



Identification of QTLs conferring resistance to begomovirus isolate of PepYLCIV in *Capsicum chinense*

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Abstract Pepper yellow leaf curl disease caused by begomoviruses has emerged as a major limitation to the cultivation of pepper (*Capsicum* spp.) production in a number of regions around the world. Although begomovirus resistance has been reported in several accessions, *pepy-1* derived from BaPep-5 (*C. annuum*) is the only begomovirus resistance gene cloned to date. In this study, we evaluated the resistance of a *C. chinense* accession GR1 against pepper yellow leaf curl Indonesia virus (PepYLCIV), which is one of the predominant begomovirus species infecting pepper plants in Indonesia. Infection of PepYLCIV to susceptible Habanero (*C. chinense*) plants induced typical yellowing symptoms, whereas the GR1 plant showed symptoms with very low severity. Moreover,

the accumulation of viral DNA was restricted in GR1 compared to Habanero. Phenotypic analyses of F₁ and F₂ populations obtained by crossing Habanero with GR1 inferred that the resistance is a dominant trait controlled by multiple genes. Linkage analysis in the F₂ population using restriction site-associated DNA sequencing data detected two significant quantitative trait loci (QTLs), one on chromosome 4 and another on chromosome 11, which explained 31.6 and 19.7% of the phenotypic variation, respectively. Moreover, QTL-seq conducted using F₃ population partially confirmed the result of F₂ population, which detected QTLs on chromosome 3 and 11, respectively. The QTLs identified for PepYLCIV resistance in this research are novel since no other resistance causing QTLs have ever been reported in these genomic regions. GR1 is a highly valuable genetic resource for the breeding of begomovirus resistance in peppers.

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Introduction

Peppers (*Capsicum* spp.) consist of several wild relatives and five domesticated species, which include *C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum*, and *C. pubescens* (Bosland and Votava, 2000). The origin of *Capsicum* is postulated in an area along the Andes of western to north-western South America, and the expansion of the genus has followed a clockwise direction around the Amazon basin, toward central and south-eastern Brazil, then back to western South America, and finally northwards to Central America, where *C. annuum* originates (Kraft et al. 2014; Carrizo García et al. 2016). Of the five domesticated species, *C. annuum* is grown all over the world with thousands of cultivars. The production of peppers for use as spices and as vegetables has consistently increased during the last 50 years. World production was estimated at 6.8 million tonnes in 1970, and it has increased to 42.3 million tonnes in 2019, which is grown on more than 3.7 million ha (FAOSTAT 2019). The largest producers of peppers (total fresh and dried) include China, Mexico, Turkey, Indonesia, and India, and together they produce approximately 70% of the world's supply.

Pepper yellow leaf curl disease (PepYLCD) caused by genus *Begomovirus* of the *Geminiviridae* family seriously affects pepper cultivation in a number of regions around the world (Devendran et al. 2021). The genus *Begomovirus* encompasses 445 virus species, which is the largest number of species in plant infecting viruses (ICTV 2020). Thus, this virus infects various crops, including pepper, tomato, okra, cucurbit, common bean, papaya, cassava, and cotton, causing devastating damage to production (Varma and Malathi 2003; Rojas et al. 2018). The remarkable emergence of begomoviruses has been driven by the whitefly (*Bemisia tabaci*) supervector (Gilbertson et al. 2015). Generally, diseases caused by begomoviruses are controlled using insecticides that target the *B. tabaci* populations; however, intensive and unregulated use of insecticides has led to insecticide

resistance in *B. tabaci* (Palumbo et al. 2001; Rojas et al. 2018). An effective alternative to control begomoviruses is the use of an integrated pest management approach, which involves the development of begomovirus resistant genotypes. Our understanding and breeding of begomovirus resistance are most progressed in tomatoes (*Solanum lycopersicum*), and six resistance loci (*Ty-1–Ty-6*) conferring resistance to the monopartite tomato yellow leaf curl virus (TYLCV) have been identified in wild tomato accessions (Zamir et al. 1994; Agrama and Scott 2006; Anbinder et al. 2009; Ji et al. 2009a, b; Hutton and Scott 2014). However, the breeding resistance to begomovirus is lesser advanced in peppers than in tomatoes (Kenyon et al. 2014b). Although naturally occurring begomovirus resistance has been reported in several *C. annuum* and *C. chinense* accessions (Kumar et al. 2006; García-Neria and Rivera-Bustamante 2011; Kenyon et al. 2014a, Rai et al. 2014; Srivastava et al. 2015, 2017; Singh et al. 2016; Adluri et al. 2017; Retes-Manjarrez et al. 2019; Barchenger et al. 2019; Thakur et al. 2019), the genes responsible for begomovirus resistance in these accessions have not been identified. In our previous study, we cloned a begomovirus resistance gene *pepy-1*, which encodes the messenger RNA surveillance factor Pelota, locating in chromosome 5 of a *C. annuum* accession BaPep-5 for the first time in peppers (Koeda et al. 2021).

Multiple begomovirus resistance loci (*Ty-1–Ty-6*) have been identified in tomatoes and pyramiding of multiple genes in a single plant is expected to gain durable and broad-spectrum resistance (Yan et al. 2018, 2021). It is significantly important for the control of PepYLCD to identify distinct begomovirus resistance loci from *pepy-1* which was identified from *C. annuum*. GR1, a *C. chinense* accession, was identified as a begomovirus-resistant genetic resource in our preliminary analyses. In the present study, we conducted genetic mapping to identify a novel begomovirus resistance gene in F₂ and F₃ populations derived from a cross between a GR1 and the begomovirus susceptible Habanero. The work presented here infers that resistance to pepper yellow leaf Indonesia virus (PepYLCIV) in *C. chinense* is controlled by quantitative trait loci (QTLs) located on chromosome 3, 4, and 11.

