Agr. Nat. Resour. 55 (2021) 654–663

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Genome scan for quantitative trait loci underlying *cucumber mosaic virus* resistance in *Capsicum annuum* 'CA2106'

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Article Info

Article history:
Received 6 May 2021
Revised 22 June 2021
Accepted 10 July 2021
Available online 31 August 2021

Keywords:
Capsicum annuum,
Cucumber mosaic virus (CMV),
DArTseq,
Disease resistance,
QTL

Abstract

Cucumber mosaic virus (CMV) is a serious disease that causes large economic losses in pepper (*Capsicum annuum*) crops globally. The genetic resistance of CMV in pepper is complex and the resistance reaction fluctuates across different pepper production areas. *C. annuum* 'CA2106', a variety from the Tropical Vegetable Research Center collection that has been identified as a new resistance source to CMV, shows a specific resistance response to a local CMV strain of Thailand (CMV_{KPS10}). The current study identified the genomic regions which control CMV resistance in 'CA2106' via a high-density genetic map constructed using single-nucleotide polymorphism (SNP) markers obtained through the DArTseq technology. A double haploid mapping population was developed from a cross between 'CA500' and 'CA2106' as susceptible and resistant parents, respectively. The genetic analysis revealed that CMV resistance in 'CA2106' is inherited quantitatively. In total, 2,398 SNP markers were mapped across the 12 pepper chromosomes covering a genetic distance of 1,310.18 cM with a mean marker distance of 0.56 cM between adjacent markers. This map was used to identify genetic regions associated with quantitative trait loci (QTLs) for CMV resistance using a restricted multiple-QTL model. Two novel QTLs (*Q1A* and *Q1B.2*), conferring resistance to the local CMV_{KPS10} strain were identified on chromosome 1, explaining 19.1% and 20.1% of the phenotypic variance, respectively. Three QTLs (*Q1B.1*, *Q6* and *Q12*) were located on chromosomes 1, 6 and 12, respectively. These findings will be helpful for developing closely linked markers to CMV resistance and facilitating the improvement of resistance to CMV in pepper.

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https://doi.org/10.34044/j.anres.2021.55.4.16

Introduction

Cucumber mosaic virus (CMV) is a member of the *Cucumovirus* genus and belongs to the *Bromoviridae* family, which is one of the five most important viruses infecting vegetable crops worldwide (Palukaitis et al., 1992). CMV has the largest host range, with over 1,200 plant species and 80 aphid species being reported as susceptible hosts (Palukaitis and García-Arenal, 2003). In pepper (*Capsicum* sp.), CMV can cause a wide range of symptoms including mosaic, mottling, yellow discoloration, leaf deformation, shoe stringing or leaf narrowing and fruit lesions, thereby markedly decreasing marketable yields (Palukaitis et al., 1992; Kapoor et al., 2018). Since CMV has a broad host range and many insect vectors, it cannot be completely controlled by chemicals. The most common method of control is the use of disease-resistant plants, which provides several advantages such as being the most economic, environmental-friendly and efficient long-term method (Yao et al., 2013).

A few decades ago, CMV resistance sources were identified from several accessions of *Capsicum* species. Most studies showed partial resistance control by multiple genes in different resistant sources such as *C. annuum* 'Perennial' (Caranta et al., 1997; Lapidot et al., 1997; Grube et al., 2000), *C. annuum* 'Vania' (Caranta et al., 2002) *C. annuum* 'Sapporo-oonaga' and 'Nanbu-oonaga' (Suzuki et al., 2003) *C. frutescens* 'BG2814-6' (Grube et al., 2000), *C. frutescens* 'LS1839-2-4' and *C. baccatum* 'PI439381-1-3' (Suzuki et al., 2003; Kang et al., 2010). These accessions have shown diverse mechanisms of resistance, including the inhibition of viral replication, cell-to-cell movement and long-distance movement of viral particles. Nevertheless, breeding for polygenic resistance is problematic because of the varying response across different environmental conditions and CMV isolates. However, a few reports have shown the resistance source was controlled by a single gene such as a dominant gene in *C. annuum* 'Bukang' (Kang et al., 2010) or a recessive gene in *C. annuum* 'Lam32' (Choi et al., 2018).

