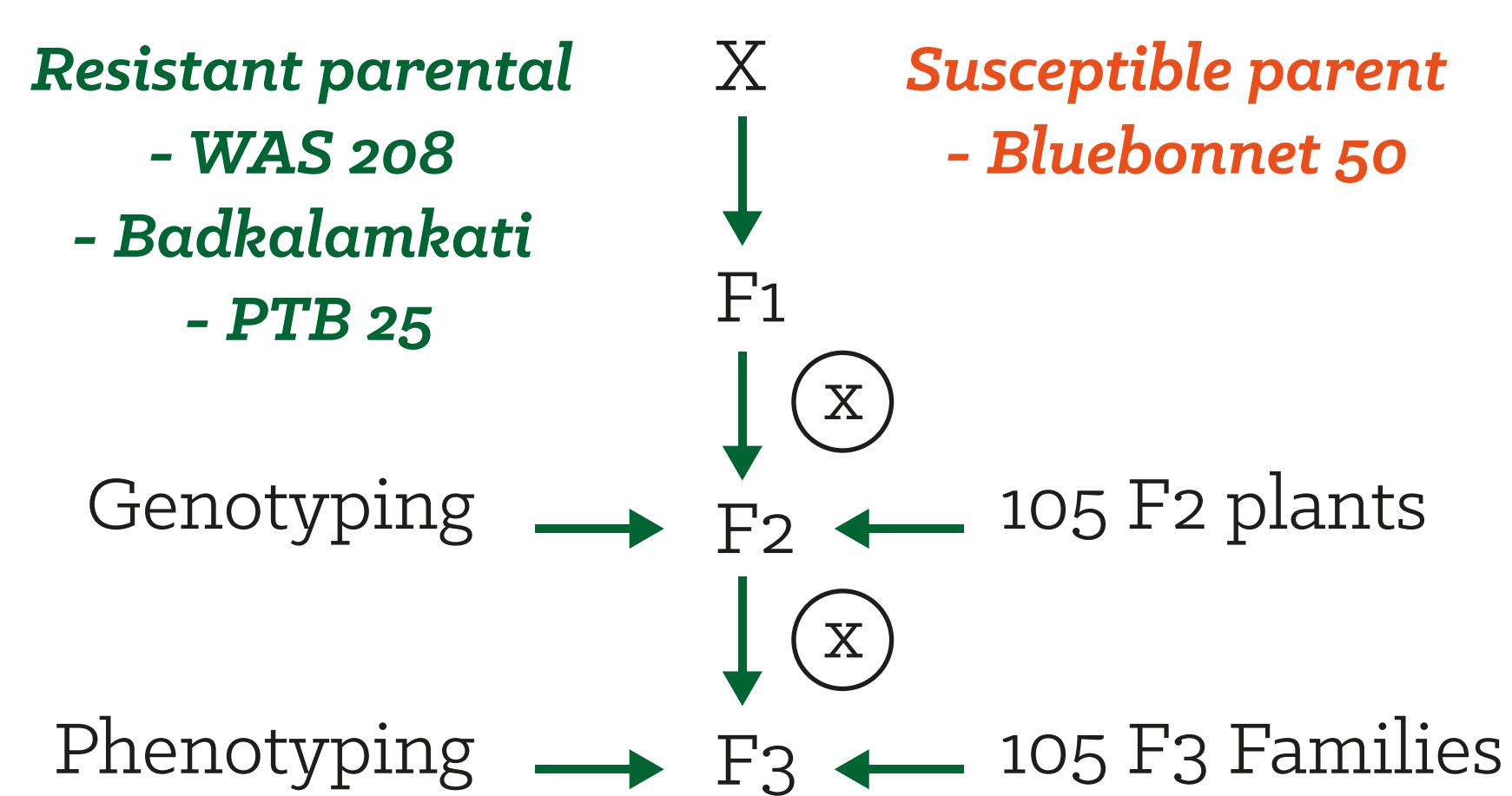


## INTRODUCTION

Rice *Hoja Blanca* (RHB) is one of the most devastating diseases in rice growing areas in tropical America. The main symptoms are chlorotic streaks that coalesce and produce the characteristic yellow or white leaf, stunting, panicle sterility, and reduced number of grains [1]. The disease is caused by the tenuivirus *Rice hoja blanca virus* (RHBV), transmitted by the planthopper *Tagosodes orizicolus*. Gene discovery for RHB resistance is so scarce that, so far, only one major QTL has been reported in Fedearroz 2000 [2], the most resistant variety in Colombia. Recently, new sources of resistance have been identified from a diverse panel of *Oryza sativa indica* germplasm [3]. The aim of this study was to perform QTL mapping to get a better understanding of the genetic bases of RHB resistance in these new sources, using two ways of evaluating the disease – incidence and severity.

