Genotyping-by-sequencing-based QTL mapping reveals novel loci for Pepper yellow leaf curl virus (PepYLCV) resistance in Capsicum annuum

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Abstract

Disease caused by Pepper yellow leaf curl virus (PepYLCV) is one of the greatest threats to pepper (Capsicum spp.) cultivation in the tropics and subtropics. Resistance to PepYLCV was previously identified in a few Capsicum accessions, but no resistance QTLs have been mapped. This study aimed to elucidate the genetics of PepYLCV resistance in C. annuum L. Augmented inoculation by the viruliferous whitefly Bemisia tabaci was used to evaluate parental lines and an F2 segregating population derived from a cross between resistant C. annuum line LP97 and susceptible C. annuum line ECW30R. Final evaluation was performed six weeks after inoculation using a standardized 5-point scale (0 = no symptoms to 4 = very severe symptoms). A high-density linkage map was constructed using genotyping-by-sequencing (GBS) to identify single-nucleotide polymorphism (SNP) markers associated with PepYLCV resistance in the F2 population. QTL analysis revealed three QTLs, peplcv-1, peplcv-7, and peplcv-12, on chromosomes P1, P7, and P12, respectively. Candidate genes associated with PepYLCV resistance in the QTL regions were inferred. In addition, single markers Chr7-LCV-7 and Chr12-LCV-12 derived from the QTLs were developed and validated in another F2 population and in commercial varieties. This work thus provides not only information for mapping PepYLCV resistance loci in pepper but also forms the basis for future molecular analysis of genes involved in PepYLCV resistance.

Introduction

Begomoviruses cause severe diseases in major vegetable crops, especially in the tropics and subtropics of Asia and America. The genus Begomovirus is the largest in the family...
Geminiviridae and contains more than 200 species. Begomoviruses have been reported to contain monopartite and bipartite genomes [1, 2]. Recently, the two begomoviruses Tomato yellow leaf curl virus (TYLCV) and African cassava mosaic virus (ACMV) were listed among the 10 most important plant viruses according to the economic losses they cause and their scientific importance [3, 4].

The begomovirus Pepper yellow leaf curl virus (PepYLCV) is a destructive pathogen that severely limits pepper production in South Asian countries. PepYLCV consists of a circular ssDNA of about 2.75 kb and is mainly transmitted via the whitefly insect vector Bemisia tabaci [5, 6]. Upon PepYLCV infection, pepper plants show cupping, the deformation of leaves and stunted plant growth, and a reduction in fruit size. Following severe infection, plants show inhibited pollen development and the dropping of flower buds, which leads to the absence of fruit set [7]. These symptoms complicate the adoption of appropriate preventative measures at early stages [4, 8]. Despite its importance, the occurrence of begomoviruses as a major pathogen of pepper is relatively recent compared with that of tomato [4]. For instance, among the five begomoviruses that cause leaf curl disease in pepper in the Americas, only one was identified in Asian countries early in the year 2000 [9]. Since then, the number of begomoviruses that infect pepper has increased dramatically in Asian regions of pepper cultivation, including Bangladesh, China, India, Indonesia and Pakistan, and about 29 species have been reported, with a vast diversity of virus strains [4, 8].

Current begomovirus management approaches depend mainly on insecticides to control the whitefly vector. However, such strategies are ineffective when whiteflies transmit the virus to other plants before disease symptoms become apparent in the field or greenhouses. Moreover, some vector insects have become resistant to specific insecticides [3, 10]. An alternative method of begomovirus management is to develop resistant cultivars. Indeed, begomoviruses resistance has been identified in accessions of several Capsicum species, such as C. chinense BG-3821, a Mexican line [11]. Resistance has also been reported in C. annuum, including EC-497636, GKC-29, BS-35 [12], Kalyanpur Chanchal [13] and breeding lines from the World Vegetable Center collection [4, 8, 14].

Screening for pepper resistance against begomoviruses is challenging because the sap inoculation method has not been successful to date [2, 4, 7]. Moreover, the presence of multiple pathogens can hinder the evaluation of resistance under conditions of natural disease incidence in the field. Screening via grafting and the micro-cage technique has also been reported, but these can be time-consuming and labor-intensive [7, 12]. The agroinfiltration method has been used to evaluate resistance using PepYLCV Indonesia strains [2, 15–17]. However, this method requires sophisticated cloning and transformation to inoculate the plants. The augmented inoculation of plants by the viruliferous whitefly B. tabaci has been used for reliable screening to evaluate resistance to YLCV in pepper and tomato [4, 8, 18, 19].

Inheritance studies are required to identify novel sources of resistance to PepLCV and to develop closely linked markers that facilitate introgression of PepYLCV resistance into commercial pepper varieties. Genetic analyses of resistance to begomoviruses has revealed complex modes of inheritance, including polygenic, monogenic and strain-specific types of resistance [7, 20–23]. For instance, some resistance against PepYLCV is governed by monogenic recessive genes with additive, dominant and epistatic effects [21, 24]. By contrast, mapping of a PepYLCV resistance locus using single sequence repeat (SSR) markers in an F2 segregating population revealed a single dominant resistance gene on pepper chromosome 6 [18]. This locus was delimit to within 15.7 cM, between two adjacent SSR markers CA516044 and PAU-LC-343-1, at genetic distances of 6.8 cm and 8.9 cm, respectively. The discrepancies concerning the modes of resistance inheritance may be due to the use of different regional virus strains and inoculation methods; for example, the use of open-field tests versus artificial
inoculation conditions based on viruliferous whitefly as a vector [25]. Furthermore, virus strains are continuously evolving from monopartite to bipartite species, which may overcome the previous resistance.

In this study, we report genetic mapping of novel resistance genes to PepYLCV Indonesia strain using genotyping-by-sequencing-based QTL mapping in an F$_2$ segregating population of pepper. Markers linked to the identified QTLs were developed and validated using several resistant pepper varieties.

