A more complete story of the genetic bases of resistance to the rice *hoja blanca* disease

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ABSTRACT

Rice *hoja blanca* (RHB) is one of the most serious diseases in rice growing areas in tropical Americas. Its causal agent is *Rice hoja blanca virus* (RHBV), transmitted by the planthopper *Tagosodes orizicolus* Müir. Genetic resistance is the most effective and environment-friendly way of controlling the disease. So far, only one major quantitative trait locus (QTL) of *Oryza sativa* ssp. *japonica* origin, *qHBV4.1*, that alters incidence of the virus symptoms in two Colombian cultivars has been reported. This resistance has already started to be broken, stressing the urgent need for diversifying the resistance sources. In the present study we performed a search for new QTLs of *O. sativa indica* origin associated with RHB resistance. We used four $F_{2:3}$ segregating populations derived from *indica* resistant varieties crossed with a highly susceptible *japonica* pivot parent. Beside the standard method for measuring disease incidence, we developed a new method based on computer-assisted image processing to determine the affected leaf area (ALA) as a measure of symptoms severity. Based on the disease severity and incidence scores in the F_3 families under greenhouse conditions, and SNP genotyping of the F_2 individuals, we identified four new *indica* QTLs for RHB resistance on rice chromosomes 4, 6 and 11, namely $qHBV4.2^{WAS208}$, $qHBV6.1^{PTB25}$, *qHBV11.1* and *qHBV11.2*. We also confirmed the wide-range action of *qHBV4.1*. Among the five QTLs, *qHBV4.1* and *qHBV11.1* had the largest effects on incidence and severity, respectively. These results provide a more complete understanding of the genetic bases of RHBV resistance in the cultivated rice gene pool, and can be used to develop marker-aided breeding strategies to improve RHB resistance. The power of joint- and meta- analyses allowed precise mapping and candidate genes identification, providing the basis for positional cloning of the two major QTLs *qHBV4.1* and *qHBV11.1*.

KEY WORDS

Rice hoja blanca virus (RHBV), Rice *hoja blanca* disease (RHB), Quantitative trait locus (QTL), candidate gene

INTRODUCTION

The rice *hoja blanca* (RHB) disease is one of the most important constraints to rice productivity in the tropical zone of Americas, causing yield losses in many countries, including Colombia,

Costa Rica, Ecuador, Guyana, Panama, Peru, Dominican Republic, Nicaragua and Venezuela (Morales and Jennings 2010). Its causal agent is the *Rice hoja blanca virus* (RHBV), a *Tenuivirus* transmitted by the planthopper *Tagosodes orizicolus* M. (Hemiptera:Delphacidae). Genetic resistance to both the virus and its vector insect is key for a successful, environment- and consumers health-friendly, integrated crop management. No real immunity has been found in the cultivated rice germplasm, and even in resistant materials, the plantlets (< 10 days old) can show susceptibility to the virus. Nonetheless, a handful of varieties – including the two Colombian cultivars, Fedearroz 50 and Fedearroz 2000 – with good resistance level have been bred in the past and have been important to stabilize rice production. Fedearroz 2000 is still the most resistant commercial variety, however, the incidence of the disease has increased under field and controlled conditions. Fedearroz 50 has turned virus-susceptible in the past years in the field, probably due to virus mutations that allowed it to overcome the resistance. Thus, there is an urgent need for diversifying the sources of genetic resistance in order to breed new varieties with more durable resistance. Genetic resistance to RHB disease can be decomposed into resistance to the virus itself, and resistance to its insect vector.

In a previous study, we reported a major QTL on the short arm of chromosome 4 for resistance to RHBV, shared by Fedearroz 50 and Fedearroz 2000, abbreviated as FD 50 and FD 2000 in this paper (Romero et al. 2014). This QTL, called here *qHBV4.1*, controlled RHB *incidence*, measured as the percentage of plants that show symptoms of the viral infection, no matter the level of the symptoms. Incidence is of course an important parameter of the epidemics of a disease. Yet, its *severity* is certainly as much as important: if severity is low, a high incidence might have no significant impact on plant viability, panicle development, or grain yield. Additional to RHBV incidence, we thus designed – and report in this study – new experiments to decipher the genetic control of RHB resistance seen as symptoms severity, measured by computer-aided image processing of the affected leaf area (ALA).

Moreover, a local ancestry analysis showed that, although FD 50 and FD 2000 are mostly of *indica* genetic background, *qHBV4.1* in those two cultivars was found to be of *japonica* origin, and was surrounded by several hundred kilobase pairs of *japonica* DNA. We found desirable to search for *indica* resistance QTLs in order to ensure better compatibility with tropical irrigated materials in breeding programs, and also to increase the diversity of the available sources of genetic resistance. Based on the encouraging screening work by the FLAR (Fondo Latinoamericano para Arroz de Riego) team (Cruz-Gallego et al. 2018), we thus designed a new QTL analysis based on four crosses involving new resistance sources.

MATERIALS AND METHODS

Plant materials

Four *O. sativa* ssp. *indica* accessions were selected amongst the most resistant materials identified in (Cruz-Gallego et al. 2018): WAS 208-B-B-5-1-1-3 (IRGC 121855) thereafter abbreviated as WAS 208), Badkalamkati (IRGC 45011) (abb. Badka), PTB 25 (IRGC 6386) and Fedearroz 2000 (IRGC 124388) (abb. FD 2000). The main selection criteria were (1) selected sources should show consistent and high resistance to RHB and (2) they should cover the

genetic diversity spectrum of the *indica* cluster. Then, the four resistance sources were crossed with the highly susceptible *japonica* line Bluebonnet 50 (IRGC 121874) (abb. BBT 50), using BBT 50 as the male parent. Candidate F_1 hybrids were checked for self-pollination of the mother plant with 48 SNP (single nucleotide polymorphism) markers distributed along the rice genome. F_2 (S_1) populations were derived from self-pollination of the verified F_1 hybrids. Each F_2 plant was self-pollinated to produce F_3 (S_2) families. All panicles involved in crosses or self-pollination were bagged to avoid out-crossing.

Population sizes

The following population sizes were used for both genotyping in F_2 plants and trait scoring in F_3 families: WAS 208 × BBT 50: 104 F_2, 1,040 F_3 (ALA), 6,240 F_3 (incidence); Badka × BBT 50: 105 F_2, 1,050 F_3 (ALA), 6,300 F_3 (incidence); PTB 25 × BBT 50: 108 F_2, 1,080 F_3, 6,480 (incidence); FD 2000 × BBT 50: 105 F_2, 1,050 F_3 (ALA), 6,300 F_3 (incidence). Populations sizes for ALA scoring are significantly lower than for incidence, however, as ALA is assessed using tube-based phenotyping (non-choice feeding test), precision is higher than for incidence measured in trays (see hereby).

Evaluation of RHB resistance

To evaluate the level of resistance – or susceptibility – of the F_3 plants, we looked at two complementary aspects of the disease: the *incidence* and the *severity* of the symptoms. *Incidence* is simply defined by the proportion of diseased plants in a population, while *severity* is the area or volume of plant tissue that is visibly diseased (Campbell and Neher 1994). The F_3 families, parents and check lines were evaluated under greenhouse conditions, at the CIAT (Centro Internacional de Agricultura Tropical, International Centre for Tropical Agriculture) headquarters in Palmira, Colombia.

Virulent insect colonies

T. orizicolus virulent insects were obtained from colonies maintained at CIAT. The RHBV-harboring colony contained insects that were fed on RHBV-infected plants and allowed to reproduce on BBT 50. To determine the percentage of virulent insects in this colony, 200 individual nymphs were tested for virulence on separate, caged, RHB-susceptible 8-day-old seedlings.

Incidence

Here we consider incidence as the percentage of plants showing any level of RHB symptoms. We consider the absence of symptoms as a sign of absence of infection, not as extreme tolerance. F_3 materials and controls were planted in plastic trays containing 17 furrows of 20 plants each. Each tray contained a furrow of each parent – BBT 50 also served as a susceptible control – and the controls FD 2000 (resistant) and Colombia 1 (intermediate). The trays were placed in a mesh cage 18 days after sowing, and infested by mass release of *T. orizicolus* with virulence between 50% and 65%, with an average of four nymphs per plant. The nymphs were allowed to feed for three days on the plants, after which they were eliminated with water rinsing. A randomized complete block design was used with three replicates (20 plants from

116 each F_3 family per replicate), where each block represented a cage. Incidence assessment was
117 made 35 days after infestation (DAI) by counting the plants showing disease symptoms, per
118 row.

119 ***Severity***

120 In order to test the severity of RHB, a novel methodology based on the affected leaf area (ALA)
121 by the virus was developed using an image processing approach. Briefly, images of the three
122 youngest leaves infected plants were taken using a reference scale (a ruler) and a contrasting
123 background under homogeneous light exposure. Raw images of plants with symptoms (Figure
124 1A) are processed using the ImageJ software (https://imagej.nih.gov/ij) in two steps: in the
125 first step, brightness is decreased to eliminate the areas affected by the disease (Figure 1B).
126 Then, the image is binarized, *i.e.*, converted to black and white (Figure 1C). The number of black
127 pixels is thus representing the healthy area of the leaf, according to the previously calibrated
128 reference scale. In the second step, a binarization process allows to calculate the total area
129 (Figure 1D). The affected area is then calculated as the difference between total and healthy
130 areas.

131 **Statistical treatment**

132 Since ALA was measured as the ratio of affected/total leaf area for each family in each block, a
133 weighted ALA was calculated with a generalized linear mixed model (MLGM) using the gamma
134 distribution. The incidence variable was analyzed with an MLGM using the binomial
135 distribution. The MLGM procedure is used for variables that are not necessarily normally
136 distributed. For each variable, an analysis of variance was performed to test for differences
137 between families and to evaluate the effect of the blocks. Subsequently, adjusted means were
138 calculated for each family and a mean comparison test (t-test) was performed to identify those
139 families that were more resistant or equal to the resistant parent. The descriptive analysis was
140 performed in the R program and the other analyses were performed in the SAS® statistical
141 program.