Materials and methods

Plant material

Two *C. chinense* accessions, GR1 and Habanero, were used in this study. F₁ populations were obtained by a single cross between Habanero and GR1, the F₂ population was used for restriction site-associated DNA sequencing (RAD-seq), and the F₃ population was used for QTL-seq. Plants were grown in a growth room with temperatures ranging from 23 to 30 °C under a 13 h light/11 h dark photoperiod.

Inoculation of pepper plants with begomovirus pepper yellow leaf curl Indonesia virus

A bipartite begomovirus, pepper yellow leaf curl Indonesia virus (PepYLCIV) isolate BA_D1-1 (Accession number of GenBank for DNA A: LC051114, DNA B: LC314794) (Koeda et al. 2016, 2018), originally isolated in Indonesia was used in this study. The infectious clone of PepYLCIV isolate BA_D1-1 was previously constructed by our group through the transformation of pGreenII-p35S-PepYLCIV-DNA-A+B into *Agrobacterium tumefaciens* strain GV2260 (Koeda et al. 2018). Inoculation experiments were conducted for graft transmission of PepYLCIV into GR1, Habanero, F₁, and F₂ plants. The begomovirus susceptible *C. annuum* accession No.218 was agroinfiltrated with pGreenII-p35S-PepYLCIV-DNA-A+B, as previously described (Koeda et al. 2018, 2021). The agroinfiltrated No.218 plants were used as scions, and uninoculated GR1, Habanero, F₁, and F₂ plants were used as rootstocks. PepYLCIV-infected scion plants were grafted onto rootstocks approximately 30 days after agroinfiltration, and the grafted plants were covered by polybags to maintain high humidity. Plants were acclimatized approximately 14 days after grafting, and most of the scion was decapitated to stimulate lateral branch growth of the rootstock. The newly developed lateral branches of GR1, Habanero, F₁, and F₂ plants were evaluated for disease symptoms, and young upper leaves were collected and stored at – 80 °C until needed for DNA extraction. The symptoms of each plant were scored on a disease severity index (DSI) ranging from 0 to 4, as follows: 0, no symptoms or very mild symptoms; 1, mild symptoms; 2, moderate symptoms; 3, heavy symptoms; 4, very

heavy symptoms. Statistical analysis of the average DSI was performed using the Mann–Whitney U test with Excel Toukei ver. 7.0 and a *p*-value less than 0.05 was considered as statistically significant.

The cotyledons of F₃ plants (*n* = 1,082) were agroinfiltrated with pGreenII-p35S-PepYLCIV-DNA-A+B, and symptoms surveys were conducted at approximately 95 dpi. Young upper leaves were collected and stored at – 80 °C until needed for DNA extraction. We used the further precise disease severity index (DSI) ranging from 0 to 6, as follows: 0, no symptoms or extremely slight symptoms; 1, very slight symptoms; 2, very mild symptoms; 3, mild symptoms; 4, moderate symptoms; 5, heavy symptoms; 6, very heavy symptoms.

Diagnosis of PepYLCIV infection

DNA was extracted from pepper leaves using the Nucleon PhytoPure Kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK) or by a simple method, as previously described (Koeda and Fujiwara 2019). The DNA A component of PepYLCIV was detected using PepYLCIV uni 2 F and uni R primers, which amplified 696-bp fragments (Koeda et al. 2018). A polymerase chain reaction (PCR) test was performed using EmeraldAmp PCR Master Mix (Takara Bio) under the following conditions: initial denaturation at 94 °C for 2 min, followed by 35 cycles at 94 °C for 30 s, 63.2 °C for 30 s, and 72 °C for 1 min, and termination with 3 min of extension at 72 °C. The amplified PCR products were subjected to electrophoresis using 1.0% (w/v) agarose gel. Primer sequences used for viral DNA detection are listed in Supplementary Table S1.

Quantification of PepYLCIV titer by real-time PCR

The PepYLCIV DNA was quantified using the CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA), as previously described (Koeda et al. 2021). The DNA A component of PepYLCIV was detected by quantitative PCR (qPCR) using PepYLCIV A real 2 F and 2R primers, which amplified 114-bp fragments. Data from the total DNA extracts were normalized relative to the 25 S ribosomal RNA gene (93 bp) amplified by qPCR using 25 S-rRNA 2 F and 2R primers. The qPCR was performed using SsoAdvanced Universal SYBR Green Supermix

(Bio-Rad) under the following conditions: initial denaturation at 95 °C for 2 min, followed by 40 cycles at 95 °C for 5 s, and 58 °C for 10 s. Statistical analysis was performed using the Student's *t*-test and a *p*-value less than 0.05 was considered as statistically significant. Primer sequences used for real-time PCR are listed in Supplementary Table S1.