C. annuum 'CA2106' was derived from a pepper collection at the Tropical Vegetable Research Center (TVRC), Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom, Thailand and comprises a valuable genetic source that has exhibited a resistance response to the CMV Thailand isolate named CMV_{KPS10} (Patarapuwadol et al., 2010). The objective of the current study was to construct the genetic linkage map using single-nucleotide polymorphism (SNP) markers through the DArTseq approach and to identify the quantitative trait loci (QTLs) for CMV resistance in *C. annuum* 'CA2106'. The disease resistance genes in pepper 'CA2106' should be useful for developing molecular marker-assisted selection in pepper breeding programs.

Materials and Methods

Plant materials

Two pepper cultivars, 'CA500' and 'CA2106', were used as parents to produce the F_1 hybrid and were acquired from the TVRC. 'CA500' is a CMV-susceptible cultivar, while the 'CA2106' cultivar has good resistance to CMV with a specific response for the Thai strain (CMV_{KPS10}). In total, 101 double haploid (DH) lines were derived from anther culture of the F_1 hybrid (CA500 × CA2106). The 101 DH lines and parental lines were analyzed for mapping populations and CMV resistance. DH lines are valuable material and an optimum population for QTL mapping because they are homozygous in nature and this allows for replicated trials across years and locations (Collard et al., 2005).

Cucumber mosaic virus inoculation

The CMV_{KPS10} strain was used as a virus source for mechanical inoculation. The virus was propagated and maintained in tobacco plants which were provided by Asst Prof. Dr Sujin Patarapuwadol (Kasetsart University, Thailand). The inoculation was performed in four different periods, January–February 2017, February–March 2017, January–February 2018 and February–March 2018, assigned as the 1st, 2nd, 3rd and 4th inoculations, respectively. The mechanical inoculation was done on pepper seedlings aged 30 d. Nine pepper seedlings for each line (CA500, CA2106, F_1 hybrid and 101 DH lines) were used for CMV inoculation and disease assessment and two healthy peppers without inoculation were maintained as a control. The inoculum of CMV_{KPS10} was prepared from the symptomatic leaves of tobacco. An amount (1 g) of infected leaves was homogenized in 10 mL of 0.05 M phosphate buffer (pH 7.0) containing 2% sodium sulfite (Na_2SO_3). The pepper plants were rub-inoculated with sap extract including carborundum. The inoculated plants were kept insect-free in a mesh greenhouse under natural sunlight. Basic statistics, correlation coefficients and histograms of frequency were calculated for disease indices using the R Studio Team (2015) software and the R software package (R Core Team, 2015).

Detection of virus using indirect plate-trapped antigen enzyme-linked immunosorbent assay

The indirect plate-trapped antigen enzyme-linked immunosorbent assay (PTA-ELISA) test was used to determine the concentration of virus at 4 wk of plant inoculation in the 1st–3rd experiments, following the method of Clark and Adams (1977) with modification. Eight inoculated plants from each line were used in PTA-ELISA reading. Leaves samples were collected from the top, middle and bottom positions of the inoculated plant and were combined into one sample of each plant for ELISA reading for three trials of inoculation. ELISA reading was not performed for the 4th trial due to technical problems, so only visual scoring was performed.

Samples were measured at an absorbance of 405 nm in an ELISA reader (Multiskan EX; LabSystems; Vantaa, Finland) and disease incidence was calculated using: Disease incidence (%) = (Number of infected plants) / (Total plants) × 100. The score system for the percentage of disease incidence was: < 20% = 1, 21–30% = 2, 31–50% = 3 and > 50% = 4.

Visual observation for disease and severity indices

Severity of infected pepper plants was determined by the visual observation in all four (1st–4th) inoculations. The symptom index was based on the severity of the mosaic and leaf distortion on a scale of 0–4, where: 0 = no symptoms; 1 = mild mosaic, no leaf distortion; 2 = strong mosaic, mild leaf distortion; 3 = severe mosaic and distortion, mild stunting; and 4 = severe mosaic, distortion, stunted plants (Monma and Sakata, 1977). The disease indices (DI) were based on the formula: DI = [(∑Number of plants in the symptom index × Symptom index) / (4 × Total number of plants)] × 100 (Yao et al., 2013). Additionally, the severity index was calculated using the formula: Severity index = ∑ (Symptom index × Number of plants with each symptom index) / Total number of plants. Visual observation for disease in the four trials was used to assess for the symptoms at days 7, 14, 21, 30, 37 and 44 after inoculation (DAI).