142 **SNP marker development**

143 Whole-genome sequences (WGS) of parental lines, available from previous studies (Li et al.
144 2014; Duitama et al. 2015; Cruz-Gallego et al. 2018) and the IRGSP 1.0 Nipponbare reference
145 genome were compared *in silico* using the Next Generation Sequencing Eclipse Plugin, NGSEP
146 (Duitama et al. 2014). Variant detection and annotation were performed with NGSEP and SNP
147 positions were determined relative to the reference genome. Informative biallelic SNPs were
148 filtered based on the following characteristics of their flanking sequence: minimum 30 bp
149 between the target SNP and other variant and GC content between 20 and 65%. The flanking
150 sequence of 100bp upstream and downstream the target SNP was retrieved and submitted to
151 D3 assay design web-based tool (https://d3.fluidigm.com) to develop Fluidigm® SNPtype
152 assays. SNPs were chosen based on their design quality score and their genomic position.

153 **DNA extraction**

154 Leaf tissue was collected from 15-days-old F_2 plants. Samples were frozen in liquid nitrogen and
155 stored at –80°C until processed. Plant DNA was isolated in 96-racked tubes using a modified
156 version of a method previously described (Risterucci et al. 2000) as follows: 480 µl of extraction
157 buffer was added to 150 mg of ground frozen leaf tissue. The buffer was 100 mM Tris (pH =
158 8.0), 2M NaCl, 20 mM EDTA (pH = 8.0), 2% MATAB, 0.5% sodium bisulfite, and 1% PEG 8000.
159 This mixture was homogenized and incubated in a water bath at 74°C for 30 min. Subsequently,
160 480 µl of chloroform:isoamyl-alcohol (24:1) was added and the mixture was centrifuged at
161 3000 rpm in a Sorvall® centrifuge with a GLA-3000 rotor. Supernatants were precipitated with
162 270 µl of isopropanol at –20°C for one hour and centrifuged at 3000 rpm. The pellets were
163 washed with 200 µl of 80% ethanol and allowed to dry at 40°C by inverting the tubes for one
164 hour. DNA was resuspended in Tris-EDTA [10 mM Tris-HCl, pH8 and 1 mM EDTA, pH8)
165 containing 40µg/ml of RNAse A and spectrophotometric quantification was done using a
166 multimode plate reader Synergy H1 (Biotek®). DNAs were normalized at 60ng/µl for
167 subsequent processing.

168 **Genotype scoring**

169 F_2 individuals and parents were scored for polymorphic SNPs in each population using the
170 Fluidigm® nanofluidic genotyping platform, according to the manufacturer's protocols. Briefly,
171 pre-amplification of targets regions was done in a 5µl multiplex-PCR mixture. The amplicons
172 were used as template for allele specific-PCR that was conducted in 48 × 48 integrated fluidic
173 circuit (IFCs) of the Fluidigm® genotyping platform. Fluorescent images of the IFCs were
174 acquired in an EP1 reader and analyzed with the Fluidigm® Genotyping Analysis Software.

175 **Linkage maps construction**

176 Genetic maps were calculated with MapDisto 2.0 (Lorieux 2012; Heffelfinger et al. 2017)
177 (http://mapdisto.free.fr). Goodness-of-fit to Mendelian segregation (1:2:1) was tested for each
178 marker by computing the chi-squared (χ^2) statistic with the 'Segregation χ^2s' function. As the
179 SNP markers were defined from WGS, we kept their order on the physical map or rice. For each
180 cross separately, we checked the data for singletons (e.g., the "B" in "AAAAAAA**B**AAAAAA" is a
181 singleton) with the 'Replace errors by flanking genotypes' function, with a maximum
182 probability of a singleton to occur of 0.001. A few missing data were inferred using the 'Replace
183 missing data by flanking genotypes' function, with the same threshold. Recombination fractions
184 were calculated with the standard EM algorithm (appendix 1 in Lorieux 2021) converted to
185 centimorgans (cM) with the Kosambi mapping function (Kosambi 1944).

186 **QTL mapping**

187 Data files were prepared using the 'Export map and data' function of MapDisto 2.0. Analyses of
188 distribution of the phenotypic traits as well as QTL detection were performed using the Qgene
189 4.0 program (Joehanes and Nelson 2008) (http://www.qgene.org). For QTL detection, the LOD
190 score statistics were calculated with different methods and then compared: single-marker
191 regression (SMR), simple interval mapping (SIM) and composite interval mapping (CIM). The
192 forward cofactor selection option was used in CIM. Additivity and dominance effects were

193 calculated according to Falconer (1960). Empirical thresholds to declare the presence of a QTL
194 were obtained using the resampling by permutation method, performing 10,000 (SMR) or
195 1,000 (CIM) iterations for each trait-chromosome combination. In order to correct for possible
196 erroneous phenotypic data corresponding to escape, mis-scoring, or incomplete penetrance, all
197 positive QTLs were additionally confirmed by analysis of outliers in the trait distribution using
198 the 'Plot trait vs. genotype' module of MapDisto. This module allows to calculate corrected
199 single-marker regression F-test values after detecting and removing outlier data in each marker
200 genotypic sub-class.

201 In the case of a secondary LOD score peak linked to a major peak of a QTL, in order to determine
202 if the secondary peak corresponded to a true QTL or to an artifact – or "fake QTL" – a detailed
203 analysis of the distribution of recombination fractions along the chromosome was performed,
204 flowing the method of Lorieux (2018). The analysis looked for restriction of recombination
205 fractions that could induce artificial linkage disequilibrium between the major and the
206 secondary LOD score peaks. If artificial linkage disequilibrium was detected, then the secondary
207 peak was declared an artifact. Interaction or epistasis was tested using the R/qtl 'scantwo'
208 function (Prins et al. 2010).

209 When a QTL is found in more than one cross, a joint or a meta-analysis can increase the
210 precision of the QTL location. As different markers segregated in the four populations, we
211 created a genotype matrix from the union of the four individual data sets. Markers with no data
212 in some of the populations were imputed using the R/qtl 'Argmax' function. For joint analysis,
213 only the population that showed a QTL in single-population analyses were pooled. Meta-
214 analysis was always ran using the four populations pooled. Joint- and meta-QTL analyses were
215 then ran using MapDisto 2.0 using centralized and normalized phenotypic data, that is

216 $$P_M0_V1_i = (P_i - M)/S$$

217 Where P_i is the phenotypic value of the individual i, M is the average value in the population,
218 and S is the standard deviation in the population. Centralized and normalized data always have
219 a mean equal to 0 and a variance equal to 1, making them more suitable for combination in joint
220 analyses.

221 To identify candidate genes in the fine-mapped regions, the MSU Rice genome annotation
222 database and the Overview of Functionally Characterized Genes in Rice Online database
223 (OGRO) (Yamamoto et al. 2012) were used. A 1-LOD drop-off support interval was used to
224 define the search region for each QTL.

225 RESULTS

226 Genetic maps

227 Table 1 summarizes the main statistics of the individual genetic maps obtained for each cross.
228 Overall, we obtained genetic map sizes coherent with their expected size according to ten high-
229 quality maps based on flexible and scalable genotyping by sequencing (fsGBS) (Heffelfinger et

230 al. 2014; Fragoso et al. 2017), indicating the good level of purity of the F_2 populations and the
231 high quality of the SNP data.

232 ***Segregation distortion (SD):*** SD was observed on chromosome 3 (11.1-21.0 Mbp) in the
233 crosses involving WAS 208, Badka and FD 2000. This region is commonly affected by SD in
234 *indica* × *japonica* crosses (Wang et al. 2009; Fragoso et al. 2017). The homozygotes for the
235 donor allele (AA genotype) were favored over both the homozygotes for the susceptible parent
236 allele (BB genotype) and the heterozygotes (AB). In the cross WAS 208 × BBT 50 cross, a
237 pattern of SD was also observed on chromosome 8 (3.53-16.44 Mbp), where the AB genotypes
238 were favored over the AA. In the cross PTB 25 × BBT 50, segregation distortion was observed
239 on chromosomes 2 (20.1 Mbp, 47AA:50AB:11BB), 6 (2.9-7.9 Mbp, 34AA:64AB:10BB) and 9
240 (10.8-12.2 Mbp, 42AA:54AB:12BB).

241 **RHB incidence and severity variation**

242 In the cross WAS 208 × BBT 50, the parental lines showed 24.2±11.9% and 98.1±3.2% of
243 incidence and 25.8±32.7% and 69.5±37.7% of RHB severity in WAS 208 and BBT 50,
244 respectively. The F_3 families (each representing one F_2 parental plant) exhibited a continuous
245 variation of incidence ranged between 21.4% and 100%, as well as a variation of severity
246 ranged between 9% and 68% (Figure 2). The high incidence score for the susceptible parent
247 BBT 50 – almost 100% – and the maximum values in the F_3 families indicate a perfect infection
248 efficiency. The similarity between the severity score for BBT 50 and the maximum scores in F_3
249 families indicates that the experimental design, and in particular the population size, were
250 adequate to capture the range of variation between the parental values. Interestingly, some F_3
251 families exhibited a significant lower severity than the resistant parent WAS 208.

252 In the cross Badka × BBT 50, the resistant and susceptible parents exhibited 32.8±12.4% and
253 98.9±2.9% of RHB incidence, respectively. In the same order, these parents displayed
254 35.3±17% and 81.6±27.7% of severity. The incidence in the F_3 families ranged between 13%
255 to 98%, and the severity varied between 11% and 80% (Figure 2). A positive correlation
256 between incidence and severity of the disease was detected (r=0.56, p<0.0001) using the entire
257 population, suggesting a common partial genetic control for both assessments of RHB disease.
258 Some F_3 families exhibited lower incidence and severity than the resistant parent Badka.

259 In the cross PTB 25 x BBT 50, the incidence of the resistant and susceptible parents was
260 3.4±3.5% and 98.2±3.3%, respectively, and ranged between 5% to 96% in the F_3 families.
261 Furthermore, PTB 25 and BBT 50 showed 11.8±22.1% and 68.1±25.1% of severity,
262 respectively. The same trait varied between 7% and 69% in the F_3 families.