**Materials and methods**

**Plant materials**

The resistant *C. annuum* line LP97 and the susceptible *C. annuum* line ECW30R were provided by EcoSeed (Gimjae, Korea) (Table 1). The F$_1$ plants derived from a cross between the two lines were self-pollinated to develop a segregating F$_2$ population named LP97-F$_2$. The 150 LP97-F$_2$ seedlings, 13 resistant (LP97) and 13 susceptible (ECW30R) control plants (Table 1) were grown in the greenhouse of East West Seeds Indonesia (EWINDO). An additional F$_2$ population derived from the resistant commercial hybrid “Eagle F$_1$” named Eagle-F$_2$ was also screened and used for marker validation (Table 1). In this screening trial, the 184 Eagle-F$_2$ seedlings with 40 resistant (Eagle F$_1$) and 40 susceptible (ECW30R) control plants were evaluated for PepYLCV resistance as described above. Additional resistant commercial F$_1$ hybrids were also used for marker validation (Table 1).

**Resistance screening**

The PepYLCV Indonesia strain was used to screen the F$_2$ segregating population and additional commercial F$_1$ hybrids. The strain was maintained on susceptible infected pepper plants in a screen house of EWINDO. The seedlings were transferred to the screen house for resistance evaluation using viruliferous whitefly as a vector [4, 18]. To maintain the whitefly population, highly susceptible *Solanum lycopersicum* (local cultivar: Tombatu) and *S. melongena* (local cultivar: Mustang) plants were placed 90 cm apart among the test plants. A screen house was formed from insect-proof nylon net to prevent whitefly escape and entry by other insects. The plants were assessed according to disease severity grades 0 to 4 (S1 Fig) as described previously [4] with slight modification. The final evaluation for resistance and susceptibility was carried out 6 weeks after the transfer of seedlings to the screen house.

| No. | Cultivar   | Type         | Expected phenotype | Source       |
|-----|------------|--------------|--------------------|--------------|
| 1   | LP97       | Double haploid | Resistant          | Eco Seeds    |
| 2   | ECW30R     | Accession    | Susceptible        | Eco Seeds    |
| 3   | LP97-F2    | F$_2$        | Segregating population | Eco Seeds   |
| 4   | Eagle-F2   | F$_2$        | Segregating population | Eco Seeds   |
| 5   | Eagle-F1   | Commercial F$_1$ | Resistant          | Eco Seeds    |
| 6   | SONAL-F1   | Commercial F$_1$ | Resistant          | Eco Seeds    |
| 7   | Sarangi-F1 | Commercial F$_1$ | Resistant          | Eco Seeds    |
| 8   | Vikrant-F1 | Commercial F$_1$ | Resistant          | Eco Seeds    |
| 9   | Armour-F1  | Commercial F$_1$ | Resistant          | Eco Seeds    |
| 10  | Romyz1 F1  | Commercial F$_1$ | Resistant          | Eco Seeds    |

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Preparation of GBS libraries and SNP identification

Genomic DNA was extracted from young leaf tissues of plants from the F$_2$ segregating population at the seedling stage using the hexadecyl trimethyl ammonium bromide (CTAB) method described by [26]. Genotyping-by-sequencing was performed as described previously [27, 28]. Briefly, genomic DNA of F$_2$ and control plants was diluted, and the concentration was adjusted to 20 ng μL$^{-1}$. The DNA was digested with EcoRI and MseI; after ligation of adaptors to the digested DNA, the libraries were amplified with selective primers. The amplified libraries consisting of 92 F$_2$ samples and two replicates of susceptible (ECW30R) and resistant (LP97) parents were pooled into a single tube. The pooled libraries were sequenced using an Illumina HiSeq2000 at Macrogen (Macrogen, Inc., Seoul, Korea). Trimming and quality control of the GBS raw data were performed using CLC Genomics Workbench v6.5 (Qiagen, Aarhus, Denmark) with a minimum read length of 80 bp and a minimum quality score of Q20. Filtered reads were aligned to the C. annuum cv. Dempsey reference genome [Unpublished] using the Burrows-Wheeler Aligner (BWA). Filtering and SNP calling were performed using the Genome Analysis Toolkit (GATK) Unified Genotyper version 3.3–0. The SNPs in the F$_2$ population were filtered with QUAL value $> 20$ and a minimum read depth of three.

Bin map construction, linkage analysis and QTL mapping

The SNPs that showed distorted and uneven segregation and more than 50% missing data were removed before linkage-map construction. To construct a linkage map, linkage bins were treated as genetic markers. The sliding window approach was used to impute the missing data and identify recombination break points as described previously [27]. To assign genetic positions to the bins, arranged bins were mapped with a LOD (logarithm of the odds) threshold of 3.0 and a distance threshold of 30 cm using CarthaGene software. The Kosambi mapping function was used to convert genetic distances between markers. CIRCOS 0.66 software v0.66 was used to compare the collinearity of the bin locations between the physical position and the genetic position [29]. QTL analysis was performed using the composite interval mapping using Windows QTL Cartographer 2.5 [30]. The 1,000-permutation test ($P < 0.05$) was performed to determine the LOD threshold for the significance of each QTL. Explanations of the phenotypic variance (PV) and additive effects for each QTL were also obtained using this software. In addition, R/qtl was used to verify the QTL results (https://cran.r-project.org/web/packages/qtl).

Candidate gene and intergenic SNP analysis

The physical positions of the QTLs in the pepper genome were marked using the genetic distance information of the corresponding bins. Candidate genes within the QTL regions were retrieved using the annotated genes from the C. annuum cv. Dempsey reference genome [Unpublished]. Functions for candidate genes were annotated using BLAST2GO version 5 [31]. To annotate the intra- and intergenic variants in the QTL regions, snpEff version 4.3t [32] was used and the SNP information in the QTL regions was classified using the results generated by snpEff.

Marker development and validation

The flanking sequences of the linked SNPs in the QTL regions were retrieved from the C. annuum cv. "Dempsey" reference genome database [Unpublished] and the online OligoAnalyzer tool (IDT International, Iowa, USA) was used to design primers. All DNA sequences containing SNPs were subjected to PCR amplification, and polymorphisms were confirmed
between the resistant and susceptible parents by Sanger sequencing. In the next step, high-resolution melt (HRM) analysis was performed to validate the SNP markers. The HRM analysis was performed as described by [33].