Disease rating

Disease rating was categorized by combining the data of disease incidence and severity. Correspondingly, disease severity was based on a scoring scale for the severity index where: 0.0–1.0 = 1, 1.1–2.0 = 2, 2.1–3.0 = 3 and > 3.0 = 4. The final scoring derived from the sum of disease incidence and disease severity data were applied for the disease-resistance rating of the DH progenies into four groups: total score ≤ 2.99 = resistant (R), 3–4.99 = moderately resistant (MR), 5–6.99 = susceptible (S) and 7–8 = highly susceptible (HS).

DNA extraction and genotyping using Diversity Arrays Technology (DArT) Pty Ltd -sequencing technology

Fresh, young leaf tissue around 5–10 g from seedlings aged 7–14 d was collected from the plant samples. The genomic DNA was extracted using a modified CTAB mini-prep procedure according to Fulton et al. (1995). DNA quantification was performed using a NanoDrop-1000 spectrophotometer (NanoDrop; Wilmington, DE, USA) and quality of DNA was determined using 1% agarose gel electrophoresis. Finally, 20 µL of 50 ng/µL genomic DNA of each sample was sent to Diversity Arrays Technology Pty Ltd (Canberra, ACT, Australia) for the generation of whole-genome genotyping (DArTseq). The genotyping of each DNA sample can be generated following the procedure described on the company website (https://www.diversityarrays.com). The sequence of each clone was aligned with *C. annuum* cv. CM334 as a reference genome from the Sol Genomics Network (https://solgenomics.net).

Genetic linkage analysis and quantitative trait loci mapping

The data quality of the total SNP markers from the DArTSeq polymorphism was evaluated based on the polymorphic information content value (PIC) in the range 0.8–1. The segregation ratios of the SNP markers in the population were examined using χ^2 analysis. SNP markers data with segregation ratios that differed from the expected ratio of 1:1 at $p < 0.05$ were classified as segregation distortion markers and removed as ambiguous SNP marker data. Then, the linkage map was constructed using JoinMap version 4.1 (Van Ooijen, 2006) with the Kosambi map function (Kosambi, 1944). Linkage groups were called at the LOD score of 4.0. The final linkage map was drawn graphically using the plotMap command from R/QTL packages by RStudio Team (2015).

The genetic linkage map was integrated with four trials of the CMV inoculation to identify and locate QTL positions using the MapQTL 4.0 software (Van Ooijen et al., 2002). The significant QTL was determined by the significant logarithm of odds (LOD) score threshold corresponding to a chromosome-wide *p* value of 0.05 (5%) by the test with 1,000 per mutations based on each trial of phenotypic data. Interval mapping (IM) was initially used to identify possible QTL markers. Subsequently, the nearest markers at the highest LOD peak of each putative QTL were used as cofactors for automatic cofactor selection option at $p < 0.02$ and significant marker

cofactors were used in restricted multiple-QTL model (rMQM) analysis. A second round of rMQM mapping was analyzed using cofactors for QTLs that were significant after the first round of rMQM mapping. The only QTLs considered significant were those having an LOD score higher than the 5% chromosome-wide LOD threshold in the second round of rMQM mapping. Visualization of the QTL locations was performed using MapChart 3.32 (Voorrips, 2002). Single marker analysis was included using Kruskal-Wallis-based one-way analysis of variance to determine the putative QTL.

Results and Discussion

Variation of cucumber mosaic virus response in doubled haploid population

In the 2017 measurement period, the resistant parent 'CA2106' had DI values of 38.89% and 33.33% in the 1st and 2nd inoculations, respectively. In 2018, the values were lower with a DI value of 25% for both the 3rd and 4th inoculation trials. The 'CA500', susceptible parent demonstrated a stable effect with DI values of 88.89% and 75% in the 1st and 2nd inoculations, respectively, whereas there were lower reactions in the 3rd and 4th inoculations in 2018, with DI values of 50% and 59.38%, respectively (Table 1). The inoculation was performed in four different periods. The 1st inoculation period was from January to February 2017 with minimum (Min T) and maximum air temperature (Max T) of 20.8°C and 30.7°C, respectively. The 2nd inoculation period was from February to March 2017 (Min T= 23°C, Max T= 38°C), the 3rd inoculation period was from January to February 2018 (Min T= 21°C, Max T= 30°C). Lastly, the 4th inoculation period was from February to March 2018 (Min T= 22°C, Max T= 34°C). These results indicated that the different air temperatures in 2017 and 2018 affected the disease development to differentiate the response of genotypes. The different response could stem from several factors during plant host-virus interaction such as plant growth stage, temperature, virus replication, virus movement and RNA silencing (Soler et al., 1998; Szittya et al., 2003; Chellappan et al., 2005; Zitter and Murphy, 2009; Zhang et al., 2012).