263 In the cross FD 2000 × BBT 50, the resistant and susceptible parents showed 20.8±8.8% and
264 75.4±14.1% of RHB incidence, and 3.4±6.5% and 50.1±33.1% of severity, respectively. In the F_3
265 families; incidence ranged between 10% and 90%, and severity between 0% and 59%. The
266 incidence and severity for the susceptible parent BBT 50 were notably lower than in the crosses
267 before mentioned, indicating a low infection efficiency that can affect the power of QTL

268 detection and the QTL effects estimation. In contrast, the incidence of FD 2000 was higher than
269 in previous works (Romero et al. 2014; Cruz-Gallego et al. 2018).

270 Incidence and severity showed correlation between 0.42 and 0.66 (Table 2), indicating either a
271 common genetic control of the two traits, or that they are interdependent.

272 **The major QTL *qHBV4.1* for RHBV incidence is present in most donors**

273 A major QTL, *qHBV4.1,* on chromosome 4 for RHBV incidence was identified by SMR, SIM and
274 CIM in the crosses Badka × BBT 50 (LOD=20.97, R^2=0.63), PTB 25 × BBT 50 (LOD=21.26,
275 R^2=0.60) and FD 2000 × BBT 50 (LOD=9.11, R^2=0.34) (Table 3). It was not detected in the cross
276 WAS 208 × BBT 50, although a fake QTL analysis (Lorieux 2018) showed that it could be
277 present in WAS 208, but with a much smaller effect (data not shown). The QTL support
278 intervals are overlapping in the three populations, suggesting that the same QTL is shared by
279 the three resistance donors. Joint analysis gave a LOD=47.00 and R^2=0.42 at the position ~3.56
280 Mbp. The *qHBV4.1* position also corresponds to a previously identified locus characterized as
281 the major contributor to RHBV resistance in FD 2000 and FD 50 (Romero *et al.*, 2014),
282 confirming the wide range of action of this QTL. It explained 34-63% of the trait variance,
283 indicating that *qHBV4.1* is a major regulating factor of incidence of RHBV infection. The
284 estimation of QTL effects for *qHBV4.1* showed that this QTL is mostly of the additive type. The
285 same genomic region was also associated with RHB severity in the same crosses, however with
286 lower LOD scores and R^2 values (LOD=6.69-8.50, R^2=25-31%).

287 **A new major QTL for RHB incidence, *qHBV4.2*, identified in WAS 208**

288 The control of RHB incidence in WAS 208 was mainly explained by a different QTL on
289 chromosome 4, designated as $qHBV4.2^{WAS208}$ (LOD=15.49, R^2=0.52) by SMR, SIM and CIM. This
290 QTL was not found in the other crosses (Table 3). This newly discovered QTL was located
291 between 21.29 and 21.81 Mbp and explained 52% of the incidence variance. *qHBV4.2* is
292 therefore another major QTL for RHB incidence that seems less frequent than *qHBV4.1* in the
293 rice germplasm.

294 **Two new QTLs for RHB incidence identified in WAS 208 and PTB 25**

295 Two additional QTLs, although of lesser effect, were detected for RHB incidence (Table 4):

296 – $qHBV6.1^{PTB25}$ on chromosome 6 (0.18-1.76 Mbp, LOD_{SIM}=3.64, LOD_{CIM}=9.71, R^2=25%), mostly
297 of the additive type effect and detected in the PTB 25 × BBT 50 cross only.

298 – *qHBV11.2* on chromosome 11 in the crosses involving WAS 208 (7.43-11.9 Mbp, LOD_{CIM}=5.02,
299 R^2=21%) and PTB 25 (7.43-16.6 Mbp, LOD_{CIM}=5.9, R^2=24.2%). However, the SMR or SIM
300 methods produced LOD scores under the retained threshold (Tables 3 and 5).

301 **A new QTL, *qHBV11.1*, controls RHB severity**

302 One of the most interesting results of this work is the discovery of a new QTL associated with
303 RHB symptoms severity (measured as ALA). It was detected on chromosome 11 in the crosses
304 WAS 208 × BBT 50 (18.0-18.8 Mbp, LOD=5.32, R^2=0.21), Badka × BBT 50 (17.8-18.5 Mbp,

305 LOD=4.68, R^2=0.19) and FD 2000 × BBT 50 (17.8-18.5 Mbp, LOD=8.98, R^2=0.33). This QTL was
306 designated as *qHBV11.1* and explained 17-33% (SIM) or 19-37% (CIM) of the trait variation,
307 depending on the cross (Tables 3 and 4). The QTL effects in the populations involving WAS 208
308 and Badka indicate an additive behavior of *qHBV11.1*, while in FD 2000 × BBT 50 it seems to be
309 more dominant (Table 3). Due to the correlation between incidence and severity, *qHBV11.1* was
310 also significant for RHB incidence in the cross FD 2000 × BBT 50 although with lower statistics
311 (LOD=7.43, R^2=0.29), confirming that this QTL is controlling primarily the RHB severity.

312 **The *qHBV4.1* and *qHBV11.1* QTLs show strong interaction**

313 Testing interaction between QTL regions with the R/qtl "scantwo" function produced a strong
314 signal between the two QTLs *qHBV4.1* and *qHBV11.1* (LOD>15) (see example for the
315 FD 2000 × BBT 50 cross in Figure S1), revealing either an epistasis relationship between the
316 two regions, or a simple interdependency between the two traits. This is coherent with the
317 positive correlation observed between severity and incidence.

318 **Joint- and meta-analyses provide good candidates for QTL cloning**

319 The joint and meta-analyses approaches allow pooling populations, providing more resolution
320 for QTL mapping. This allowed us identifying candidate genes that could underlie two of the
321 QTLs we discovered.

322 ***The qHBV4.1 region contains the AGO-4 Argonaute gene***

323 In the *qHBV4.1* region, 33 genes encoding proteins with unknown function, five hypothetical
324 genes, four genes encoding different types of kinases, two genes of the MEG (maternally
325 expressed gene) family involved in the translocation of nutrients to the seed, and one *Argonaute*
326 gene were identified. The most interesting gene within the *qHBV4.1* region is probably the
327 *Argonaute* AGO-4 (MSU: LOC_Os04g06770, Chr4:3,562,793-3,555,220bp). AGO proteins are
328 effector proteins of RNA silencing pathways, which regulate gene expression in a sequence-
329 specific manner (Duan et al. 2015). RNA silencing is also the main antiviral defense mechanism
330 possessed by plants. It can be post-transcriptional (PTGS) or transcriptional (TGS) (Carbonell
331 and Carrington 2015).

332 ***The qHBV11.1 region contains a gene for durable resistance to Rice stripe virus***

333 The genomic region of *qHBV11.1* contains many nucleotide binding site-leucine-rich repeat
334 (NBS-LRR) genes, which are broadly known to confer resistance to multiple diseases (McHale
335 et al. 2006). Other types of genes are also found in the interval, and remarkably the *STV11* gene
336 (MSU:LOC_Os11g30910, Chr11:17,984,964-17,986,719b; RAP-DB: Os11g0505300), which
337 confers durable resistance to *Rice stripe virus* (RSV), one of the most devastative viral diseases
338 of rice in Asia (Wang et al. 2014). Interestingly, this virus belongs to the same genus as RHBV
339 and it is also transmitted by planthoppers (*Laodelphax striatellus* Fallen). It should be also
340 noted that, very close to the *qHBV11.1* support interval, there are several paralog histidine
341 kinase/Hsp90-like ATPase genes (MSU:LOC_Os11g31480, MSU:LOC_Os11g31500) that also
342 confer resistance to RSV (Hayano-Saito and Hayashi, 2020).

343 ## DISCUSSION

344 Successful genotype-phenotype association studies require precise methodologies to assess
345 phenotypic variables. The latest technological advances in image acquisition have allowed the
346 development of computer tools for picture processing and analysis that provide accurate data.
347 Image processing for quantifying damage caused by plant diseases is particularly useful as it
348 advantageously replaces traditional scoring scales, which are too dependent on the observer.
349 The RHB disease has been commonly evaluated using the percentage of diseased plants at a
350 given time – that is, the incidence. However, the main drawback of this approach is that it
351 classifies all the plants with symptoms in the same class, without considering the level of
352 damage – the severity. Understanding the genetic mechanisms of resistance to the *hoja blanca*
353 disease therefore requires proper assessment of not only its incidence but also its severity. In
354 this sense, we proposed for the first time the use of digital images to evaluate the severity of
355 damage caused by RHB by estimating the affected leaf area. We showed that severity varies
356 continuously among F_3 families, a characteristic behavior of quantitative features. Severity and
357 incidence evaluation allowed us to identify different QTLs, allowing us to better explain the
358 complexity of the genetics of resistance to RHB. Keeping in mind that the parents of the four
359 populations were among the most resistant of a larger panel (Cruz et al. 2018), and although
360 significant differences between incidence and severity were found among the parents of the
361 four populations, complete resistance was not observed, confirming a previous observation by
362 (Morales and Jennings 2010) about the lack of immunity to RHB in the *O. sativa* genepool.

363 ### RHB resistance is regulated by multiple QTLs

364 The QTLs found in this study show that resistance to RHB, assessed both by affected leaf area
365 and incidence, is controlled by multiple genes. The co-location of LOD score peaks for incidence
366 in the same region of chromosome 4 in three different crosses suggests that it is likely the same
367 QTL, namely *qHBV4.1*. Although *qHBV4.1* was associated to both phenotypic variables, its
368 association was greater with incidence where it explained up to 63.6% of the phenotypic
369 variance. These results suggest that the main action of *qHBV4.1* could take place in the first
370 phase of the interaction of the virus with the plant, preventing the virus to enter and propagate
371 in the plant, which is reflected in a smaller number of plants with symptoms. In addition, the
372 little variation in nucleotides – less than 1% – in the region of *qHBV4.1*, between Badka and PTB
373 25 indicates a probable common local ancestry (Figure S2). The differences in the LOD score
374 (5.83 *vs.* 8.26) and in R^2 values (21.4% *vs.* 36.6%) for ALA, in these two populations, could be
375 explained by the difference in the percentage of virulence of the vector colonies, which were
376 ~69% and ~46% for Badka × BBT 50 and PTB 25 × BBT 50, respectively. Also, the phenotypic
377 evaluation trials were performed at different times of the year for the different populations.
378 This involves the use of different vector colonies, and implies variations in microenvironmental
379 factors such as day and night temperature, radiation or humidity that might affect the behavior
380 of the insect and its ability to transmit the virus.