RAD-seq

DNA was extracted from pepper leaves collected from F₂ individuals before grafting using the Nucleon PhytoPure Kit (GE Healthcare). The RAD-seq libraries of F₂ individuals (*n* = 100) and their parents were constructed as previously described (Koeda et al. 2019) and sequenced with HiSeq 2500 (Illumina, Hercules, CA, USA). Three independent replicates of parental RAD-seq libraries were constructed to minimize any bias due to PCR amplification. Trimming and mapping of the sequence reads, followed by variant calling were performed as described in Koeda et al. (2021). The whole-genome sequence of *C. chinense* PI159236 (Kim et al. 2017) was used as a reference. Linkage analyses were conducted by composite interval mapping (CIM) of R/qtl (Broman et al. 2003).

QTL-seq

The DSI score of the F₃ population (*n* = 1,082) was evaluated and plants with DSI of 0 (highly resistance) and 6 (highly susceptible) were initially screened. Accumulating PepYLCIV titer was measured for further screening of the individuals, and ten plants were pooled for each of the extreme bulks: R_bulk (bulk with DSI score of 0) and S_bulk (bulk with DSI score of 6). Equal amounts of DNA from each individual were pooled into the bulks. The pooled DNA bulks were converted into DNA libraries for Illumina paired-end sequencing on the NovaSeq 6000 platform. QTLseq analysis was conducted according to Takagi et al. (2013). Whole genome-sequence of PI159236 (*C. chinense*) was used as a reference (Kim et al. 2017), and sequence of Habanero was used as a parent sequence data. The delta (Δ) SNP-index was calculated by subtracting the SNP-indices of the S_bulk from R_bulk at each locus, and candidate QTL regions were identified by using a 2 Mb sliding window with 100 kb step size.

Results

GR1 shows resistance against PepYLCIV

The transmission of PepYLCIV from symptomatic No.218 scions to healthy GR1 or Habanero rootstocks was evaluated approximately 75 days after grafting. We confirmed by PCR-based diagnosis that the successfully grafted plants were all infected by PepYLCIV. The PepYLCIV-infected Habanero (*n* = 11) plants showed symptoms of typical yellowing in the newly developed mature leaves with average DSI of 3 (Fig. 1a, c). In contrast, the PepYLCIV-infected GR1 plants (*n* = 17) showed symptoms with very low severity (DSI = 0.4) (Fig. 1b, c). Moreover, plants infected with begomovirus via graft-inoculation were randomly selected for the quantification of PepYLCIV DNA by qPCR using DNA extracted from young upper leaves at approximately 50 and 65 days from grafting. The accumulation of PepYLCIV DNA in GR1 plants (*n* = 5) was consistently lower than that in Habanero (*n* = 5), and at 65 days from grafting this difference was statistically significant (Fig. 1d). These results indicate GR1 is resistant against PepYLCIV.

Inheritance of PepYLCIV resistance to F₁ and F₂ progenies

To explore the inheritance of PepYLCIV resistance, F₁ and F₂ populations obtained by crossing Habanero with GR1 were graft-inoculated with PepYLCIV. We confirmed by PCR-based diagnosis that the successfully grafted plants were all infected by PepYLCIV. F₁ (*n* = 5) plants showed symptoms with very low severity (DSI = 0.4), equivalent to the parental GR1 (Fig. 2). This result showed that PepYLCIV resistance in GR1 is a dominant trait. At approximately 100 days from grafting, the F₂ individuals (*n* = 100) showed the following phenotypic segregation: *n* = 8, no symptoms or extremely slight symptoms (DSI = 0); *n* = 24, mild symptoms (DSI = 1); *n* = 18, moderate symptoms (DSI = 2); *n* = 28, heavy symptoms (DSI = 3); and *n* = 22, very heavy symptoms (DSI = 4) (Fig. 2). The segregation ratio of resistant individuals (DSI 0–1) to susceptible individuals (DSI 2–4) did not fit the 3:1 for F₂ populations by the χ^2 test ($\chi^2 = 98.61$, *p* = 3.1E-23), indicating that PepYLCIV resistance is controlled by multiple genes.