## METHODS

### Development of mapping population

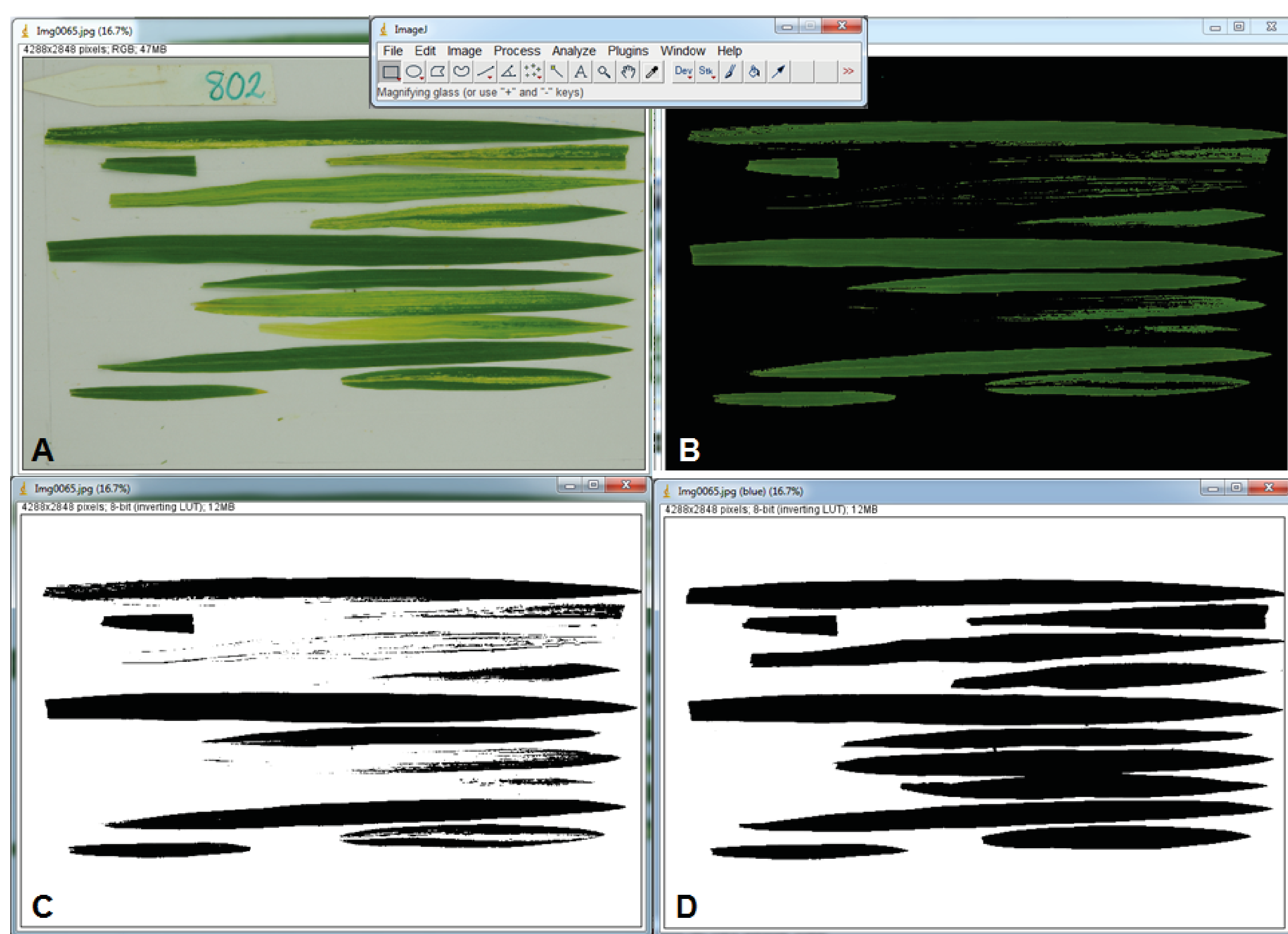


**Figure 1.** Development of F<sub>2:3</sub> mapping population for QTL detection

**Genotyping:** Genetic maps for the three F<sub>2</sub> biparental populations (WAS 208/Bluebonnet 50, Badkalamkati/Bluebonnet 50 and PTB 25/Bluebonnet 50) were constructed using 178, 185 and 139 SNP markers, respectively. SNP markers were distributed on the 12 chromosomes of the rice genome.

**QTL mapping:** QTL detection was carried out using Mapdisto software [4] for single marker analysis, and R-QTL [5] for simple and composite interval mapping. We