381 A major QTL had been previously identified in the region of *qHBV4.1* in the varieties FD 2000
382 and Fedearroz 50 by incidence assessment (Romero et al. 2014), which suggests a
383 predominant, common mechanism of resistance to RHBV in the different clades of *Oryza sativa*.

384 The genetic variation between the parents observed in this region shows that they carry
385 different alleles, of *japonica* and *indica* origins. This has implications for crop improvement,
386 allowing breeders to broaden the genetic base of resistance in elite germplasm. A QTL for
387 resistance to *Rice stripe virus* (RSV) has been identified between 4.4 and 6.9 Mpb in the N22 Aus
388 variety (Wang et al. 2013). The *qHBV4.1* QTL region might thus contain at least two tenuivirus
389 resistance genes. As mentioned above, in the region of *qHBV4.1* there is a putative gene that
390 encodes for the AGO-4 Argonaute protein (LOC_Os04g06770). AGO proteins, in addition to be
391 regulatory factors of endogenous gene expression, also play a critical role in the defense against
392 viruses through interference with small RNA of viral origin which bind to AGOs and serve as a
393 guide for it to cut new viral RNA particles (Mallory and Vaucheret 2011)(Silva-Martins et al.,
394 2020). This system is a common defense mechanism against pathogens, and AGO-4 might well
395 be associated with resistance to RHBV. We are currently carrying CRISPR-Cas9 knock-out
396 experiments in AGO-4 in order to test this hypothesis.

397 A distinct QTL for RHB incidence on chromosome 4, $qHBV4.2^{WAS}$, was identified in the WAS 208
398 × BBT 50 cross. This QTL was also a major one, explaining ~50% of the phenotypic variation.
399 The region contains genes associated with cold tolerance (SAPK7) (Basu and Roychoudhury,
400 2014) and photo-oxidative stress (OsAPX7)(Caverzan et al. 2014), and a more interesting gene
401 encoding for a zinc finger-type C3H protein (LOC_Os04g35800). This class of proteins has been
402 found to be associated with resistance viruses in animals through degradation of viral RNA (Gao
403 et al. 2002; Mao et al. 2013). Although there are no reports so far of its involvement in resistance
404 to viruses in plants, it has been found to be associated with response to other types of pathogens
405 (and abiotic stress) (Deng et al. 2012; Jan et al. 2013), which makes it an interesting candidate
406 for RHB resistance. Another candidate is a gene encoding for a MAPKKK kinase, which belongs
407 to a signaling cascade that plays a major role in disease resistance in eukaryotes. This gene is
408 also an interesting candidate because the activation of MAPK proteins occurs in the earliest
409 stages after the interaction of the pathogen with the plant (Meng and Zhang 2013), which
410 coincides well with the identification of this QTL through the assessment of incidence, but not
411 severity, indicating that it – like *qHBV4.1* – could exert its action in the early stages of the
412 interaction of RHBV with the plant.

413 In contrast with the two QTLs identified on chromosome 4 that are predominantly associated
414 with incidence, *qHBV11.1* has a greater effect on disease severity. This could suggest a later
415 action, in a second stage of the interaction of the plant with the virus or the insect vector, after
416 the virus manages to enter the cells and to propagate. In this scenario *qHBV11.1* would have a
417 key role in diminishing the damage caused by the virus. This is thus more a "tolerance QTL".

418 It has been proposed that the resistance to RHB comes mostly from *japonica* germplasm
419 (Morales and Jennings 2010). If this was the case, and to explain that the resistance sources
420 studied here are of *indica* type, one would need to assume that the region of *qHBV11.1* was
421 inherited from *japonica* through introgression into *indica*. This is not supported by the
422 clustering analysis, which showed that the WAS 208 and Badka parents, for this region, are
423 grouped together with *indica* type accessions such as IR8. Therefore, this QTL does not
424 originate from the *japonica* cluster.

425 A search for candidate genes for *qHBV11.1* evidenced a co-localization with a QTL for resistance
426 to *Rice stripe virus* (RSV) found in different genetic backgrounds: in the *indica* varieties Teqing
427 (*qSTV11*^{TQ}) (Wu et al. 2011) and Shingwang (*qSTV11*^{SG}) (Kwon et al. 2012), the Aus varieties
428 Kasalath (*qSTV11*^{KAS}) (Zhang et al. 2011), N22 (*qSTV11.1*) (Wang et al. 2013) and Dular (Wu et
429 al. 2011), and in the *japonica* Kanto 72 (Maeda et al. 2006). Whether those QTLs correspond to
430 the same locus or to different, linked loci still needs to be clarified. The hypothesis of a common
431 defense mechanism acting against RHBV and RSV seems plausible, as both are RNA viruses of
432 the same genus, although it has been found that RHBV is more related to other tenuiviruses like
433 *Echinochloa hoja blanca virus* (EHBV) and *Urochloa hoja blanca virus* (UHBV) than to RSV
434 (Fauquet et al., 2005), besides not being serologically related (Morales and Jennings 2010).
435 Further studies of fine mapping and cloning of *qSTV11*^{KAS} showed that the gene responsible for
436 resistance to RSV encodes a sulfotransferase that catalyzes the conversion of salicylic acid to its
437 sulfated form. Although it is not clear how this process confers resistance against the virus, it
438 was found that the susceptible allele of this gene is not capable of inducing this conversion
439 (Wang et al. 2014). Salicylic acid has been found to be essential in the initiating signal to activate
440 systemic resistance against *Tobacco mosaic virus* (Zhu et al. 2014), and also plays a central role
441 in the hypersensitive response against *Potato virus Y* (PVY) (Baebler et al. 2014). It has even
442 been reported as a direct inhibitor of the replication of *Tomato bush dwarf virus* (TBSV) (Tian
443 et al. 2015). It is thus plausible that the STV11 gene is involved in resistance to RHBV. We are
444 currently analyzing knock-out lines obtained by CRISPR-Cas9 in STV11 to verify this
445 hypothesis.

446 A second QTL on chromosome 11, *qHBV11.2*, was identified for disease incidence in the two
447 crosses involving WAS 208 and PTB 25. Based on the variation found in the clustering analysis
448 – and assuming the QTL is the same in the two crosses – it is possible that WAS 208 and PTB 25
449 have different allelic variants of *qHBV11.2* with a different effect on disease resistance, which
450 could explain that in PTB 25 it was also associated with severity. The region could also
451 correspond to two distinct, linked QTLs in the two populations, since their support intervals
452 are quite large (> 3 Mpb). To answer this question, a fine mapping study of the region using
453 larger F_2 populations would be needed.

454 An additional QTL for RHB incidence, *qHBV6*, was identified In the PTB 25 × BBT 50 cross on
455 chromosome 6. In a previous GWAS experiment, the same region was identified in the
456 genotypes PTB 25 and Pokkali (Cruz-Gallego et al. 2018), also supported by a biparental QTL
457 analysis in the $F_{2:3}$ cross FD 50 × WC 366 (our unpublished data). A search for genes in this
458 region found the OsBBI1 gene that participates in various biological processes, among which is
459 the innate immune response. OsBBI1's expression is induced by the fungus *Magnaporthe oryzae*
460 and chemical inducers such as salicylic acid (Li et al. 2011), which, as discussed above, is also
461 associated with systemic resistance to viruses. OsBBI1 is thus a good candidate gene for *qHBV6*.

462 **Resistance QTLs in the susceptible parent**

463 In the PTB 25 × BBT 50 population, two QTLs for RHB incidence were identified on
464 chromosomes 9 and 10, of which the allele that reduces the affected leaf area is brought by
465 BBT 50. One explanation could be that PTB 25 carries susceptibility alleles at these QTLs.

466 However, this is unlikely since inactivation of a susceptibility gene by the resistant allele results
467 in increased resistance. Genetically, the resistant allele of a susceptibility gene is therefore
468 generally recessive. This is not what we observed, since the mean ALA of the heterozygous class
469 AB is more similar to the susceptible class AA (PTB 25) than to the BB class (BBT 50). Therefore,
470 the most likely explanation is that the three other resistant parents (FD 2000, WC 366 and
471 Badka) and the susceptible parent (BBT 50) carry resistant alleles at these QTL, while the
472 PTB 25 parent does not.

473 **Integration of QTLs into a RHB disease resistance model**

474 Based on the QTLs associated with RHB resistance, we propose a simplistic model that draws
475 the possible processes involving these loci. The QTLs *qHBV4.1* and *qHBV4.2* were found to be
476 most associated with disease incidence, so it is likely that they are involved in the first phase of
477 virus-plant interaction. These QTL may be inhibiting the translation of viral RNA, either by
478 direct action on them or through other genes for degradation. The effect of these QTLs on the
479 incidence, indicates that this mechanism might be the most important for the resistance of the
480 plant to the virus. In the scenario where the favorable alleles of *qHBV4.1* or *qHBV4.2* are not
481 present or that the virus manages to overcome this barrier and to propagate in the plant, an
482 additional mechanism involving *qHBV11.1* would be hampering the multiplication of the virus.
483 This could occur by direct action, inhibiting the synthesis of new viral particles, or more likely,
484 serving as a trigger signal for the activation of defense genes of the systemic resistance system.
485 Although this mechanism would not provide complete resistance to the virus, it would
486 considerably reduce the damage – meaning less leaf area affected. The *qHBV6* QTL is also
487 related to the severity of the disease, so it is possible that it also participates in this same
488 mechanism. Although the role of the susceptibility QTLs *qHBV9* and *qHBV10* is more difficult to
489 interpret, it is likely that they are negatively regulating the activation of defense genes against
490 the virus.