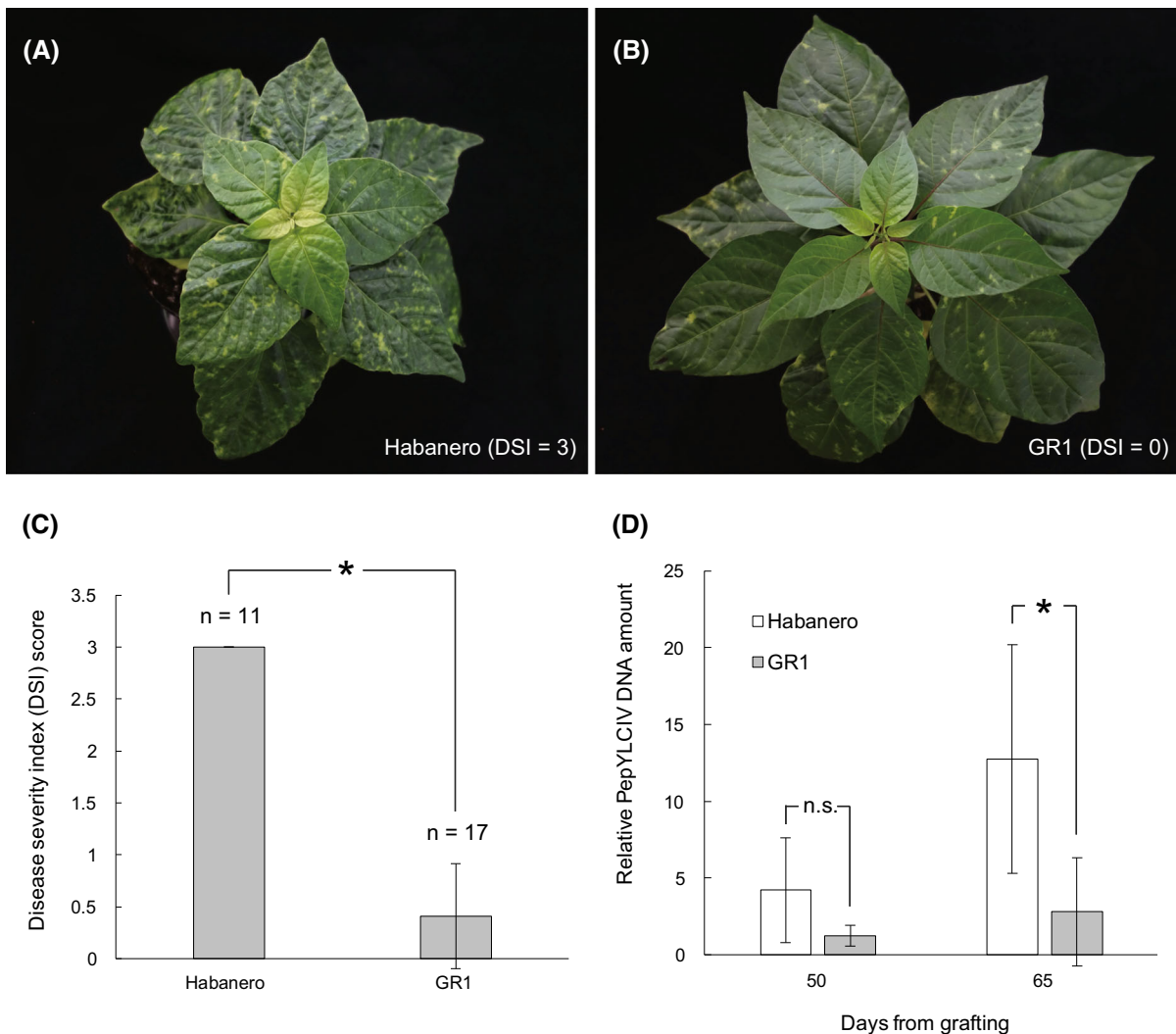


Fig. 1 GR1 and Habanero plants with single inoculation of pepper yellow leaf curl Indonesia virus (PepYLCIV). **a** Habanero and **b** GR1 plants infected with PepYLCIV approximately 75 days from grafting. **c** Disease severity index (DSI) of Habanero and GR1 plants approximately 75 days from grafting. **d** Accumulation of PepYLCIV viral DNA in PepYLCIV-infected Habanero ($n = 5$) and GR1 ($n = 5$)

plants at approximately 50 and 65 days post-grafting. Viral DNA values were normalized relative to the 25 S rRNA gene. Biological replicates are indicated on each bar. Data represent mean \pm standard deviation (SD). Asterisk indicates significant differences and n.s. indicates not significant among means of Habanero and GR1 by **c** Mann–Whitney U test ($p < 0.05$) and **d** Student's *t*-test ($p < 0.05$)

RAD-seq based genetic mapping of PepYLCIV resistance using an F_2 population

Linkage analysis of PepYLCIV resistance in the F_2 population was conducted using 279 SNPs obtained from RAD-seq data. The linkage map consisted of 12 linkage groups, which was equivalent to the

chromosome number of pepper (*C. chinense*) (Fig. 3). The overall size of the linkage map was 992.8 centimorgans (cM) (average marker distance = 3.6 cM), with a maximum spacing of 23.5 cM on chromosome 1. The CIM analysis of the Habanero \times GR1 F_2 population detected two significant QTLs, one on chromosome 4 and another on chromosome 11, accompanied by small peaks below the threshold on chromosomes 6 and 12 (Fig. 4 a). The newly found

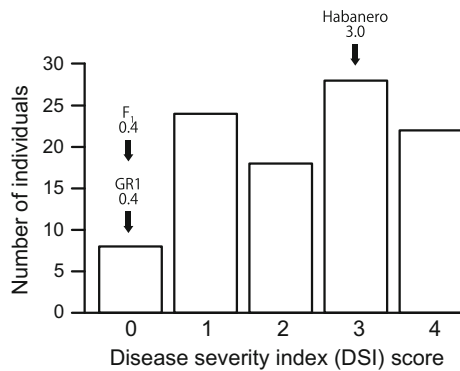


Fig. 2 Phenotypic segregation of the F_2 population ($n = 100$) derived from a single cross between Habanero and GR1. Histogram of disease severity index (DSI) scores at approximately 100 days from grafting

QTLs in GR1 were denoted as Pepy-*chr4* and Pepy-*chr11*, respectively. The peaks and intervals of the Pepy-*chr4* QTL (logarithm of the odds [LOD] score = 15.0), which explained 31.6% of the total phenotypic variation, was located at 39.1 cM on chromosome 4, and the Pepy-*chr11* QTL (LOD score = 8.3), which explained 19.7% of the total phenotypic variation, was located at 14.9 cM on chromosome 11 (Table 1). Moreover, epistatic interaction between Pepy-*chr4* and Pepy-*chr11* was detected ($p = 0.0001$) (Fig. 4b). The effect of the GR1 allele at Pepy-*chr11* to increase resistance (i.e., decrease DSI score) was observed in two genotype classes, homozygous for the Habanero allele at the Pepy-*chr4* locus and heterozygous, but not in the class homozygous for the GR1 allele at the Pepy-*chr4* locus (Fig. 4b). The intervals of Pepy-*chr4* and Pepy-*chr11* comprised 114 and 572 genes, respectively, in a whole-genome sequence of PI159236 (Table S2). In the intervals of Pepy-*chr4* and Pepy-*chr11*, six genes encoding leucine-rich repeat-containing (LRR) proteins were predicted as candidates. Also, a germin-like protein subfamily 1 member 17 (CC.CCv1.2.scaffold99.15) located in the interval of Pepy-*chr11* was predicted as a candidate.