Table 1 *Cucumber mosaic virus* response based on disease index (DI) and disease resistance rating of double haploid (DH) population from a cross between CA500 and CA2106

Inoculation	Year	Period	Plant	DI (% ± SD)	Disease rating				
					Disease incidence (1–4)	Severity index	Disease severity (1–4)	Total score (2–8)	Disease-resistant rating
1	2017	Jan–Feb	CA500 (S)	88.89±0.53	4	3.56	4	8	HS
			CA2106 (R)	38.89±0.53	4	1.56	2	6	S
			F_1(CA500 × CA2106)	50±0.0	4	2	2	6	S
			DH population mean	48.26	3.67	1.9	2	5.67	S
			DH population range	8.33–75	1–4	0.33–3	1–3	2–7	R–HS
2	2017	Feb–Mar	CA500 (S)	75±0.0	4	3	3	7	HS
			CA2106 (R)	33.33±0.5	1	1.33	2	3	MR
			F_1(CA500 × CA2106)	41.67±0.5	1	1.67	2	3	MR
			DH population mean	57.03	1.95	2.27	3	4.95	MR
			DH population range	16.67–100	1–4	0.67–4	1–4	2–8	R–HS
3	2018	Jan–Feb	CA500 (S)	50±3.54	4	2	2	6	S
			CA2106 (R)	25±0.87	3	1	1	4	MR
			F_1(CA500 × CA2106)	20±0.84	3	0.8	1	4	MR
			DH population mean	46.02	3.40	1.84	2	5.4	S
			DH population range	0–75	1–4	0–3	1–3	2–7	R–HS
4	2018	Feb–Mar	CA500 (S)	59.38±0.92	ND	2.38	3	ND	ND
			CA2106 (R)	25±0.0	ND	1	1	ND	ND
			F_1(CA500 × CA2106)	36.11±0.53	ND	1.44	2	ND	ND
			DH population mean	54.09	ND	2.16	3	ND	ND
			DH population range	25–80.56	ND	1–3.22	1–4	ND	ND

R = resistant; MR = moderately resistant; S = susceptible; HS = highly susceptible; ND = no data.

The frequency distributions of DI in the DH population revealed continuous and normal distributions with a wide range of variation (Fig. 1 and Table 1). The mean values of the population for each trial showed that the data were close to the mid-parent. In addition, most F_1 hybrids had intermediate resistance, with DI values of 50%, 41.67%, 20% and 36.11% for the 1st, 2nd, 3rd and 4th trials respectively. The data indicated that the CMV resistance of the 'CA2106' line is partially resistant and is controlled by many genes or quantitative inheritance.

During virus transmission, air temperature affects the efficiency of pathogen multiplication and the establishment of infection in the host (Feil and Purcell, 2001). The effect of temperature was reported by Chung et al. (2015) in Chinese cabbage inoculated with *turnip mosaic virus* (TuMV). Their results showed that the optimal temperature for symptom expression of TuMV was 18–28°C and the accumulation of virus coat protein was better in plants grown at 23–28°C based on quantitative real-time polymerase chain reaction analysis. Nevertheless, with TuMV, they reported that the appearance of symptoms was delayed at high temperature (33°C). Similar results have been reported for other viruses, including *potato leafroll virus* (Tamada and Harrison, 1981) and *tomato bushy stunt virus* at elevated temperature (Jones et al., 1990), where the researchers mentioned that extreme temperatures could restrict viral replication and movement.

Disease resistance rating of double haploid progeny

The disease rating was based on the results of ELISA and visual observation. The resistant parent showed a low degree of disease incidence in the 2nd inoculation; however, there were increased levels of symptoms in the 1st and 3rd experiments with scores of 4 and 3, respectively. The susceptible parent had a score of 4 in all three experiments. All experiments produced similar results with the resistant parents having less CMV severity than the susceptible parents. However, the DH populations had variation in the severity index in the ranges 0.33–3, 0.67–4, 0–3, and 1–3.22 for the 1st, 2nd, 3rd and 4th inoculation trials, respectively (Table 1).