491 ## CONCLUSION

492 Altogether these results show that resistance to RHB disease is controlled by multiple
493 quantitative genetic factors of different origins, with varying effects and action mode. The
494 identification of strong candidate genes underlying the detected QTLs supports the idea that
495 resistance is mediated by different defense mechanisms such as viral gene silencing and the
496 salicylic acid pathway. This hypothesis has proven true for other study models such as in the
497 *Citrus tristeza virus* (CTV) where it has been found that silencing of key genes in these defense
498 pathways increases the spread of the virus and its accumulation in the plant (Gómez-Muñoz et
499 al. 2017). A considerable amount of work is still needed to understand the fine mechanisms
500 behind defense against RHB. In particular, it will be necessary to clarify whether some of the
501 QTLs found in this study are actually acting against the insect vector, and not the virus itself.
502 Indeed, insect resistance by antixenosis and/or antibiosis are known to be present in rice (see
503 Figure 1 in Fujita et al. 2013), and overlap with some of our QTLs.

504 ## LIST OF ABBREVIATIONS

505 ALA: affected leaf area
506 Badka: Badkalamkati variety, accession number IRGC 45011
507 BBT 50: Bluebonnet 50 variety
508 CIAT: Centro Internacional de Agricultura Tropical (now Alliance Bioversity-CIAT)
509 CIM: composite interval mapping
510 cM: centimorgan
511 CTV: *Citrus tristeza virus*
512 DAI: days after infestation
513 DNA: deoxyribonucleic acid
514 EDTA: ethylenediaminetetraacetic acid
515 EHBV: *Echinochloa hoja blanca virus*
516 FD 50: Fedearroz 50 variety, accession number IRGC 1799-1-1
517 FD 2000: Fedearroz 2000 variety, accession number IRGC 124388
518 FLAR: Fondo Latinoamericano para Arroz de Riego
519 fsGBS: flexible and scalable genotyping by sequencing
520 IFC: integrated fluidic circuit
521 LOD: logarithm in base 10 of likelihoods odds
522 MATAB: mixed alkyltri-methylammonium bromide
523 MEG: maternally expressed gene
524 MLGM: generalized linear mixed model
525 NBS-LRR: nucleotide binding site-leucine-rich repeat
526 PCR: polymerase chain reaction
527 PTGS: post-transcriptional gene silencing
528 PTB 25: PTB 25 variety, accession number IRGC 6386
529 PVY: *Potato virus Y*
530 QTL: quantitative trait locus
531 RHB: rice *hoja blanca* disease
532 RHBV: *Rice hoja blanca virus*
533 RNA: ribonucleic acid
534 RSV: *Rice stripe virus*
535 SD: segregation distortion
536 SIM: simple interval mapping
537 SMR: single-marker regression
538 SNP: single nucleotide polymorphism
539 TBSV: *Tomato bush dwarf virus*
540 TGS: transcriptional gene silencing
541 UHBV: *Urochloa hoja blanca virus*
542 WAS 208: WAS 208-B-B-5-1-1-3 variety, accession number IRGC 121855
543 WGS: whole-genome sequencing

DECLARATIONS

Ethical Approval and Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of supporting data

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

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

AS wrote the draft manuscript and performed the SNP design and QTL analysis, MEM set up the disease severity experiments, CQ supervised the SNP genotyping, JQ did the statistical treatment of phenotype data, JT led the Agrobiotechnology laboratory, EG led the FLAR, MC supervised the phenotyping experiments, ML designed the QTL experiments and edited the manuscript, MC and ML supervised AS work.

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TABLES

Table 1 Summary of genetic maps characteristics in the four populations. Map sizes are expressed in centimorgans (cM) calculated with the Kosambi mapping function

Cross	*Population size*	*Number SNPs*	*Number SSRs*	*Total markers*	*Map size (cM)*
WAS 208 × BBT 50	104	207	2	209	1,296
Badka × BBT 50	105	226	1	227	1,570
PTB 25 × BBT 50	108	184	1	185	1,617
FD 2000 × BBT 50	105	217	0	217	1,630

Table 2 Trait correlation values in each population, and in the four populations combined. The '_M0' and '_V1' suffixes stand for normalized and centralized data, respectively

A. WAS 208 × BBT50

	SEVERITY
INCIDENCE	0.42

B. Badka × BBT50

	SEVERITY
INCIDENCE	0.625

C. PTB 25 × BBT50

	SEVERITY
INCIDENCE	0.458

D. FD 2000 × BBT50

	SEVERITY
INCIDENCE	0.655

E. Four crosses combined

	INCIDENCE	*INCIDENCE_ M0_V1*	*SEVERITY*	*SEVERITY _M0_V1*
INCIDENCE	1	0.891	0.613	0.481
INCIDENCE_M0_V1	0.891	1	0.449	0.538
SEVERITY (ALA)	0.613	0.449	1	0.829
SEVERITY_M0_V1	0.481	0.538	0.829	1

696 **Table 3** Summary statistics for QTLs detected with Simple Interval Mapping (SIM) analysis in the four crosses, and joint analysis, with Qgene version
697 4.40. Positions in Mbp on Nipponbare genome (MSU v7). Empirical threshold $\alpha_{0.01}$ calculated with 10,000 permutations. R^2: part of the trait variance
698 explained by the QTL. A: Additivity effect. D: Dominance effect. QTLs that were detected with Composite Interval Mapping (CIM) but not with SIM
699 appear in pale gray

Trait	*QTL name*	*WAS 208*	*Bad-ka*	*PTB 25*	*FD 2000*	*Joint (4 pop.)*	*Chro mo-some*	*Peak (Mbp)*	R^2	*Additivity (A)*	*Dominance (D)*	*|D /A |*	*LOD score*	*Permut. threshold α = 0.01*
Incidence	*qHBV4.1*		✓				4	3.56-6.47	0.63	-24.14	-1.17	0.05	20.97	4.43
				✓				0.22-4.19	0.60	-24.47	-5.24	0.21	21.26	4.55
					✓			3.56-3.57	0.34	-14.69	-0.46	0.03	9.11	4.40
						✓		3.56-3.57	0.42	-17.51	-2.51	0.14	47.00	4.50
Incidence	*qHBV4.2* ^{WAS208}	✓					4	21.8	0.52	-18.44	4.90	0.27	15.49	4.47
Incidence	*qHBV6.1* ^{PTB25}			✓			6	1.87-3.08	0.15	-16.79	-3.69	0.22	3.64	4.55
Incidence	*qHBV11.1*				✓		11	18.78-18.87	0.29	-14.25	-3.50	0.25	7.43	4.40
						✓		18.5	0.11	-9.33	-4.11	0.44	10.40	4.37
Incidence	*qHBV11.2*	✓					11	7.43-17.23	0.09	-8.51	1.98	0.23	2.08	4.47
				✓				7.43-8.60	0.09	-9.58	-6.68	0.70	2.16	4.55
ALA	*qHBV4.1*		✓				4	3.56-6.47	0.31	-13.90	0.43	0.03	8.31	4.49
				✓				0.22-4.19	0.30	-10.86	-5.41	0.50	8.50	4.29
					✓			3.71-4.19	0.25	-9.16	-3.73	0.41	6.69	4.40
						✓		3.71-4.19	0.23	-9.46	-3.95	0.42	23.40	4.30
ALA	*qHBV11.1*	✓					11	18.06	0.21	-8.82	-3.17	0.36	5.32	4.56
			✓					17.62-17.83	0.19	-10.70	-2.33	0.22	4.68	4.49
					✓			18.06	0.33	-9.36	-7.18	0.77	8.98	4.69
						✓		18.06	0.17	-8.65	-3.62	0.42	17.29	4.37

700

701 **Table 4** Summary statistics for QTLs detected with Composite Interval Mapping (CIM) analysis in the four crosses, and joint analysis, with Qgene
702 version 4.40. Cofactor selection method: Forward selection, automatic, QTL cofactors instead of markers = no. Positions in Mbp on Nipponbare
703 genome (MSU v7). Empirical threshold $\alpha_{0.01}$ calculated with 10,000 permutations. R^2: part of the trait variance explained by the QTL. A: Additivity
704 effect. D: Dominance effect.

Trait	*QTL name*	*WAS 208*	*Bad-ka*	*PTB 25*	*FD 2000*	*Joint (4 pop.)*	*Chro mo-some*	*Peak (Mbp)*	R^2	*Additivity (A)*	*Dominance (D)*	*|D /A |*	*LOD score*	*Permut. threshold α = 0.01*
Incidence	*qHBV4.1*		✓				4	3.56-4.46	0.6	-22.37	-0.67	0.03	23.46	5.57
				✓				3.5-4.19	0.73	-25.38	-6.84	0.27	30.45	5.10
					✓			3.5-3.7	0.501	-14.95	1.34	0.09	17.27	4.95
						✓		3.5-3.7	0.42	-16.78	-1.09	0.06	47.82	4.85
Incidence	*qHBV4.2* ^{WAS208}	✓					4	21.29-21.81	0.57	-18.75	3.15	0.17	18.31	5.47
Incidence	*qHBV6.1* ^{PTB25}			✓			6	0.18-1.76	0.25	-10.52	0.16	0.02	9.71	5.11
Incidence	*qHBV11.1*				✓		11	17.8-18.52	0.44	-13.83	-5.4	0.39	14.08	5.29
						✓		17.8-18.52	0.08	-7.154	-3.314	0.46	7.26	4.32
Incidence	*qHBV11.2*	✓					11	7.43-17.23	0.21	-8.16	2.66	0.33	5.02	4.11
				✓				7.43-16.6	0.242	-10.21	2.45	0.24	5.90	4.97
ALA	*qHBV4.1*		✓				4	3.56-4.46	0.26	-10.65	-2.86	0.27	8.98	5.26
				✓				3.56-4.19	0.26	-9.53	-1.07	0.11	9.81	5.43
					✓			3.56-4.46	0.29	-7.87	-2.48	0.32	9.93	5.32
						✓		3.56-4.46	0.222	-8.958	-2.899	0.32	23.00	5.33
ALA	*qHBV11.1*	✓					11	18.0-18.8	0.19	-8.71	-2.94	0.34	5.32	4.84
			✓					17.8-18.5	0.23	-10.27	-3.19	0.31	6.09	4.95
					✓			17.8-18.5	0.37	-8.74	-7.01	0.80	11.52	5.47
						✓		18.06	0.19	-8.03	-2.92	0.36	18.84	4.62