QTL-seq based genetic mapping of PepYLCIV resistance using an F_3 population

To confirm the QTLs detected in F_2 population, 40 F_2 recombinants on target regions of chromosomes 4 and

11 were screened and self-pollinated to obtain the F_3 population. At approximately 95 days from inoculation, highly resistant F_3 individuals with DSI of 0 ($n = 74$) and highly susceptible F_3 individuals with DSI of 6 ($n = 100$) were observed out of whole F_3 individuals ($n = 1,082$). Ten F_3 individuals deriving from different F_2 recombinants were pooled for each of the extreme bulks: R_bulk (bulk with DSI score of 0) and S_bulk (bulk with DSI score of 6). The accumulation of PepYLCIV DNA in R_bulk plants was significantly lower than that in S_bulk plants (Fig. 5a).

To identify QTL, the SNP index was calculated for each bulk by aligning the sequences to the *C. chinense* reference genome. After the (Δ) SNP index with a statistical confidence of $p < 0.01$ was calculated between the two extreme phenotypic bulks, a 9.3-Mb (224.3–233.6-Mb) genomic region on chromosome 3 and a 12.4-Mb (217.5–229.9-Mb) genomic region on chromosome 11 were identified (Fig. 5b). We designated these QTLs as Pepy-*chr3*-qtlseq and Pepy-*chr11*-qtlseq. Analysis of SNP-index of R_bulk and S_bulk inferred that QTL Pepy-*chr3*-qtlseq derived from Habanero and Pepy-*chr11*-qtlseq derived from GR1 (Fig. S1). The intervals of Pepy-*chr3*-qtlseq and Pepy-*chr11*-qtlseq comprised 152 and 108 genes, respectively, in a whole-genome sequence of PI159236 (Table S2). Candidate regions of QTL-seq partially overlapped with the candidate region of RAD-seq at 217.5–227.7-Mb genomic region on chromosome 11. A germin-like protein subfamily 3 member 2 (CC.CCv1.2.scaffold279.31) was located in the interval of Pepy-*chr11*-qtlseq.

Discussion

Begomoviruses, transmitted by whiteflies, have emerged as major limitations to the cultivation of a wide variety of crops worldwide (Navas-Castillo et al. 2011; Rojas et al. 2018), and PepYLCD seriously affects pepper production in a number of regions around the world (Devendran et al. 2021). Naturally occurring begomovirus resistance has been reported in several *Capsicum* accessions (Kumar et al. 2006; García-Neria and Rivera-Bustamante 2011; Kenyon et al. 2014a; Rai et al. 2014; Srivastava et al. 2015, 2017; Singh et al. 2016; Adluri et al. 2017; Retes-Manjarrez et al. 2019; Barchenger et al. 2019;