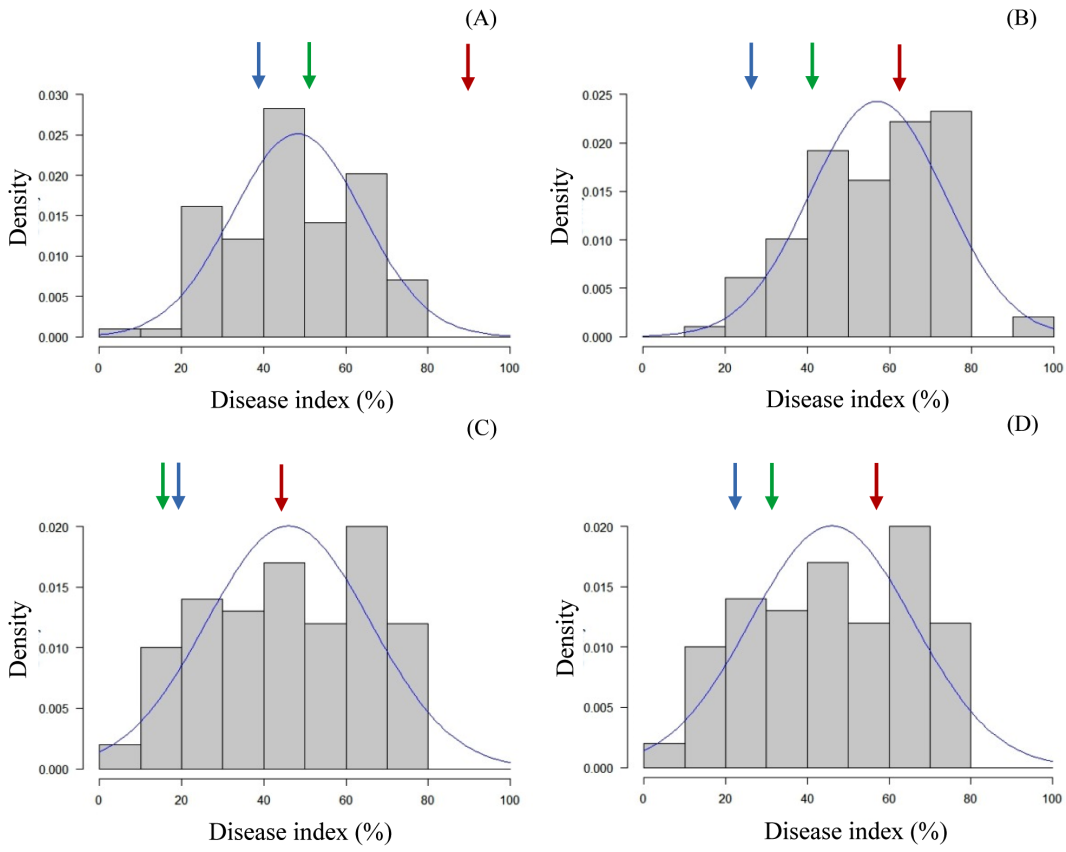

Fig. 1 Frequency distribution of disease index in double haploid population for: (A) 1st inoculation at 37 d after inoculation (DAI); (B) 2nd inoculation at 30 DAI; (C) 3rd inoculation at 30 DAI; (D) 4th inoculation at 37 DAI, where bars represent density in each DI class set at 10% intervals, parental value and F_1 indicated by blue, green and red arrows representing CA2106, F_1 and CA500, respectively, and blue curve indicates normal distribution

The DH progenies were categorized into one of four groups: resistant (R), moderately resistant (MR), susceptible (S) and highly susceptible (HS), with the parents' susceptibility for all experiments summarized in a total score. There was a wide range of variation in the CMV response among the DH populations during the three experiments (Table 1 and Fig. 2). High proportions of resistant groups (R and MR) were found specifically in the 2nd experiment, whereas high proportions of susceptible groups (S and HS) were found in the 1st and 3rd inoculations. In the 1st trial, 3 DH lines were identified as resistant, 13 as moderately resistant, 73 as susceptible and 4 as highly susceptible and there were 8, 26, 59 and 6 for R, MR, S and HS, respectively, in the 3rd inoculation. These results indicated that the pathogenicity of the CMV_{KPS10} isolate could be influenced by environmental conditions such as the air temperature and humidity.