Results
Phenotypic evaluation of parental lines and the mapping population

The resistance of the F$_2$ plants to PepYLCV was evaluated following inoculation by viruliferous whiteflies (B. tabaci). The plants were assessed according to disease severity grades 0 to 4 from June to September 2018 (S1 Fig). Thirty-day-old seedlings were moved to the screen house and final evaluations were carried out six weeks after the plants were transferred to the screen house. Out of 150 F$_2$ plants, 27 plants were assessed between grades 0 to 2, and 123 plants were scored as 3 or 4 (Fig 1A). Among 13 plants of the resistant control LP97, 9 were scored between grades 0 to 2, and 4 plants were scored at grade 3, however, no plant showed complete susceptibility (Figs 1A and S2). All 13 plants of the susceptible control line ECW30R were scored at grade 4 at the final evaluation (Figs 1A and S2). The frequency distribution curve showed a negatively skewed distribution, indicating the involvement of QTLs in controlling resistance (Fig 1A).

An additional F$_2$ population, “Eagle-F$_2$”, which contained 184 individuals derived from the commercial resistant F$_1$ hybrid “Eagle-F$_1$” was also evaluated under the same screen house conditions in 2019 from March to June. Out of 184 F$_2$ plants, 69 plants were scored at grades 0 or 1 and 115 were scored between grades 2 and 4 (Figs 1B and S2). For marker validation, F$_2$ plants scored at grades 0 or 1 were considered to be resistant “R” and plants between grades 2 to 4 were designated as susceptible “S”. Eagle-F$_1$ and ECW30R were used as resistant and

![Fig 1. Frequency distribution of disease severity. Evaluation of resistance of the LP97-F$_2$ population and control plants (A) and of Eagle-F$_1$ and Eagle-F$_2$ plants (B) to Pepper yellow leaf curl virus (PepYLCV) Indonesia strain performed 6 weeks after transfer of seedlings to the screen house. The disease severity index from 0 to 4 was used.](https://doi.org/10.1371/journal.pone.0264026.g001)
susceptible controls, respectively. Out of 40 Eagle-F₁ plants, 11 were scored at grades 0 or 1 and 29 plants were scored at grades 2 or 3 (Figs 1B and S2). However, no plant was scored at grade 4, whereas all susceptible control “ECW30R” plants were scored at grade 4 (Fig 1B). These results also indicated that the resistance against PepYLCV is governed by multiple loci.

**SNP discovery and linkage-map construction**

We performed genotyping-by-sequencing to obtain the single nucleotide polymorphism (SNPs) markers to construct a genetic linkage map of LP97-F₂ population. The genotyping of 92 randomly selected F₂ samples and two replicates for each control was performed using genotyping-by-sequencing (GBS) following digestion with EcoRI and MseI. The Illumina paired-end sequencing of the GBS libraries and controls generated 247.8 million raw reads. After the trimming of raw reads with quality filters and alignment of the reads to the reference genome, 55,460 SNPs were obtained (Fig 2). The SNP density distribution revealed that the SNPs were uniformly distributed across the chromosomes (Fig 2). In the next step, after parental calling between resistant and susceptible controls, the removal of more than 50% of missing data and filtering unequally distributed SNPs, a total of 3,249 high-quality SNPs were obtained, which were used for the construction of bins (Fig 2).

The sliding window method was employed to estimate the genotyping error and missing data before the construction of a genetic linkage map [27, 28]. First, recombination breakpoints were ascertained by sliding 15 SNPs sequentially as a single window, which resulted in 1,140 bins (Fig 2 and Table 2). A recombination bin map of the F₂ population showed the admixture of resistant, susceptible and heterozygote segments (S3 Fig). A high-density genetic linkage map was then constructed using 3,249 high-quality SNPs, which yielded 1,140 bins representing a total genetic distance of 1,737.1 cM in length (Table 2). Among the 12 linkage groups, maximum and minimum genetic distances of 214.9 and 72.9 cM were obtained for chromosomes P₁ and P₈, respectively (Table 2). To assess the quality of the genetic map, collinearity analysis was conducted to compare the physical and genetic positions of the bin (Fig 2).
Most of the bins showed the same order on the corresponding chromosomes of the reference genome, except for P10 and P12, which deviated slightly in the collinearity analysis (Fig 3). This high-density genetic linkage map was used in further QTL analysis for PepYLCV resistance.

### Identification of QTLs for resistance to PepYLCV

The genetic map of the LP92-F$_2$ population was used to detect the QTLs for resistance to PepYLCV. In total, three QTLs for PepYLCV resistance that explained phenotypic variation ($R^2$) ranging from 6.3 to 31.7% were identified across the pepper genome (Fig 4 and Table 3). These QTLs for PepYLCV resistance were detected on chromosomes P1, P7 and P12 (Fig 4 and Table 3). The major QTL was on P7 at 21.01 cM, corresponding to 26–32 Mb in the pepper genome.

### Table 2. Summary of the bins and genetic linkage map for the LP97-F$_2$ population.

| Chromosome | Number of SNPs | Number of bins | Physical length of bin (Mb) | Genetic distance of Bin (cM) |
|------------|----------------|----------------|-----------------------------|-----------------------------|
| P1         | 269            | 125            | 332.7                       | 214.9                       |
| P2         | 233            | 91             | 175.7                       | 128.4                       |
| P3         | 273            | 117            | 291.1                       | 154.2                       |
| P4         | 141            | 60             | 248.3                       | 90.6                        |
| P5         | 221            | 82             | 250.1                       | 120.8                       |
| P6         | 274            | 116            | 249.4                       | 183.6                       |
| P7         | 238            | 93             | 262.8                       | 147                         |
| P8         | 84             | 39             | 173.4                       | 72.9                        |
| P9         | 388            | 85             | 271.6                       | 123.6                       |
| P10        | 396            | 124            | 240.1                       | 186.7                       |
| P11        | 550            | 139            | 271.1                       | 193.2                       |
| P12        | 182            | 69             | 257.6                       | 121.2                       |
| Total      | 3,249          | 1,140          | 3,023.90                    | 1,737.10                    |