employed a LOD (Logarithm of the Odds) threshold of 3.0 to declare the significance of a QTL.

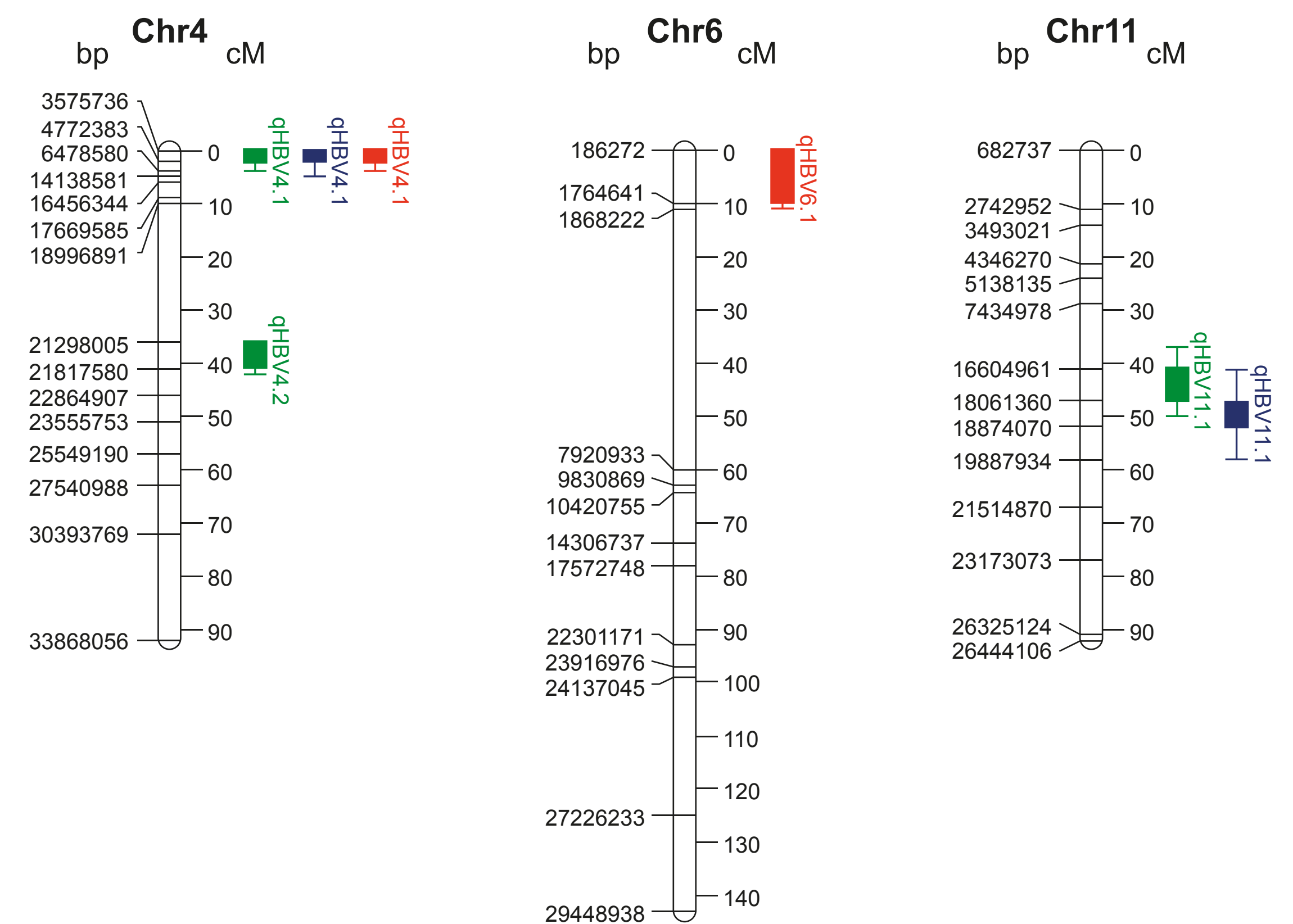
**Phenotyping:** F<sub>3</sub> families were evaluated for severity and incidence of RHB disease under greenhouse conditions. Severity was estimated with a novel image analysis strategy as the affected leaf area (ALA) in 10 plants of each F<sub>3</sub> family using the ImageJ software (Fig. 2). ALA was calculated as the difference between total and healthy leaf area. The incidence was estimated as the ratio of the number of plants with RHB symptoms to 60 plants of each F<sub>3</sub> family.