705

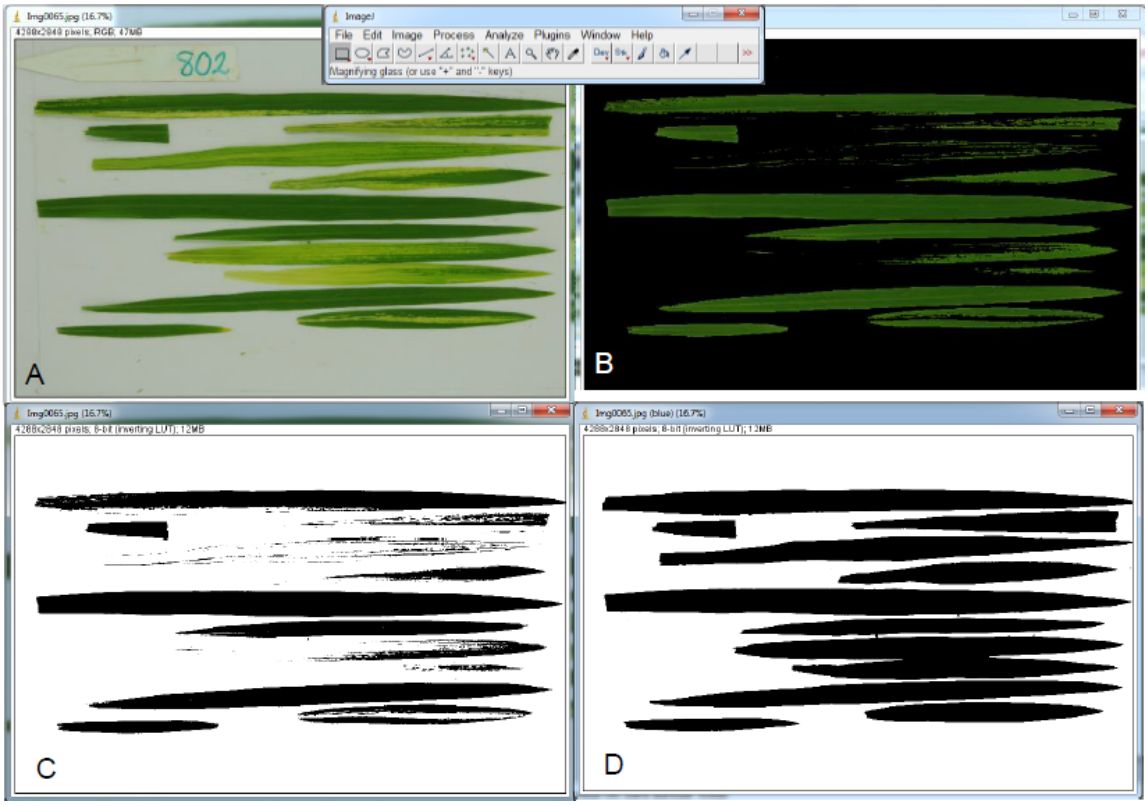
706 **Table 5** Summary statistics for QTLs detected with Simple Marker Regression (SMR) analysis in the four crosses, joint- and meta-analyses, with
707 MapDisto version 2.0. Positions in Mbp on Nipponbare genome (MSU v7). Empirical threshold $\alpha_{0.01}$ calculated with Qgene 4.40, with 10,000
708 permutations*. R^2: part of the trait variance explained by the QTL. A: Additivity effect. D: Dominance effect. QTLs that were detected with Composite
709 Interval Mapping (CIM) but not with SMR appear in pale gray

Trait	*QTL name*	*WAS 208*	*Bad-ka*	*PTB 25*	*FD 2000*	*Joint (4 pop.)*	*Meta (4 pop.)*	*Chro mo-some*	*Peak (Mbp)*	R^2	*Additivity (A)*	*Dominance (D)*	*|D /A |*	*F-test*	*-Log $_{10}$ (p-value of test)*	*Permut. threshold α = 0.01*
Incidence	*qHBV4.1*		✓					4	3.56-6.47	0.61	22.43	-1.65	0.07	74.18	19.33	4.35
				✓					0.22-4.19	0.60	24.47	-5.24	0.21	77.85	20.67	4.18
					✓				3.56-3.57	0.34	14.69	-0.46	0.03	25.29	8.84	4.20
						✓			3.56-3.57	0.42	17.51	-2.51	0.14	142.30	46.65	4.29
							✓		3.56-3.57	-	-	-	-	-	47.00	*
Incidence	*qHBV4.2* ^{WAS208}	✓						4	21.8	0.50	17.67	4.40	0.25	48.04	14.42	4.28
Incidence	*qHBV6.1* ^{PTB25}			✓				6	2.97-3.08	0.13	15.04	-6.76	0.45	7.82	3.16	4.31
Incidence	*qHBV11.1*				✓			11	18.78-18.87	0.29	14.09	-2.92	0.21	19.45	7.10	4.22
						✓			18.52-18.87	0.11	9.33	-4.11	0.44	25.24	10.32	4.29
							✓		18.52-18.87	-	- `	-	-	-	8.91	*
Incidence	*qHBV11.2*	✓						11	7.43-17.23	0.10	7.55	2.05	0.27	5.12	2.11	4.29
				✓					7.43-8.60	0.09	9.15	-6.09	0.67	5.03	2.08	4.25
ALA	*qHBV4.1*		✓					4	3.56-6.47	0.29	12.85	-1.09	0.09	21.12	7.67	4.23
				✓					0.22-4.19	0.30	10.54	-2.48	0.24	22.09	8.01	4.18
					✓				3.71-4.19	0.25	8.86	-3.19	0.36	17.23	6.45	4.38
						✓			3.56-3.71	0.23	9.46	-3.95	0.42	60.94	23.23	4.22
							✓		3.70-4.19	-	-	-	-	-	20.75	*
ALA	*qHBV11.1*	✓						11	18.06	0.21	8.82	-3.17	0.36	13.43	5.17	4.31
			✓						17.62-17.83	0.19	10.70	-2.33	0.22	11.62	4.55	4.16
					✓				18.06	0.32	9.22	-6.66	0.72	23.72	8.46	4.38
						✓			18.06	0.17	8.65	-3.62	0.42	43.49	17.16	4.30
							✓		18.06	-	-	-	-	-	15.40	*

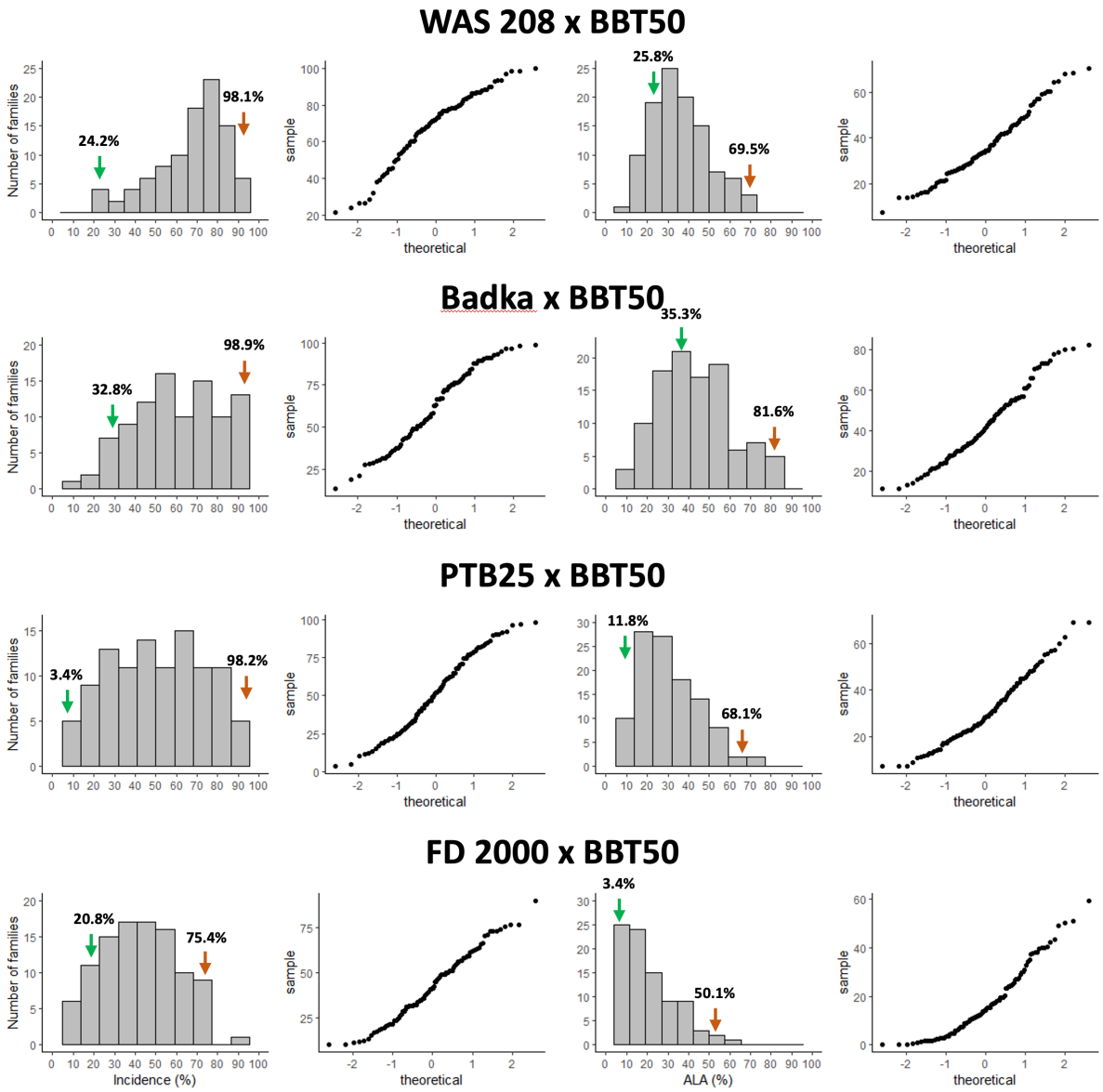
710
711 *Meta-analysis not available in Qgene

FIGURES

Figure 1 RHB disease severity (% of affected leaf area, ALA) measurement workflow. A: Image acquisition of rice leaves affected by RHB. B: Image segmentation to separate healthy from affected leaf areas. C: Healthy leaf area binarization. D: Total leaf area binarization



718 **Figure 2** Distribution of the means of RHB incidence (% of plants with symptoms) and severity (% of
719 affected leaf area, ALA) in F_3 families, in four *indica* × *japonica* rice mapping populations. Associated
720 quantile-quantile plot to the right of every distribution. Means in F_3 families were calculated from 60
721 plants (incidence) or 10 plants (ALA). The green and orange arrows represent the average of the
722 resistant and susceptible parents, respectively.