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![Fig 3. Collinearity between the genetic map and the physical map of pepper.](https://doi.org/10.1371/journal.pone.0264026.g003)
reference genome, and was named `peplcv-7`. The QTL `peplcv-7` explained 31.7% of the phenotypic variation ($R^2$) with a LOD score of 8.6 (Fig 4). Two minor QTLs, `peplcv-1` and `peplcv-12`,

![Bin based linkage map showing the locations of QTLs for resistance to PepYLCV.](https://doi.org/10.1371/journal.pone.0264026.g004)

Table 3. QTL analysis for resistance to PepYLCV Indonesia strain in the LP97-F$_2$ population using composite interval mapping.

| QTLs   | Chromosome | Genomic position (cM) | Flanking bins        | Physical position (Mbp) | LOD | $R^2$ (%) | Dominant effect |
|--------|------------|-----------------------|----------------------|-------------------------|-----|-----------|-----------------|
| peplcv-1 | 1          | 185.41                | Chr1-LCV-102–103     | 254–256                 | 2.7 | 9.9       | 6.483           |
| peplcv-7 | 7          | 21.01                 | Chr7-LCV-16–21       | 26–32                   | 8.6 | 31.7      | 3.799           |
| peplcv-12 | 12         | 113.51                | Chr12-LCV-94–96      | 252–256                 | 4.2 | 6.3       | 10.936          |

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were detected at 185.41 cM and 113.51 cM, corresponding to 254–256 Mb and 252–256 Mb on P1 and P12, respectively. The peplcv-1 and peplcv-12 QTLs explained 9.9% and 6.3% of the phenotypic variation (R$^2$), respectively, with LOD scores of 2.7 and 4.2, respectively (Fig 4 and Table 3). The QTL results were verified using an alternative software R/qtl, which also detected similar QTLs at the same locations (S4 Fig). These QTLs were used for further investigation to develop single markers and predict candidate genes linked to PepYLCV resistance.

**Potential candidate genes that confer resistance to PepYLCV**

We searched for candidate genes related to PepYLCV resistance within the detected QTL regions. The flanking sequences of the QTL regions were retrieved from the C. annuum cv. “Dempsey” reference genome database [Unpublished] using upper and lower delimiting bin markers (Table 3). The QTL peplcv-7 on P7 was inferred to contain 141 genes (S1 Table), including four genes that encode leucine-rich repeat domain-containing proteins, which are known to be associated with disease resistance (Table 4). By contrast, the QTL peplcv-12 on P12 was inferred to contain 300 genes (S1 Table), including seven genes that encode leucine-rich repeat domain-containing proteins, two R1 gene for resistance to late blight, and one gene that encodes a protein with an Rx N-terminal domain, which is also known to be associated with disease resistance (Table 4). These genes represent potential candidate resistance genes for resistance to PepYLCV in pepper.

**Validation of SNPs linked to PepYLCV resistance**

The single markers linked to the QTLs detected for resistance to PepYLCV were developed and validated. The flanking bin markers of QTL peplcv-7 included the bins from Chr7-LCV-16 to Chr7-LCV-21 (Table 3). These bin markers were used for box-plots. For the bin marker Chr7-LCV-16, the level of PepYLCV resistance of the F$_2$ plants that harbored the homozygous resistance allele was significantly higher than that for plants carrying the heterozygous and the homozygous alleles of the susceptible parents (Fig 5A). Similarly, a significant difference in the level of PepYLCV resistance was observed among F$_2$ plants that carried a homozygous resistance allele for the Chr12-LCV-94 bin marker in the QTL peplcv-12 (Fig 5B).

### Table 4. Candidate genes associated with PepYLCV resistance and their functions.

| Chromosome | Start position | End position | GO description                      |
|------------|----------------|--------------|-------------------------------------|
| P7         | 31363005       | 31372454     | Leucine-rich repeat N-terminal domain |
| P7         | 29255606       | 29258419     | Leucine-rich repeat                  |
| P7         | 29241237       | 29245815     | Leucine-rich repeat                  |
| P7         | 29162748       | 29164059     | Leucine-rich repeat                  |
| P12        | 252998942      | 253002525    | Late blight resistance protein R1    |
| P12        | 253709733      | 253714975    | Leucine-rich repeat                  |
| P12        | 253006918      | 253025995    | Rx N-terminal domain                 |
| P12        | 255842999      | 255845707    | Leucine-rich repeat                  |
| P12        | 253283936      | 253289693    | Leucine-rich repeat N-terminal domain |
| P12        | 252990677      | 252994517    | Late blight resistance protein R1    |
| P12        | 255451163      | 255451900    | Leucine-rich repeat                  |
| P12        | 253304077      | 253307441    | Leucine-rich repeat N-terminal domain |
| P12        | 255851047      | 255854299    | Leucine-rich repeat                  |
| P12        | 255367051      | 255373032    | Leucine-rich repeat                  |

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To validate these results further, the flanking sequences of SNPs in those bin markers were retrieved from the reference genome and additional markers were developed (Table 5). The high-resolution melt (HRM) genotyping assay was used to genotype the additional Eagle-F2 population and commercial F1 hybrids. The developed markers (Chr7-LCV-7 and Chr12-LCV-12) clearly distinguished the resistant and susceptible controls in a parental polymorphism survey (Fig 6A and 6B). The markers Chr7-LCV-7 and Chr12-LCV-12 were validated using commercial PepYLCV-resistant F1 hybrids (Table 1 and S2 Table). Among these, Sonal and Sarangi were determined to be resistance genotypes, whereas Eagle, Vikrant, Armour and Romyz1 were classified as heterozygous genotypes by the Chr7-LCV-7 marker on P7 (Fig 6C and 6D and S2 Table). The commercial F1 hybrids Eagle, Armour and Romyz1 were genotyped as resistant, whereas Sonal and Vikrant were determined to be the susceptible genotype for the Chr12-LCV-12 marker on P12 (Fig 6C and 6D and S2 Table).