**Figure 2.** Image processing to estimate affected leaf area (severity). A: Leaves with RHB symptoms, B: Healthy leaf area, C: Image binarization for healthy leaf area, D: Image binarization for total leaf area.

## RESULTS

### RHB resistance is controlled by multiple QTLs



**Figure 3.** QTLs identified for RHB resistance in F<sub>2:3</sub> mapping populations. bp: base pairs, cM: centiMorgan. Green: QTLs detected in WAS 208/Bluebonnet 50, Blue: QTLs in Badkalamkati/Bluebonnet 50, and Red: QTLs detected in PTB 25/Bluebonnet 50

**Table 1.** Phenotypic variance explained by the QTLs for RHB resistance detected in F<sub>2:3</sub> populations

QTL	Population	Severity		Incidence	
		LOD	Variance explained (%)	LOD	Variance explained (%)
qHBV4.1	WAS 208/Bbt 50	3.2	13.6	3.6	16.0
	BKT/Bbt 50	5.8	21.4	19.9	61.0
	PTB 25/Bbt 50	8.3	36.6	21.1	63.6
qHBV4.2	WAS 208/Bbt 50	ND	ND	14.9	50.0
qHBV6.1	PTB 25/Bbt 50	ND	ND	8.4	8.1
qHBV11.1	WAS 208/Bbt 50	5.2	20.0	ND	ND
	BKT/Bbt 50	5.9	18.1	4.2	10.0

ND: QTL not detected, BKT: Badkalamkati, Bbt 50: Bluebonnet 50, LOD: Logarithm of odds

- Two major QTLs associated with RHBV resistance on chromosome 4 were identified (Fig. 3). The QTL qHBV<sub>4.1</sub>, detected earlier in Fedearroz 2000 [2], was found in the three populations and it was more associated with incidence than severity (Table 1). The QTL qHBV<sub>4.2</sub> was only detected in the population WAS 208/Bluebonnet 50.
- One novel major QTL for RHB resistance was identified on chromosome 11, named qHBV<sub>11.1</sub>, and it was more associated with severity than incidence of RHB.
- In the population PTB 25/Bluebonnet 50, besides qHBV<sub>4.1</sub>, a minor QTL for RHB resistance on chromosome 6 was detected and designated as qHBV<sub>6.1</sub> (Fig. 3).
- Some QTL were in fact more associated with incidence and others with severity of RHB disease, suggesting that more than one mechanism of resistance is involved at the different stages of the interaction plant-virus.

## CONCLUSIONS AND PERSPECTIVES

Our results showed that RHB resistance is controlled by multiple QTLs. The evaluation of both severity and incidence was a successful phenotyping strategy to identify a greater number of genomic regions associated with resistance. The QTL qHBV<sub>4.1</sub> was detected in our three mapping populations and, previously, in Fedearroz 2000 and Fedearroz 50 [2], suggesting it is a key factor in RHB resistance. Future fine mapping studies will allow us to identify candidate genes for these QTLs.

We are developing genetic markers to allow breeders apply marker-assisted selection with the aim to obtain new elite germplasm with high RHB resistance.

## REFERENCES

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