Quantitative trait loci analysis and genetic linkage map

In total, 2,398 SNP markers were assigned into 12 linkage groups covering a genetic distance of 1,310.18 cM. The linkage groups were rearranged to chromosomes according to their order in the pepper genome and as inferred from *C. annuum* cv. CM334 at the Sol Genomics Network (https://solgenomics.net). The average marker distance was 0.56 cM per marker with a range of 0.47–0.71 cM, as shown in Table 2. The two largest chromosomes (1 and 3) contained 276 and 343 markers, respectively, with total distances of 165.56 cM and 162.13 cM, respectively. The smallest chromosome (8) consisted of 46 markers covering 30.86 cM. (Table 2 and Fig. 3). The genetic map had a high level of saturation compared to the same DArT genetic map in other studies, such as the genetic map of pea with an average distance between markers of 1.49 cM (Aznar-Fernandez et al., 2020) and the map for rust resistance in *Pisum sativum* having an average distance of 1.85 cM (Barilli et al., 2018). Additionally, the current study used filtration of the sequence error rate together with a reference genome to exclude suspicious markers and prevent false positive results. This genetic map was slightly dense with high quality markers that were sufficient for conducting QTL of CMV resistance.

Identification of quantitative trait loci for cucumber mosaic virus resistance

The identification of QTLs for CMV resistance was based on the DI using visualization for each trial independently to compare the phenotypic data with the genotypic data. The QTLs associated with resistance to CMV_{KPS10} are summarized in Table 3 and Fig. 4. In total, five significant QTLs located on chromosomes 1, 6 and 12 were identified. High LOD scores with values of 4.57, 4.83 and 4.74 were found on *Q1A*, *Q1B.2* and *Q12*, respectively, explaining 19.1%, 20.1% and 19.2%, respectively, of the phenotypic variation response to 1st_37DAI, 4th_21DAI and 1st_44DAI, respectively. In the QTLs, there was a small effect in *Q1B.1* and *Q6* consistently detected in the three trials of inoculation. *Q1B.1* responded to the 1st inoculation trial and *Q6* was a significant QTL for the 3rd inoculation trial. However, there were no QTLs detected in all inoculation trials, as the LOD scores were above the threshold.

The region of *Q1B* (*Q1B.1* and *Q1B.2*) had a large effect and was located in the range 90.4–95.4 cM. This QTL explained

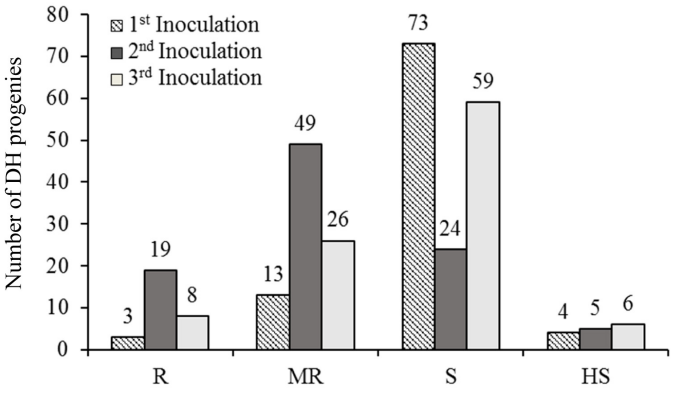

Fig. 2 Number of double haploid (DH) progenies for each disease rating categorized by total scores for 1st, 2nd and 3rd inoculations obtained from disease incidence and severity index, where R = resistant, MR = moderately resistant, S = susceptible and HS = highly susceptible

Table 2 Summary statistics of chromosome genetic linkage mapping

Chromosome	Total markers	Total distance (cM)	Average distance/marker (cM)
1	276	165.562	0.60
2	155	80.847	0.52
3	343	162.132	0.47
4	182	92.266	0.51
5	188	108.08	0.58
6	170	92.345	0.55
7	251	123.91	0.50
8	46	30.858	0.69
9	227	112.026	0.50
10	197	105.492	0.54
11	192	115.453	0.60
12	171	121.209	0.71
Mean	200	109.18	0.56
Total	2,398.00	1,310.18	na