The marker genotyping results for the Eagle-F2 population are shown in the (Fig 6E and 6F and S3 Table). The single marker Chr7-LCV-7 from the QTL peplcv-7 showed a genotype and phenotype matching rate of 22% among F2 plants (Fig 6G and S3 Table). However, when the heterozygotic genotype was considered as susceptible, the marker genotype and phenotype matching rate increased to 55% (Fig 6G and S3 Table). Another single marker (Chr12-LCV12) developed within QTL peplcv-12 on P12 showed a genotype and phenotype matching rate of 20% among F2 plants (Fig 6H and S3 Table). Similarly, when the heterozygous genotype was considered as susceptible, the genotype and phenotype matching rate of the marker increased to 65% (Fig 6H and S3 Table). Furthermore, annotation of the intra- and intergenic

Table 5. Marker sequence information used for QTL validation and genotyping.

| Primer     | Chromosome | Physical position (bp) | Sequence (5’ to 3’)            |
|------------|------------|------------------------|--------------------------------|
| Chr7-LCV-7_F | P7         | 26705882               | CTGATAACTGACAGTTTAGATAGGAATTGG |
| Chr7-LCV-7_R | P7         | 26706033               | CAACTCAGTCTATAACCGGTGTATG      |
| Chr7-LCV-12_F | P12        | 256125396              | TTTTAAAGTCTCGTGGAAGGACCGCA     |
| Chr7-LCV-12_R | P12        | 256125557              | CTATTAAAAGCGGAGTTGGTTTGCA      |

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variants in the QTL regions performed by snpEff revealed the presence of 366 intergenic SNPs, including 12 upstream gene variants and 8 downstream gene variants in QTL peplcv-7 on P7 (S4 Table). By contrast, 102 intergenic SNPs, including 27 upstream variants and 43 downstream variants, were annotated in the QTL peplcv-12 on P12 (S4 Table). These SNPs can be useful to develop molecular markers for PepYLCV resistance in pepper.

**Discussion**

In this study, we mapped the novel QTL linked to PepYLCV resistance through viruliferous whitefly-mediated artificial screening of an F₂ segregating population and using genotyping-by-sequencing based QTL mapping. To the best of our knowledge, this is the first report of QTLs associated with resistance to PepYLCV in pepper. We also developed and validated single markers linked to the resistant QTLs using a different F₂ segregating population and commercial cultivars.
Previous reports described that resistance to PepYLCV is controlled by single recessive and dominant genes [17, 18, 34]. Genetic studies of virus resistance against leaf curl viruses during 1989–1990 showed that resistance was governed by monogenic recessive genes in pepper (C. annuum) [35]. Resistance to PepYLCV was evaluated using six genotypes that were resistant in the field. This revealed that PepYLCV resistance in ‘Punjab Lal’ pepper variety was recessive, because F1 plants were susceptible in artificially challenged conditions as well as in field conditions [20]. In a research report, germplasm that was resistant against Chilli leaf curl virus (ChiLCV-VNS; Varanasi isolate) and markers that were significantly linked to ChiLCV-VNS resistance were revealed that, resistance was governed by major recessive genes [21]. In another study which was carried out to elucidate the inheritance of resistance all types of gene actions including additive, dominant and epistatic gene interactions for virus resistance were revealed in interspecific crosses between C. annuum L. and C. frutescens L. [24]. An inheritance study of resistance to PepLCV in a partially compatible interspecific cross (C. annuum PBC-535 x C. chinense Bhut Jolokia) revealed the monogenic recessive nature of PepLCV resistance [7]. A recent study using the C. annuum BaPep-5 resistant accession inoculated with PepLCV by graft transmission and agroinfiltration showed that resistance to PepLCV was governed by a single recessive locus on chromosome P5, although susceptible and resistant plants did not segregate in a 3:1 ratio [17]. However, the evaluation of resistance against PepYLCV Indonesia strain mediated by whiteflies in the present study showed that resistance is controlled by QTL.

Tomato is a closely related species to pepper and TYLCV shares a high sequence similarity with PepYLCV [1, 2]. Several QTLs for resistance against TYLCV have been mapped in wild species of tomato, including Solanum chilense (Ty-1, Ty-3, Ty-4, and Ty-6), S. habrochaites (Ty-2), and S. peruvianum (ty-5) [3, 36–41]. Among these loci, Ty-1 was initially mapped to chromosome 6 using a backcrossed population from a cross between S. chilense and S. lycopersicum [41]. Ty-3 was mapped in S. chilense accessions (LA1932, LA2779, and LA1938) to chromosome 6 [37, 42]. Another resistance gene, Ty-2, was identified on chromosome 11 [39, 43] and was recently shown to encode a protein containing a nucleotide-binding domain and leucine-rich repeat (NB-LRR) [44]. A previous study on resistant gene analogues in pepper reported 5 different resistant gene classes including NB-LRR conferring resistance against multiple viruses such as Cucumber mosaic virus (CMV), Chilli veinal mottle potyvirus (ChiVMV), Chilli leaf curl virus (ChiLCV) [45]. Here, we identified a total of 11 (NB-LRR) genes as candidate resistant genes in the QTL regions. Among these, four were in QTL peplcv-7 and seven were in QTL peplcv-12 vicinity. In tomato, the TYLCV resistance locus Ty-4 was mapped to chromosome 3 and a locus for recessive resistance, ty-5, was mapped to chromosome 4 and encodes a messenger RNA surveillance factor Pelota (Pelo) that is associated in the ribosome recycling-phase of protein synthesis [46]. Recently, a resistant locus pepy-1 on pepper chromosome 5 was fine mapped in C. annuum and found to encode an RNA surveillance factor Pelota in BaPep-5 variety [17]. In tomato, a major begomovirus-resistant QTL, Ty-6, was mapped to chromosome 10 and strong resistance against TYLCV was obtained when Ty-6 was pooled with Ty-3 or ty-5 [3, 38]. Further synteny and collinearity analyses would identify any conserved QTL region between pepper and tomato that is responsible for PepYLCV resistance.