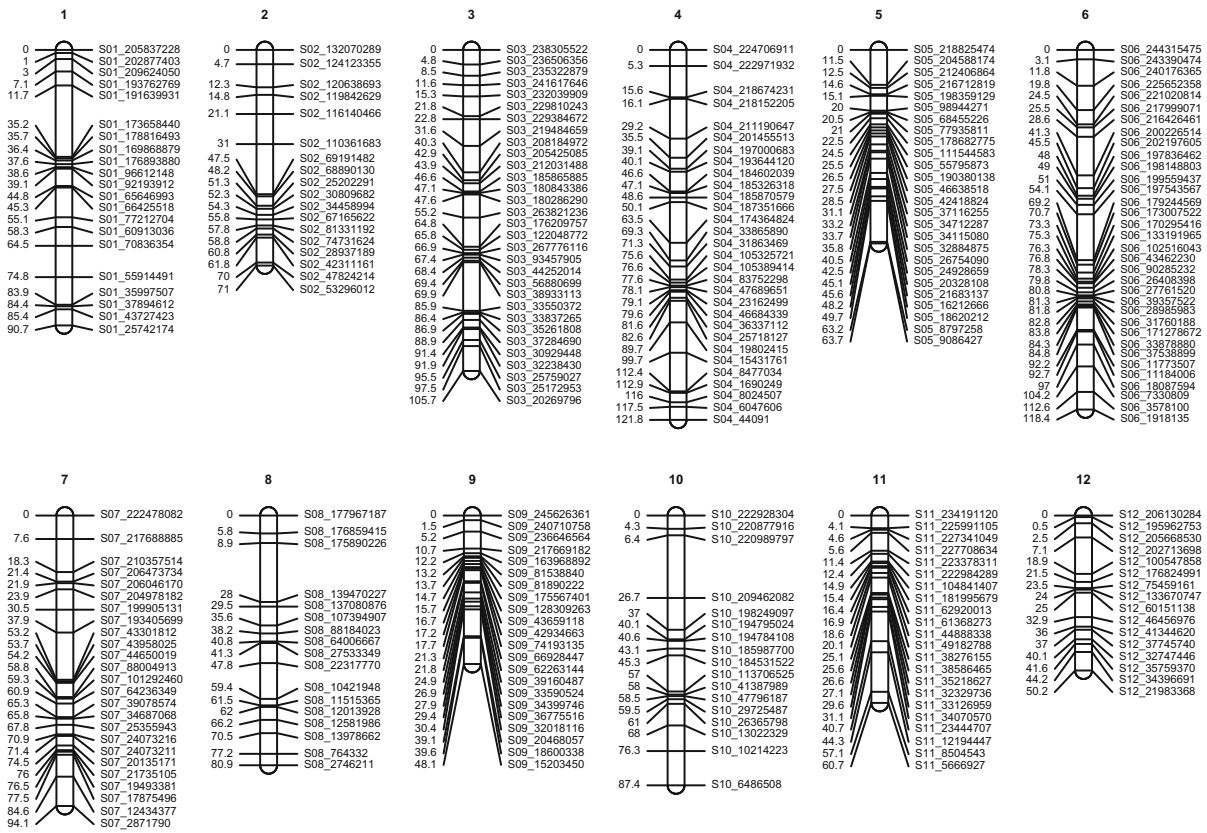


Fig. 3 Linkage map constructed from restriction site-associated DNA sequencing (RAD-seq)-derived 279 SNPs in 100 F₂ individuals

Thakur et al. 2019), but *pepy-1* derived from BaPep-5 (*C. annuum*) is the only begomovirus resistance gene cloned to date in *Capsicum* (Koeda et al. 2021). In this study, we analyzed the resistance of *C. chinense* accession GR1 against one of the predominant bipartite begomovirus PepYLCIV infecting pepper plants in Indonesia (Koeda et al. 2016).

Most of the virus resistance genes in plants either prevent viral replication or restrict viral replication to the cells targeted by the virus to enter the host (Kang et al. 2005). The *Ty-1/Ty-3*, *Ty-2*, *ty-5* of tomato and *pepy-1* of pepper provide a level of virus tolerance rather than immunity against begomovirus (Verlaan et al. 2013; Lapidot et al. 2015; Yamaguchi et al. 2018; Koeda et al. 2021). This is consistent with the results of this study wherein PepYLCIV-infected GR1 showed symptoms with very low severity (DSI = 0.4) (Fig. 1B, C), and a low level of PepYLCIV DNA accumulation was detected in systemic leaves (Fig. 1d).

Analysis of F₁ individuals indicated that the resistance derived from GR1 seemed to be a dominant trait, but the segregation ratio in the F₂ population did not fit the expected 3:1 by χ^2 test ($\chi^2 = 98.61$, $p = 3.1E-23$), which indicated that resistance is controlled by multiple genes. This is consistent with the linkage analysis results conducted by CIM using F₂ population, which detected a major QTL Pepy-chr4 on chromosome 4 and a minor QTL Pepy-chr11 on chromosome 11, explaining 31.6% and 19.7% of the phenotypic variation, respectively (Fig. 4 a; Table 1). Further analysis using F₃ population with QTL-seq detected QTL Pepy-chr3-qt1seq on chromosome 3 and a QTL Pepy-chr11-qt1seq on chromosome 11 (Fig. 5b). A major QTL located on chromosome 4 and a minor QTL located on chromosome 11 were detected in the F₂ population which all the segregating individuals ($n = 100$) were phenotyped, and epistatic interaction was detected between the QTLs ($p = 0.0001$) (Fig. 4). On the other hand, a QTL located on chromosome 11 was detected in the F₃

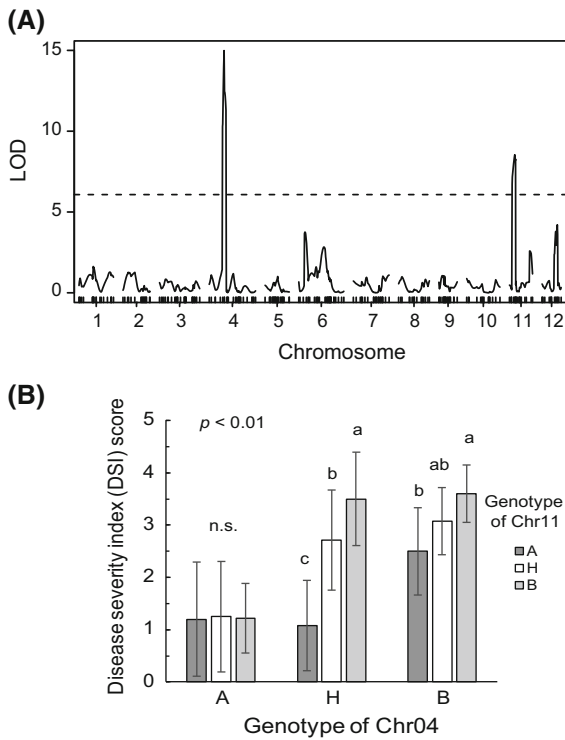


Fig. 4 QTL analysis of PepYLCIV resistance in F₂ population. **a** The logarithm of the odds (LOD) scores determined by linkage analysis of PepYLCIV resistance in the F₂ population (*n* = 100) across 12 pepper chromosomes. **b** Differences in mean disease severity index (DSI) for nine genotype classes in the F₂ population derived from crosses between Habanero and GR1. A, B, and H indicate homozygous for the GR1 allele, homozygous for the Habanero allele, and heterozygous, respectively. Data represent mean ± standard deviation (SD). The *p*-value was calculated by using a 2-way analysis of variance. Different letters within a row indicate significant differences and n.s. indicates not significant among means by Bonferroni–Dunn test (*p* < 0.05)