723

724

Figure 3 Whole-genome QTL plots (SIM) in the four crosses analyzed independently (A-D), and jointly (E). Red:incidence; purple: severity (ALA)

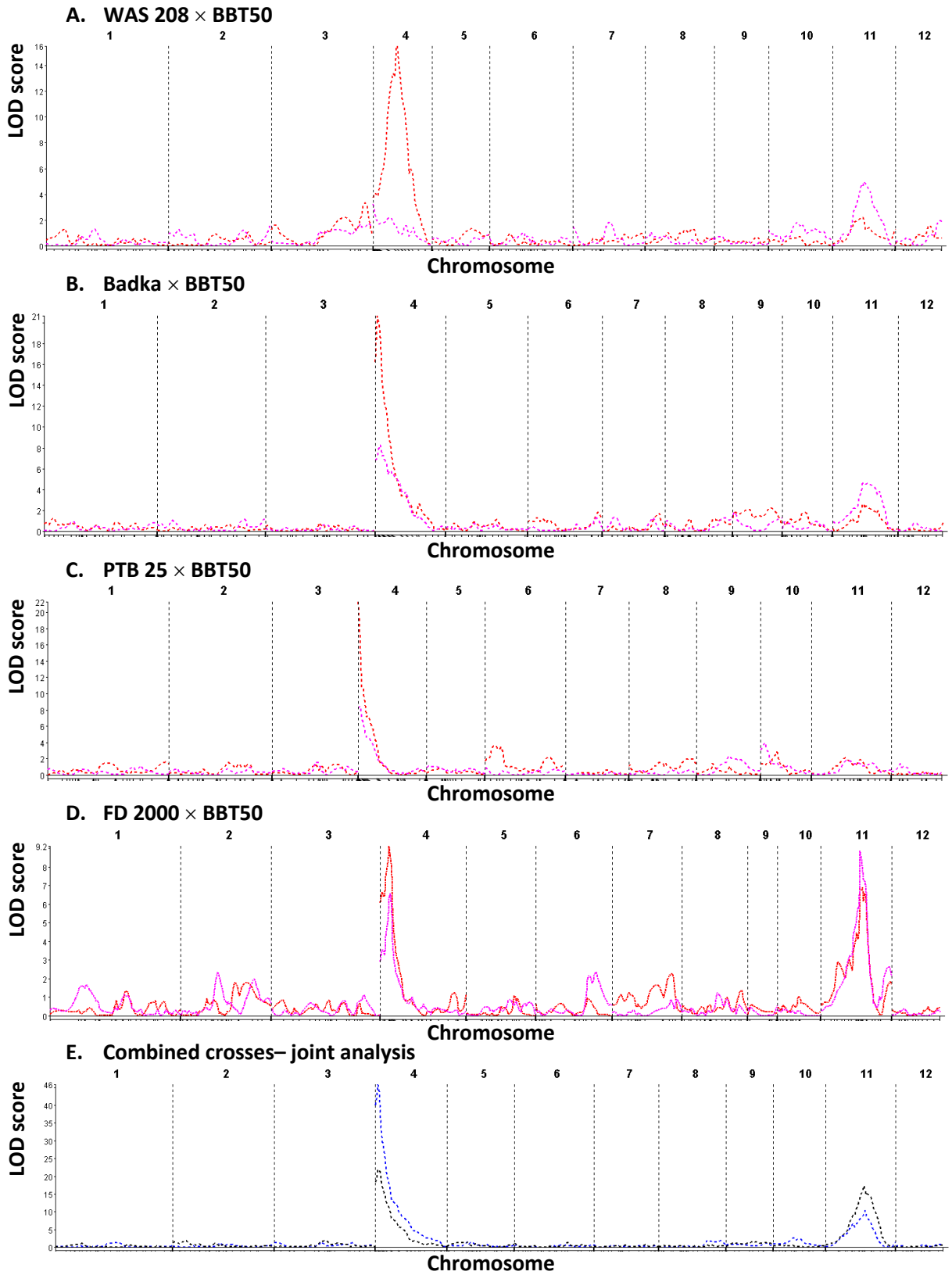
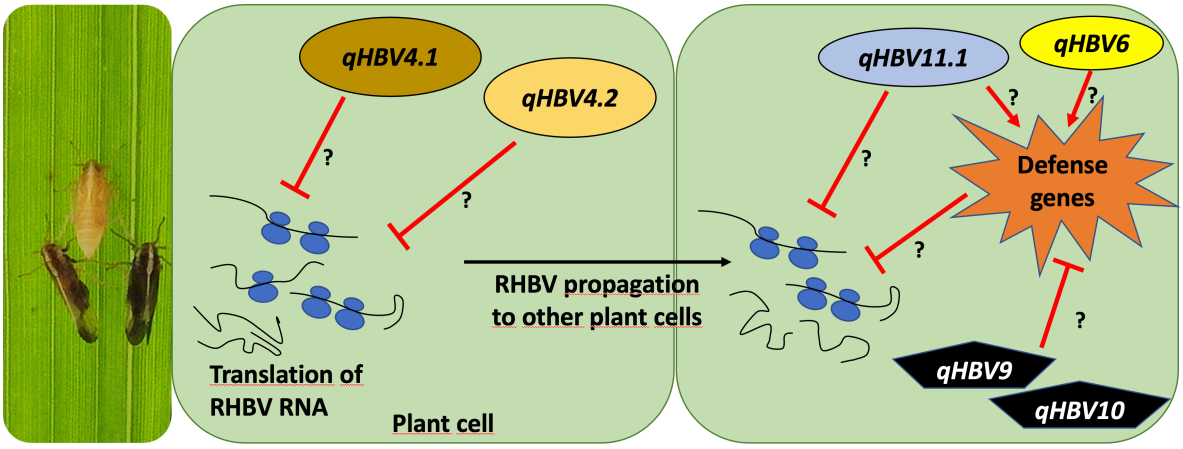


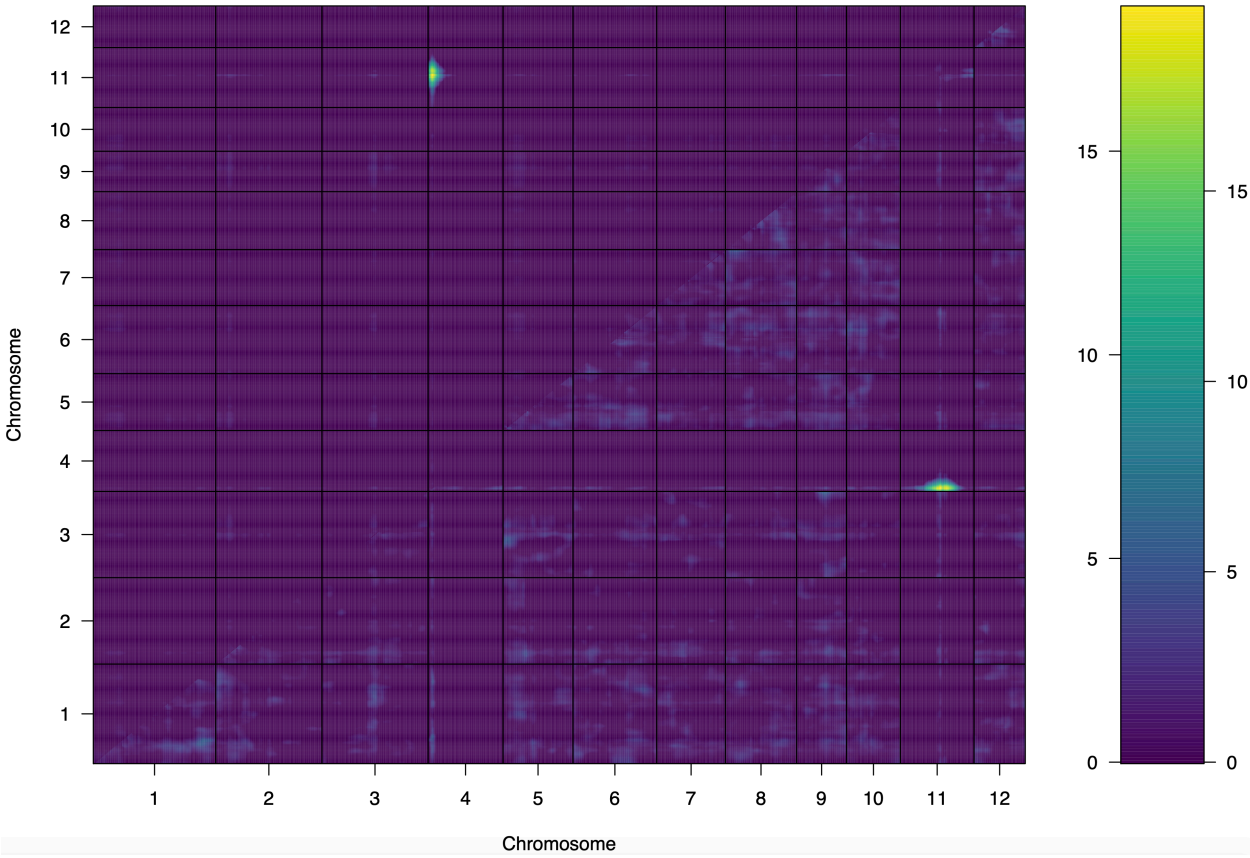
Figure 4 Hypothetical model of plant resistance mechanisms to RHBV