Molecular markers linked to potential resistance loci can provide information for early selection and are useful for breeding horticultural crops resistant to viruses [47]. Several molecular markers associated with virus resistance loci in pepper and tomato has been developed to expedite marker-assisted breeding [48, 49]. Introgression of resistance genes into cultivated varieties has been the principal route of breeding cultivars that are resistant to viruses such as Tomato yellow leaf virus (TYLCV), Tomato spotted wilt virus (TSWV), Pepper yellow leaf curl virus resistance in pepper.
*Leaf curl virus* (PepYLCV) and *Chilli veinal mottle virus* (ChiVMV) [49, 50]. The major markers linked to tomato yellow leaf disease (TYLCD) that have been developed to date include a closely linked molecular marker, SCAR1, for screening of the Ty-1 locus [51], P6-25 and FLUW25 for evaluation of Ty-3, Ty-3a, and Ty-3b loci [37, 50, 52], and SCAR2 and P1-16 for the detection of Ty-2 [39]. Despite this progress, fewer markers for resistance to PepYLCV have been reported.

Genotyping-by-sequencing (GBS)-based SNP markers represent an advancement in marker-assisted selection. In this study, we developed and validated new markers based on GBS-SNPs, named Chr7-LCV-7 and Chr12-LCV-12, for the marker-assisted selection for PepYLCV resistance breeding in pepper. The single marker Chr7-LCV-7 developed for the QTL *peplcv*-7 on P7 showed a genotype and phenotype matching rate in F2 plants of 22%. However, when the heterozygous genotype was considered as susceptible, the marker genotype and phenotype matching rate increased to 55%. Another single marker, Chr12-LCV-12, which was developed for the QTL *peplcv*-12 on P12 showed a genotype and phenotype matching rate in F2 plants of 20%. However, when the heterozygous genotype was considered susceptible, the marker genotype and phenotype matching rate increased to 65%. These markers can therefore improve the accuracy of selection during PepYLCV-resistance breeding in pepper. In this study, the validation of markers using commercial resistant pepper genotypes revealed a correlation between the phenotypic and genotypic data. The commercial hybrids Sonal and Sarangi showed a resistant genotype with marker Chr7-LCV-7, whereas the marker Chr12-LCV-12 defined them as susceptible. This discrepancy might be because the resistance for PepYLCV from different sources has been incorporated in these commercial F1 hybrids.

**Conclusions**

This study provides information regarding the genetic mapping of loci that confer resistance against PepYLCV. We reported the first PepYLCV-resistance QTLs and mapped them to pepper chromosomes P1, P7 and P12 using whitefly-mediated artificial screening and GBS-based linkage mapping approaches. The detected QTLs explained in total 47.9% of the phenotypic variation (R2) in a segregating F2 population. We further developed single markers linked to the resistance QTLs on P7 and P12 and validated them in an additional F2 population and commercial resistant F1 hybrids. The novel resistance loci and markers developed here will accelerate breeding programs for PepYLCV resistance in pepper.

**Supporting information**

S1 Fig. Disease scale used for resistance evaluation. (TIF)

S2 Fig. Resistant and susceptible controls. (TIF)

S3 Fig. Bin map of LP97-F2 population. (TIF)

S4 Fig. QTLs verification using Rqtl and comparison of WinQTL Cartographer results. (TIF)

S1 Table. Candidate genes in QTL regions associated with resistance to PepYLCV. (XLSX)

S2 Table. Marker validation using resistant commercial F1 hybrids. (XLSX)
S3 Table. Marker validation using an additional F$_2$ population.
(XLSX)

S4 Table. Intergenic SNPs in QTL regions.
(XLSX)

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References
1. Shafiq M, Asad S, Zafar Y, Briddon RW, Mansoor S. Pepper leaf curl Lahore virus requires the DNA B component of Tomato leaf curl New Delhi virus to cause leaf curl symptoms. Virol J. 2010; 7: 367. https://doi.org/10.1186/1743-422X-7-367 PMID: 21144019

2. An JW, Lee JH, Choi S, Venkatesh J, Kim JM, Kwon JK, et al. Identification of the determinant of tomato yellow leaf curl Kanchanaburi virus infectivity in tomato. Virus Res. 2021; 291: 198192. https://doi.org/10.1016/j.virusres.2020.198192 PMID: 33058965

3. Gill U, Scott JW, Shekasteband R, Ogundiwin E, Schuit C, Francis DM, et al. Ty-6, a major begomovirus resistance gene on chromosome 10, is effective against Tomato yellow leaf curl virus and Tomato mottle virus. Theor Appl Genet. 2019; 132: 1543–1554. https://doi.org/10.1007/s00122-019-03298-0 PMID: 30758531

4. Barchenger DW, Yule S, Jeeatid N, Lin SW, Wang YW, Lin TH, et al. A novel source of resistance to pepper yellow leaf curl Thailand virus (PepYLCThV) (Begomovirus) in Chile pepper. HortScience. 2019; 54: 2146–2149. https://doi.org/10.21273/HORTSCII14484-19

5. Chattopadhyay B, Singh AK, Yadav T, Fauquet CM, Sarin NB, Chakraborty S. Infectivity of the cloned components of a begomovirus: DNA beta complex causing chilli leaf curl disease in India. Arch Virol. 2008; 153: 533–539. https://doi.org/10.1007/s00705-007-0017-2 PMID: 18175042

6. Kushwaha N, Sahu PP, Prasad M, Chakraborty S. Chilli leaf curl virus infection highlights the differential expression of genes involved in protein homeostasis and defense in resistant chilli plants. Appl Microbiol Biotechnol. 2015; 99: 4757–4770. https://doi.org/10.1007/s00253-015-6415-6 PMID: 25693670

7. Rai VP, Kumar R, Singh SP, Kumar S, Kumar S, Singh M, et al. Monogenic recessive resistance to Pepper leaf curl virus in an interspecific cross of Capsicum. Sci Hortic (Amsterdam). 2014; 172: 34–38. https://doi.org/10.1016/j.scienta.2014.03.039
8. Kenyon L, Tsai WS, Shih SL, Lee LM. Emergence and diversity of begomoviruses infecting solanaceous crops in East and Southeast Asia. Virus Res. 2014; 186: 104–113. https://doi.org/10.1016/j.virusres.2013.12.026 PMID: 24440320

9. Pernezny KL, Roberts PD, Murphy JF, Goldberg NP. Compendium of pepper diseases. APS Press, St. Paul, MN. 2003.

10. Moriones E, Navas-Castillo J. Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. Virus Res. 2000; 71: 123–134. https://doi.org/10.1016/s0168-1702(00)00193-3 PMID: 11137167

11. Anaya-López JL, Torres-Pacheco I, González-Chavira M, Garzon-Tiznado JA, Pons-Hernández JL, Guevara-González RG, et al. Resistance to geminivirus mixed infections in Mexican wild peppers. HortScience. 2003; 38: 251–255.