population, whereas the major QTL detected on chromosome 4 in the F₂ population was not detected in the F₃ population (Fig. 5b). Because a subset of individuals (*n* = 20) was selected for QTL-seq analysis in the F₃ population, there might be some unexpected bias for the genotype of Pepy-*chr4* locus in F₃ individuals which resulted in non-detection of QTL in chromosome 4. Interestingly, we detected Pepy-*chr3*-qtlseq on chromosome 3 in the F₃ population, which was assumed to be derived from susceptible parent Habanero (Fig. 5b; Fig. S1). The effect of QTL on chromosome 3 could have been masked in the F₂ population by the larger effects of chromosomes 4 and 11 QTLs. PepYLCIV and PepYLCAV resistance gene

Table 1 Quantitative trait loci (QTL) identified in the F₂ populations of Habanero × GR1

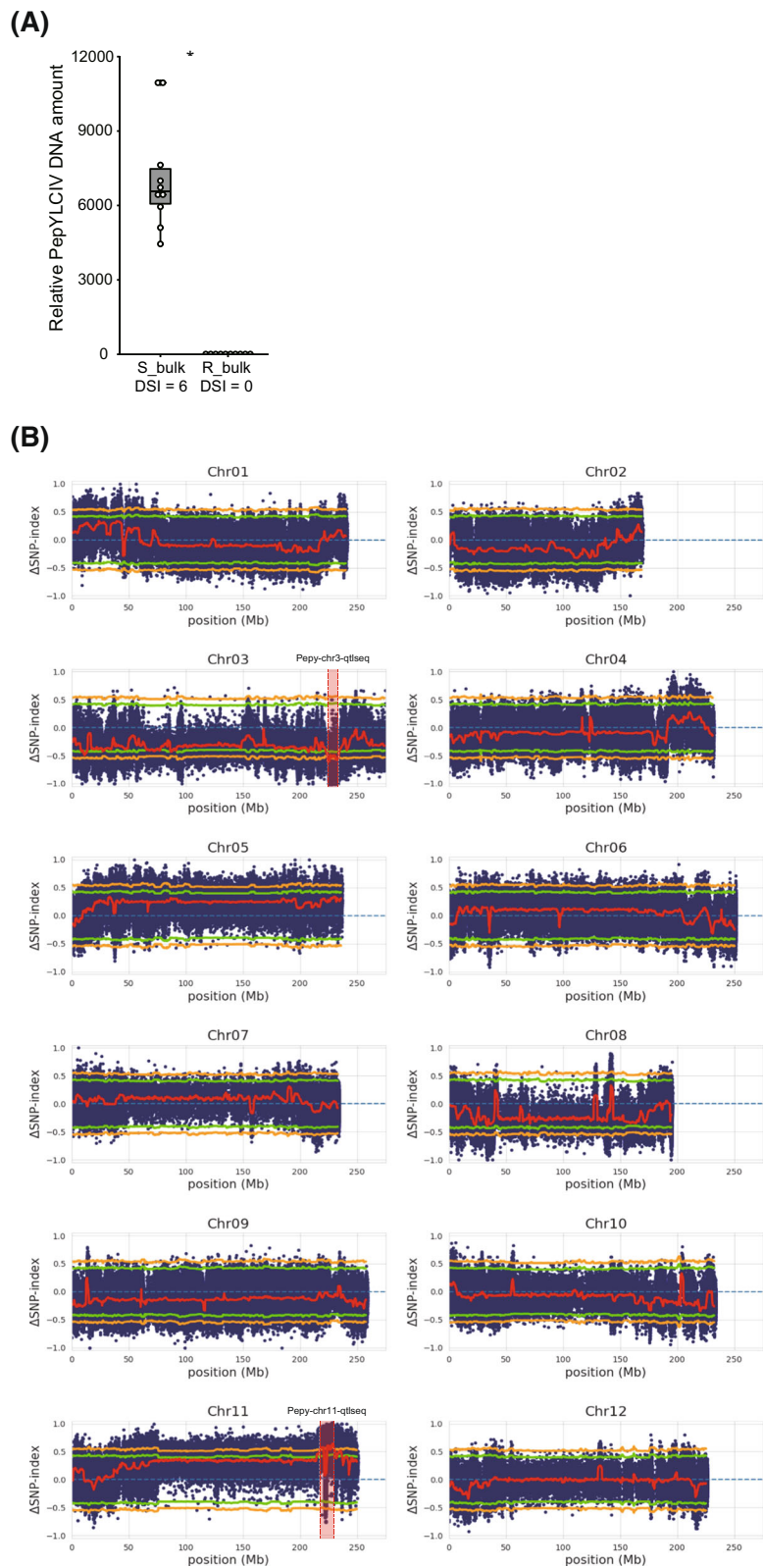
Chr	QTL name	Contributing parent	LOD	PVE ^x %	Additive effect	Dominant effect	Peak Marker	1.8-LOD support interval			Genes ^z		
								Position ^y	Left marker	Right marker			
4	Pepy- <i>chr4</i>	GR1	15.0	31.6	0.905	0.404	S04_197000683	197000683	S04_193644120	S04_201455513	201455513	35.5–40.1	114
11	Pepy- <i>chr11</i>	GR1	8.3	19.7	0.699	0.280	S11_104841407	104841407	S11_62920013	S11_227708634	227708634	5.6–16.4	572