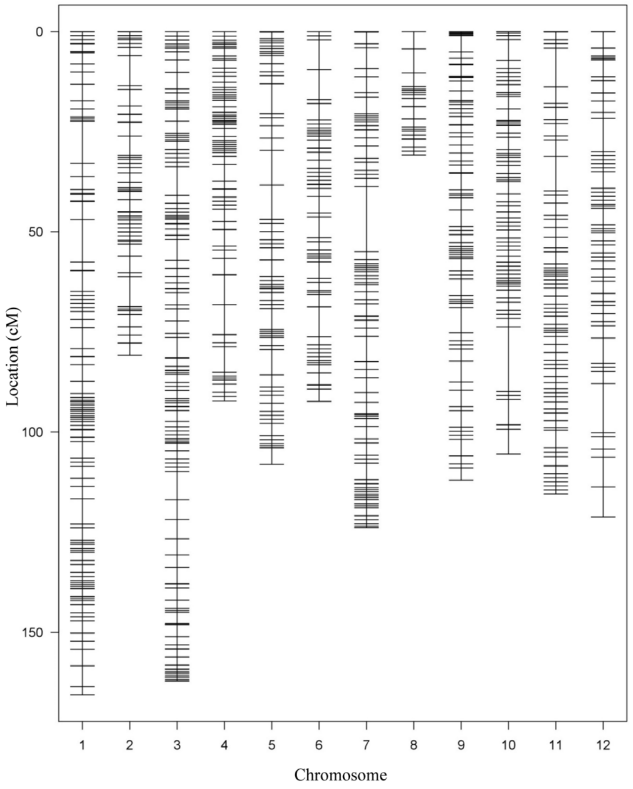

Fig. 3 Genetic length and marker distribution of 12 pepper chromosomes constructed from cross between CA500 and CA2106

genetic variation in the range 14.1–20.1%, corresponding to the 1st and 4th inoculation trials. The other region (*Q12*) was identified in chromosome 12 and was composed of marker c12p2044 at a genetic distance of 104.2 cM. The QTL peak at marker c12p2044 explained 19.2% of the phenotypic variance corresponding to 1st_44DAI with an LOD score of 4.74. This result revealed that the separate analysis of the different scoring dates and trials allowed investigation for further opportunities for resistance variation.

CMV resistance has been identified from several sources using different CMV strains and evaluation methods. Almost all of these studies have found partial inheritance controlled by multiple loci located on different chromosomes (Grube et al., 2000; Suzuki et al., 2003; Yao et al., 2013). Similarly, in the current study, all five CMV-resistant QTLs against the local isolate (CMV_{KPS10}) in *C. annuum* 'CA2106' were composed of three major effect (*Q1A*, *Q1B.1*, *Q1B.2* and *Q12*) and one minor effect (*Q6*) QTL. Two QTL regions (*Q1A* and *Q1B.2*) located on chromosome 1 had not been detected in previous studies. Thus, the current research revealed that *Q1A* and *Q1B.2* are novel QTLs specifically responsible for resistance to the local strain of Thailand (CMV_{KPS10}). Additionally, the *Q1B* region distributed near the position of the protein kinase represented the R gene. Consequently, this position is a pinpoint area that deserves further study for developing more closely linked markers. The QTL detected on chromosome 12 explained about 19.2% of the phenotypic variation commonly detected and represented the major effect QTL for resistance to CMV from other sources. Caranta et al. (2002) identified the major QTL on chromosome 12 (*cmv12.1*) associated with the partial restriction of CMV long-distance movement from the pepper cultivar 'Vania'. The next QTL on chromosome 12 was detected in the resistant pepper cultivar 'BJ0747' by Li et al. (2018).

The current study identified a minor effect QTL, *Q6,* which had an LOD score near the threshold level. The detected chromosomes in the current study were consistent with other studies of CMV-resistant QTLs identified in different resistant sources and viral strains. Chaim et al. (2001) identified the CMV-resistant QTL *cmv6.1* on chromosome 6, as the major effect presented in the CMV-resistant breeding line 3990 (*C. annuum* var. Perennial). In addition, Min et al. (2014) detected two recessive genes, *cmr3E* and *cmr3L*, that responded to CMV_{P1} in *C. annuum* 'I7339' were located on chromosome 6.

Table 3 Quantitative trait loci related to *cucumber mosaic virus* disease in double haploid population from F_1(CA500 × CA2106) in four inoculations

QTLs	Chr	Position	Marker at QTL peak[a]	Trait	LOD	LOD threshold[b]	PVE	KW[c]
Q1A	1	68.9	c1p229, c99p2545, c1p169	1st_37DAI	4.57	2.1	19.1	*******
Q1B.1	1	90.4	c1p128	1st_37DAI	3.36	2.1	14.5	-
Q1B.2	1	95.4	c1p230, c1p211, c1p233	1st_37DAI	3.28	2.1	14.1	******
Q1B.2	1	95.4	c1p230, c1p211, c1p233	4th_21DAI	4.83	2	20.1	*******
Q6	6	46.3	c6p1146, c6p1019	3rd_30DAI	3.09	1.8	10.7	*******
Q12	12	104.2	c12p2044	1st_44DAI	4.74	1.9	19.2	*******