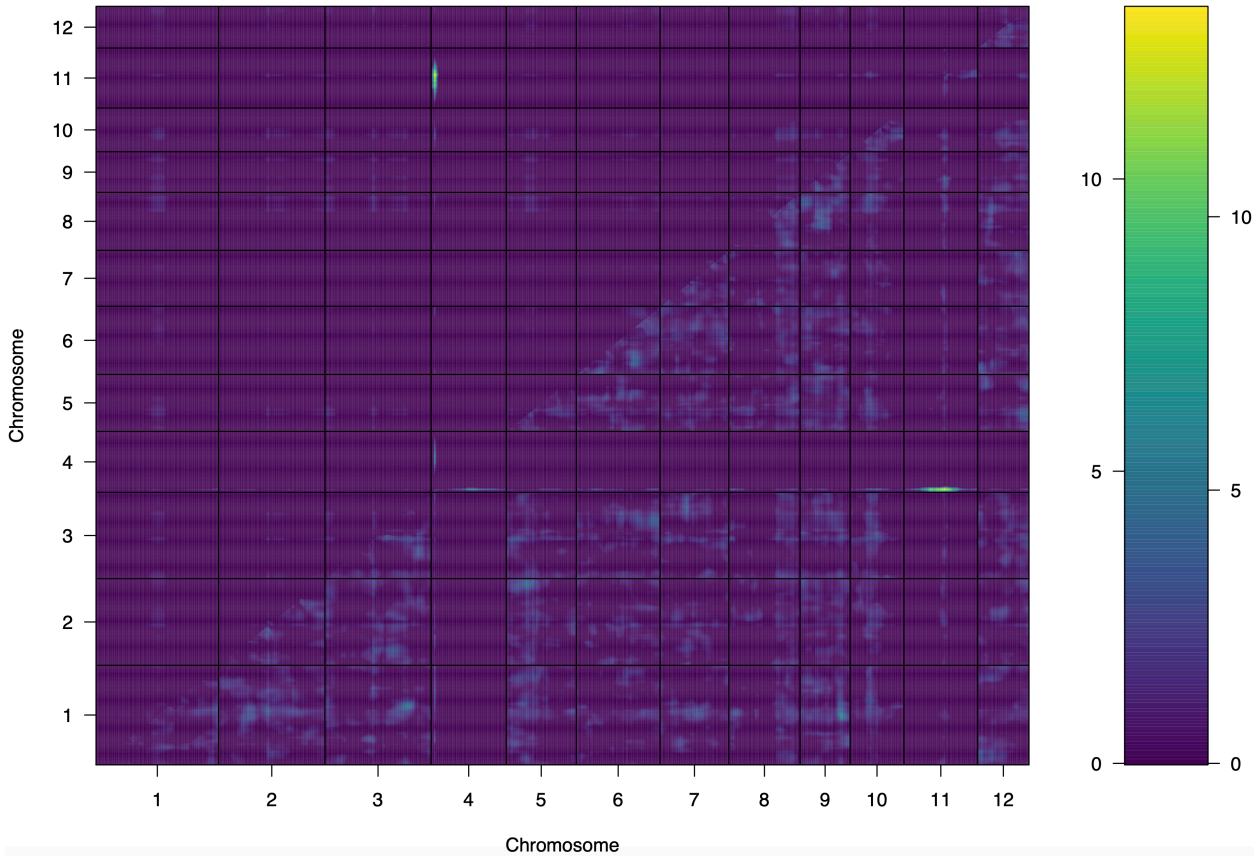
SUPPLEMENTAL FIGURES

Figure S1 2-D plot for interaction analysis between genomic regions calculated in the FD2000 × BBT50 cross. A: RHBV incidence. B: RHBV severity

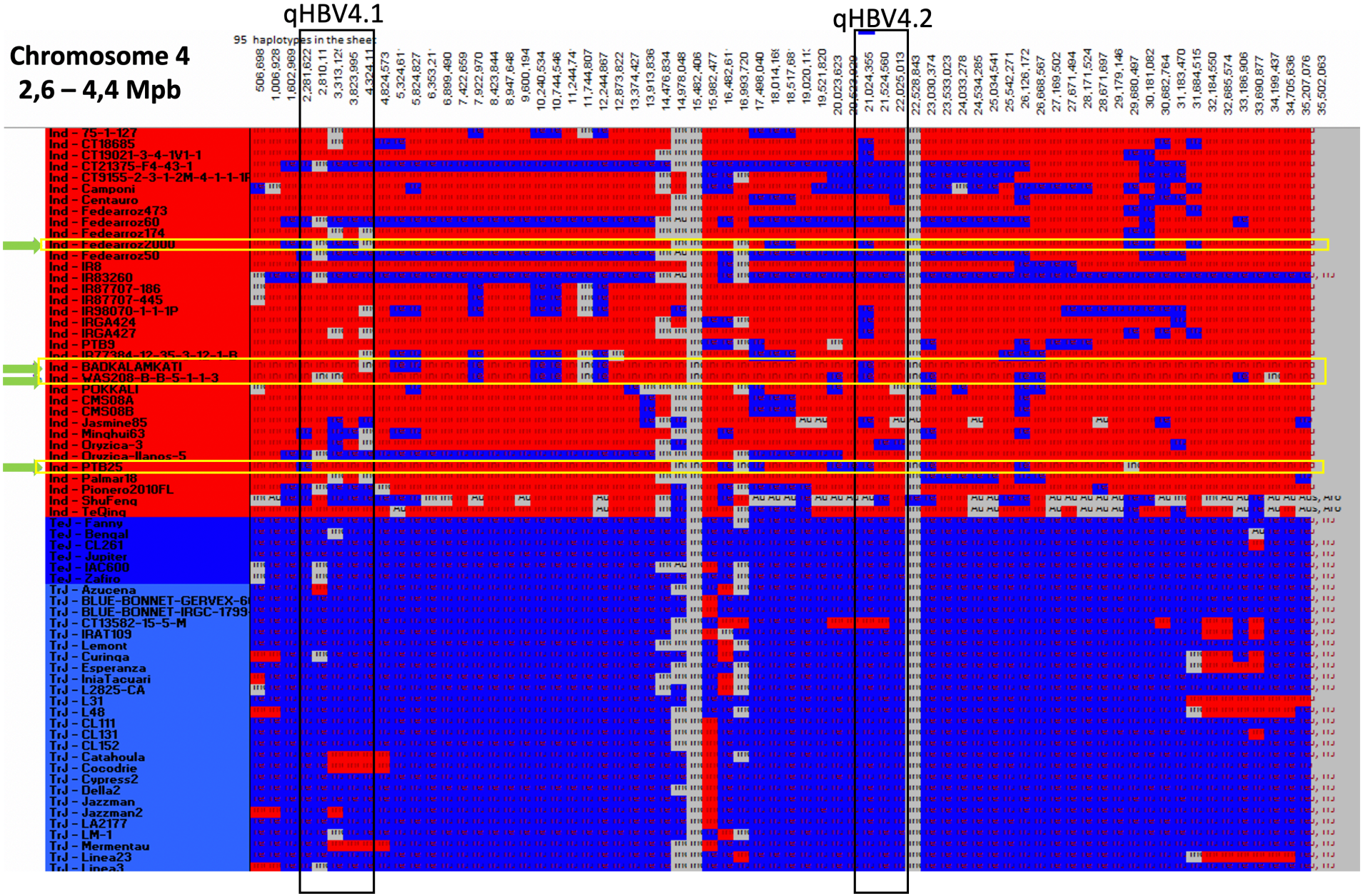
A



B



755 **Figure S2** Graphical genotyping showing probable local ancestry for the four RHB-resistant parents
756 (green arrows), in the two QTL regions *qHBV4.1* and *qHBV4.2* on chromosome 4. Red: *indica* origin,
757 including the Aus cluster. Blue: *japonica* origin, including temperate, sub-tropical and tropical
758 clusters.


Chromosome 4
2,6 – 4,4 Mpb
qHBV4.1
qHBV4.2
95 haplotypes in the sheet
Ind - 75-1-127
Ind - CT18685
Ind - CT19021-3-4-1W1-1
Ind - CT21375-F4-43-1
Ind - CT9155-2-3-1-2M-4-1-1-1P
Ind - Camponi
Ind - Centauro
Ind - Fedearroz473
Ind - Fedearroz60
Ind - Fedearroz174
Ind - Fedearroz2000
Ind - Fedearroz50
Ind - IR8
Ind - IR83260
Ind - IR87707-186
Ind - IR87707-445
Ind - IR98070-1-1-1P
Ind - IRGA424
Ind - IRGA427
Ind - PTB9
Ind - IR77384-12-35-3-12-1-B
Ind - BADKALAMKATI
Ind - WAS208-B-B-5-1-1-3
Ind - POKKALI
Ind - CMS08A
Ind - CMS08B
Ind - Jasmine85
Ind - Minghui63
Ind - Oryzica-3
Ind - Oryzica-llanos-5
Ind - PTB25
Ind - Palmar18
Ind - Pionero2010FL
Ind - ShuFeng
Ind - TeQing
TeJ - Fanny
TeJ - Bengal
TeJ - CL261
TeJ - Jupiter
TeJ - IAC600
TeJ - Zafiro
TrJ - Azucena
TrJ - BLUE-BONNET-GERVEX-6
TrJ - BLUE-BONNET-IRGC-1799
TrJ - CT13582-15-5-M
TrJ - IRAT109
TrJ - Lemont
TrJ - Curinga
TrJ - Esperanza
TrJ - IniaTacuari
TrJ - L2825-CA
TrJ - L31
TrJ - L48
TrJ - CL111
TrJ - CL131
TrJ - CL152
TrJ - Catahoula
TrJ - Cocodrie
TrJ - Cypress2
TrJ - Della2
TrJ - Jazzman
TrJ - Jazzman2
TrJ - LA2177
TrJ - LM-1
TrJ - Mermentau
TrJ - Linea23
TrJ - Linea3