12. Kumar S, Kumar S, Singh M, Singh AK, Rai M. Identification of host plant resistance to pepper leaf curl virus in chilli (Capsicum species). Sci Hort (Amsterdam). 2006; 110: 359–361. https://doi.org/10.1016/j.scienta.2006.07.030

13. Singh AK, Kushwaha N, Chakraborty S. Synergistic interaction among begomoviruses leads to the suppression of host defense-related gene expression and breakdown of resistance in chilli. Appl Microbiol Biotechnol. 2016; 100: 4035–4049. https://doi.org/10.1007/s00253-015-7279-5 PMID: 26780359

14. Srivastava A, Mangal M, Saritha RK, Kalia P. Screening of chilli pepper (Capsicum spp.) lines for resistance to the begomoviruses causing chilli leaf curl disease in India. Crop Prot. 2017; 100: 177–185. https://doi.org/10.1016/j.cropro.2017.06.015

15. Koeda S, Homma K, Tanaka Y, Kesumawati E, Zakaria S, Kanzaki S. Highly efficient agroinoculation method for tomato plants with tomato yellow leaf curl kanchanaburi virus. Hort J. 2017; 86: 479–486. https://doi.org/10.2503/hortj.OKD-049

16. Koeda S, Homma K, Tanaka Y, Onizaki D, Kesumawati E, Zakaria S, et al. Inoculation of capsicums with Pepper Yellow Leaf Curl Indonesia virus by combining agroinoculation and grafting. Hort J. 2018; 87: 364–371. https://doi.org/10.2503/hortj.OKD-137

17. Koeda S, Onouchi M, Mori N, Pohan NS, Nagano AJ, Kesumawati E. A recessive gene pepy-1 encoding Pelota confers resistance to begomoviruses isolates of PepYLCIV and PepYLCAV in Capsicum annuum. Theor Appl Genet. 2021; 134: 2947–2964. https://doi.org/10.1007/s00122-021-03870-7 PMID: 34081151

18. Thakur H, Jindal SK, Sharma A, Dhaliwal MS. Molecular mapping of dominant gene responsible for leaf curl virus resistance in chilli pepper (Capsicum annuum L.). 3 Biotech. 2020; 10: 1–10. https://doi.org/10.1007/s13205-019-1978-z PMID: 31815083

19. Vijeth S, Sreelathak I, Aiswarya CS, Kaushik P. Screening of Popular Indian Chili Pepper (Capsicum annuum L.) Genotypes Against the Chili leaf curl virus Disease. Plant Pathol J. 2020; 19: 121–131. https://doi.org/10.3923/ppj.2020.121.131

20. Kumar S, Kumar R, Kumar S, Singh M, Rai AB, Rai M. Reaction of Pepper leaf curl virus field resistance of chilli (C. annuum L.) genotypes under challenged condition. Veg Sci. 2009; 36: 230–232.

21. Rai VP, Rai AC, Kumar S, Kumar R, Kumar S, Singh M, et al. Emergence of new variant of chilli leaf curl virus in North India. Veg Sci. 2010; 37: 124–128.

22. Kumar S, Kumar R, Kumar S, Singh AK, Singh M, Rai AB, et al. Incidence of leaf curl disease on Capsicum germplasm under field conditions. Indian J Agric Sci. 2011; 81: 187–189.

23. Thakur H, Jindal SK, Sharma A, Dhaliwal MS. Chilli leaf curl virus disease: a serious threat for chilli cultivation. J Plant Dis Prot. 2018; 125: 239–249. https://doi.org/10.1007/s41348-018-0146-8

24. Anandhi K, Abdul Khader KM. Gene effects of fruit yield and leaf curl virus resistance in interspecific crosses of chilli (Capsicum annuum L. and C. frutescens L.). J Trop Agric. 2011; 49: 107–109.

25. Thakur H, Jindal SK, Sharma A, Dhaliwal MS. A monogenic dominant resistance for leaf curl virus disease in chilli pepper (Capsicum annuum L.). Crop Prot. 2019; 116: 115–120. https://doi.org/10.1016/j.cropro.2018.10.007

26. Yang HB, Liu W yee, Kang WH, Kim JH, Cho HJ, Yoo JH, et al. Development and validation of L allele-specific markers in Capsicum. Mol Breed. 2012; 30: 819–829. https://doi.org/10.1007/s11032-011-9666-7

27. Han K, Jeong HJ, Yang HB, Kang SM, Kwon JK, Kim S, et al. An ultra-high-density bin map facilitates high-throughput QTL mapping of horticultural traits in pepper (Capsicum annuum). DNA Res. 2016; 23: 81–91. https://doi.org/10.1093/dnares/dsv038 PMID: 26744365

28. Siddique Mi, Lee H-Y, Ro N-Y, Han K, Venkatesh J, Solomon AM, et al. Identifying candidate genes for Phytophthora capsici resistance in pepper (Capsicum annuum) via genotyping-by-sequencing-based QTL mapping and genome-wide association study. Sci Rep. 2019; 9: 1–15. https://doi.org/10.1038/s41598-018-37186-2 PMID: 30626917
29. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, et al. Circos: An information aesthetic for comparative genomics. Genome Res. 2009; 19: 1639–1645. https://doi.org/10.1101/gr.092759.109 PMID: 19541911

30. Wang S, Basten C, Zeng Z. Windows QTL cartographer 2.5. Raleigh, NC: Department of Statistics, North Carolina State University, USA. 2011.