^xPercent of the phenotypic variation explained by the QTL

^yPhysical position in reference genome sequence of P1159236 (*C. chinense*)

^zNumber of potential candidate genes located in the 1.8-LOD interval of the QTL

Fig. 5 QTL-seq analysis of PepYLCIV resistance in F_3 population. **a** Accumulation of PepYLCIV viral DNA in PepYLCIV-infected S_{bulk} ($n = 10$) and R_{bulk} ($n = 10$) plants at approximately 105 days post-agroinfiltration. Asterisk indicates significant differences among means by Student's t -test ($p < 0.05$). **b** (Δ) SNP-index plots of 12 pseudomolecules representing the F_3 populations used in the analysis of Fig. 5a. A bulk of 10 resistant and 10 susceptible PepYLCIV F_3 individuals was analyzed, a statistical confidence interval under the null hypothesis of no QTLs (orange, $p < 0.01$; green, $p < 0.05$) is indicated in each plot. The red line represents the sliding window average of the (Δ) SNP index (window size, 2 Mb; slide size, 100 kb). The identified QTL regions on chromosome 3 and 11 (Pepy-*chr3-qtlseq* and Pepy-*chr11-qtlseq*) are highlighted



pepy-1 of BaPep-5 (*C. annuum*) is located on chromosome 5 (Koeda et al. 2021). Thakur et al. (2019, 2020) reported that the chili leaf curl virus disease resistance gene of a *C. annuum* accession S-343 is linked to a DNA marker located on chromosome 6. These results indicate that the QTLs identified for PepYLCIV resistance in this research are novel since no other resistance causing QTLs have ever been reported in these genomic regions.

To the best of our knowledge, tomato *Ty-1/Ty-3/Ty-3a* (encoding RDR), *Ty-2* (encoding an NB-LRR protein), *ty-5* (encoding Pelota), and pepper *pepy-1* (encoding Pelota) are the only begomovirus resistance genes that have been cloned to date (Verlaan et al. 2013; Lapidot et al. 2015; Yamaguchi et al. 2018; Koeda et al. 2021). *RDR* and *Pelota* were not found among the genes located in the candidate regions (Table S2). In contrast, a total of six LRR proteins were found in chromosomes 4 and 11, which are candidate genes (Table S2). Resistance against bipartite begomoviruses pepper huasteco yellow vein virus (PHYVV) and pepper golden mosaic virus (PepGMV) of a *C. chinense* accession BG-3821 is reported to be controlled by two genes which have a duplicate recessive epistatic relation (García-Neria and Rivera-Bustamante 2011). Transcriptomic studies with BG-3821 identified a germin-like protein (*CchGLP*) gene as involved in resistance to PHYVV and PepGMV (Barrera-Pacheco et al. 2008). Furthermore, transgenic expression of *CchGLP* in begomovirus susceptible tobacco (*Nicotiana tabacum*) cultivar provided resistance against PHYVV and PepGMV, and virus-induced gene silencing of *CchGLP* in BG-3821 increased susceptibility against PHYVV and PepGMV (Guevara-Olvera et al. 2012; Mejía-Teniente et al. 2015). BLASTN analysis identified CC.CCv1.2.scaffold401.11 located in chromosome 3 (position: 260,061,510-260,062,139) in the genome sequence of PI159236 (*C. chinense*), which is outside the region of Pepy-chr3-qtlseq, with the highest similarity (Identities 99%, E-value 0.0) to *CchGLP* (DQ677335.2). However, because a germin-like protein subfamily 1 member 17 (CC.CCv1.2.scaffold99.15) was found to locate in the candidate region of Pepy-chr11, and a germin-like protein subfamily 3 member 2 (CC.CCv1.2.scaffold279.31) was located in the interval of Pepy-chr11-qtlseq, these genes are candidates controlling the PepYLCIV resistance (Table S2). A more saturated molecular

map and a larger mapping population are required for the fine mapping and cloning of the resistance genes.

The most economically important species belong to the *Capsicum annuum* complex (*C. annuum*, *C. chinense*, and *C. frutescens*), two other species (*C. baccatum* and *C. pubescens*) are cultivated predominantly in Latin America (Pickersgill, 1997). Moreover, interspecific hybridization between *C. annuum* and *C. chinense* is reported to be relatively easy compared to *C. baccatum* or *C. pubescens* (Bosland and Votava 2000). Multiple begomovirus resistance loci (*Ty-1-Ty-6*) have been identified in tomatoes and pyramiding of multiple genes in a single plant is expected to gain durable and broad-spectrum resistance (Yan et al. 2018, 2021). Introgression of the resistance genes from GR1 (*C. chinense*) into *C. annuum* and pyramiding the resistance genes derived from GR1 with *pepy-1* will contribute to the control of PepYLCD. Fine mapping using a larger mapping population is currently underway. GR1 is a highly valuable genetic resource with PepYLCIV resistance, and the identification of resistance genes will greatly facilitate marker-assisted breeding of begomovirus resistance in pepper.

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Author contributions NM and SK designed experiment, interpreted the results, and wrote the manuscript. NM, SH, RT, RH, CW, and DO evaluated the begomovirus resistance. NM, HS, AJN, and SK conducted RAD-seq. EK provided the resources. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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