represses defense gene expression, negatively regulating rice blast resistance [84]. To our knowledge there are no previous reports for blast resistance loci in the LD block regions where we detected QTL S1\_1631976 and S10\_17378459 in *tropical japonica*, nor in the LD block for QTL S1\_3350405 in *indica*.

In this study, we found statistical evidence of an epistatic interaction between S9\_9786203, a QTL colocalizing in the *Pii/Pi3/Pi5* locus, and newly found QTL for blast resistance in chromosomes 1 and 8 in a *tropical japonica* subpopulation. These interactions consisted in a loss of the expected resistance effect of S9\_9786203 when combined with certain alleles of the interacting QTL. The molecular basis behind these interactions may correspond with regulation of expression and/or activity of NBS-LRR genes by trans acting elements [85,86]. Similar QTL by QTL interactions have been reported for other pathosystems, such as resistance of pea (*Pisum sativum* L.) to *Ascochyta* blight disease [87]. Furthermore, several biparental blast resistance QTL studies have reported digenic epistatic interactions involving rice blast resistance genes: between *Pi5(t)* and the *Pi7(t)* locus in chromosome 11 [9]; between a QTL close to the *Pi5(t)* locus and a QTL in chromosome 7 [88]; two digenic interactions between partial blast resistance QTL located in chromosomes 7 and 8 [89]; and 2 to 7 digenic epistatic interactions among major- and minor-effect QTL for blast resistance across different developmental stages of rice [90]. Other indications of epistatic interactions between blast resistance genes in rice have been derived from differences in resistance phenotypes found among different susceptible genotypes used as recipients of blast resistance genes [31]. Therefore, our findings show that blast resistance QTL by QTL interactions

found in the specific genetic background of biparental populations, can also be detected within the more diverse genetic background offered by GWAS mapping populations.

On the other hand, the triple interaction between the *Piz/Pi2/Pi9* linked QTL S6\_10469906, the QTL S4\_31419616, and the genetic background, is probably not attributable to epistasis, but to the additive effect of multiple small effect blast resistance QTL that collinearly segregate with the genetic background in the *indica* subpopulation. That is, genotypes with low proportion of allele sharing with the susceptible cultivar INIA Olimar may carry a higher number of favorable alleles additional to S6\_10469906 and S4\_31419616 attributable to their more diverse ancestry.

## 5. Conclusions

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

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2077-0472/10/12/622/s1>, Figure S1: Manhattan plots, Table S1: Genotypic data for *indica* subpopulation; Table S2: Genotypic data for *tropical japonica* subpopulation; Table S3: Phenotypic data for *indica* subpopulation; Table S4: Phenotypic data for *tropical japonica* subpopulation.

**Author Contributions:** J.E.R. designed and performed data analyses and wrote the manuscript; M.E. grew rice plants and inoculum, and performed blast inoculations and disease evaluation, and original data analysis; S.M. supervised inoculum production, blast inoculations and disease evaluation; P.B. and F.P. developed, selected and provided rice genotypes; G.Q. designed and supervised experiments and edited the manuscript; L.G. designed and supervised data analyses and edited the manuscript; V.B. designed and supervised the experiments and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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