31. Gölz S, García-Gómez JM, Terol J, Williams TD, Nueda MJ, et al. High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acids Res. 2008; 36: 3420–3435. https://doi.org/10.1093/nar/gkn176 PMID: 18445632

32. Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w118; iso-2; iso-3. Fly (Austin). 2012; 6: 80–92. https://doi.org/10.4161/fly.19695 PMID: 22728672

33. Kim N, Kang WH, Lee J, Yeom SL. Development of Clustered Resistance Gene Analogs-Based Markers of Resistance to Phytophthora capsici in Chili Pepper. Biomed Res Int. 2019;2019. https://doi.org/10.1155/2019/1093186 PMID: 30719438

34. Maurya PK, Srivastava A, Mangal M, Talukdar A, Mondal B, Solanki V, et al. Genetic analysis for resistance to leaf curl disease in chilli peppers (Capsicum annum L.) under specific situations. Indian J Genet Plant Breed. 2019; 79: 741–748. https://doi.org/10.31742/JGPB.79.4.13

35. Bai SS, Singh J, Dhanju KC. Genetics of resistance to mosaic and leaf curl viruses in chilli (C. annuum L.). Indian J Virol. 1995; 11: 77–79

36. Anbinder I, Reuveni M, Azari R, Paran I, Nahon S, Shlomo H, et al. Molecular dissection of Tomato leaf curl virus resistance locus on chromosome 3 of Tomato. J Am Soc Hortic Sci. 2009; 134: 281–288. https://doi.org/10.21273/jashs.134.2.281

37. Ji Y, Schuster DJ, Scott JW. Ty-3, a begomovirus resistance locus near the Tomato yellow leaf curl virus resistance locus Ty-1 on chromosome 6 of tomato. Mol Breed. 2007; 20: 271–284. https://doi.org/10.1007/s11032-007-9089-7

38. Ji Y, Scott JW, Schuster DJ, Maxwell DP. Molecular mapping of Ty-4, a new Tomato yellow leaf curl virus resistance locus on chromosome 3 of Tomato. J Am Soc Hortic Sci. 2009; 134: 281–288. https://doi.org/10.21273/jashs.134.2.281

39. Scott JW, Hutton SF, Freeman JH. Fla. 8638B and Fla. 8624 tomato breeding lines with begomovirus resistance loci Ty-5 plus Ty-6 and Ty-6, respectively. HortScience. 2015; 50: 1405–1407. https://doi.org/10.1097/01horts.50.9.1405

40. Yang X, Caro M, Hutton SF, Scott JW, Guo Y, Wang X, et al. Fine mapping of the tomato yellow leaf curl virus resistance gene Ty-2 on chromosome 11 of tomato. Mol Breed. 2014; 34: 749–760. https://doi.org/10.1007/s11032-014-0072-9 PMID: 25076841

41. Zamir D, Ekstein-Michelson I, Zakay Y, Navot N, Zeidan M, Sarfatti M, et al. Mapping and introgression of a tomato yellow leaf curl virus tolerance gene, Ty-1. Theor Appl Genet. 1994; 88: 141–146. https://doi.org/10.1007/BF00225889 PMID: 24185918

42. Verlaan MG, Szinay D, Hutton SF, De Jong H, Kornelink R, Visser RGF, et al. Chromosomal rearrangements between tomato and Solanum chilense hamper mapping and breeding of the TYLCV resistance gene Ty-1. Plant J. 2011; 68: 1093–1103. https://doi.org/10.1111/j.1365-313X.2011.04762.x PMID: 21883550

43. Hanson P, Green S, Kuo G. Ty-2, a gene on chromosome 11 conditioning geminivirus resistance in tomato. Tomato Genet Coop Rep. 2006; 56: 17–18

44. Yamaguchi H, Ohashi J, Saito A, Ohyama A, Nishio T, Miyatake K, et al. An NB-LRR gene, TYNBS1, is responsible for resistance mediated by the Ty-2 Begomovirus resistance locus of tomato. Theor Appl Genet. 2018; 131: 1345–1362. https://doi.org/10.1007/s00122-018-3082-x PMID: 29532116

45. Naresh P, Krishna Reddy M, Reddy AC, Lavanya B, Lakshmana Reddy DC, Madhavi Reddy K. Isolation, characterization and genetic diversity of NBS-LRR class disease-resistant gene analogs in multiple virus resistant line of chilli (Capsicum annum L.). 3 Biotech. 2017; 7: 1–10. https://doi.org/10.1007/s13205-016-0582-8 PMID: 28398995

46. Lapidot M, Karniel U, Gelbart D, Fogel D, Evenor D, Kutsher Y, et al. A Novel Route Controlling Begomovirus Resistance by the Messenger RNA Surveillance Factor Pelota. PLoS Genet. 2015; 11: 1–19. https://doi.org/10.1371/journal.pgen.1005338 PMID: 26448569

47. Kang BC, Yeam I, Jahn MM. Genetics of plant virus resistance. Annu Rev Phytopathol. 2003; 43: 581–621. https://doi.org/10.1146/annurev.phyto.43.011205.141140 PMID: 16078696

48. Mangal M, Srivastava A, Sharma R, Kalia P. Conservation and dispersion of genes conferring resistance to tomato begomoviruses between tomato and pepper genomes. Front Plant Sci. 2017; 8: 1–10. https://doi.org/10.3389/fpls.2017.00001 PMID: 28220127
49. Lee JH, Chung DJ, Lee JM, Yeam I. Development and application of gene-specific markers for tomato yellow leaf curl virus resistance in both field and artificial infections. Plants. 2021; 10: 1–18. https://doi.org/10.3390/plants10010009 PMID: 33374801

50. Lee JH, An JT, Siddique MI, Han K, Choi S, Kwon JK, et al. Identification and molecular genetic mapping of Chili veinal mottle virus (ChiVMV) resistance genes in pepper (Capsicum annum). Mol Breed. 2017;37. https://doi.org/10.1007/s11032-017-0717-6

51. Nevame AYM, Xia L, Nchongboh CG, Hasan MM, Alam MA, Yongbo L, et al. Development of a new molecular marker for the resistance to tomato yellow leaf curl virus. Biomed Res Int. 2018;2018. https://doi.org/10.1155/2018/8120281 PMID: 30105248

52. Dong P, Han K, Siddique MI, Kwon J-K, Zhao M, Wang F, et al. Gene-Based Markers for the Tomato Yellow Leaf Curl Virus Resistance Gene Ty-3. Plant Breed Biotechnol. 2016; 4: 79–86. https://doi.org/10.9787/pbb.2016.4.1.79