QTLs = quantitative trait loci; Chr = chromosome location; LOD = logarithm of the odds;
PVE = the percentage of the variance explained; KW = Kruskal-Wallis analysis;
DAI = day after inoculation

[a] Peak QTL position on linkage group (LG) of maximum LOD (cM);

[b] Chromosome-wide LOD threshold determined using 1,000 permutations at $p < 0.05$;

[c] Significance levels: * < 0.1; ** < 0.05; *** < 0.01; **** < 0.005; ***** < 0.001; ****** < 0.0005; *******< 0.0001

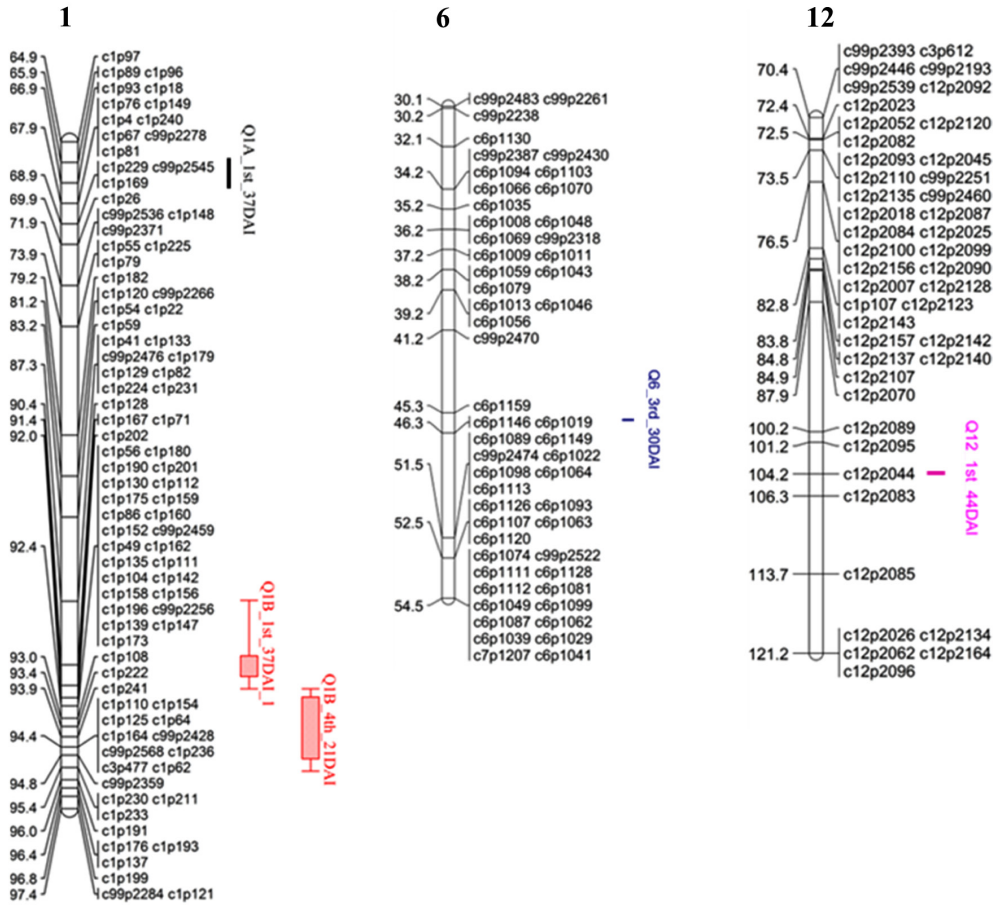

Fig. 4 Quantitative trait loci for *cucumber mosaic virus* resistance detected from double haploid population derived from F_1(CA500 × CA2106.), with four significant QTLs shown as black, red, blue and pink bars and texts, corresponding to *Q1A*, *Q1B*, *Q6* and *Q12*, respectively

The disease resistance genes that lie within the QTL regions were identified using the allele sequence of each significant QTL by blasting the queries against *C. annuum* cv. CM334. The matched sequence of *C. annuum* cv. CM334 was used to identify the presence of candidate genes by blasting using the public databases (NCBI). In total, four genes were collocated in three regions containing QTLs. Two candidate genes containing the kinase domain at genetic distances of 92.4 and 94.4 cM on chromosome 1 were present within the interval of *Q1B*. Additionally, the QTL region of *Q6* locates a nematode resistant-like protein and a TMV resistance protein on chromosome 6 (Table 4). The polymorphic SNPs between parents were A/T, G/A, C/T, C/T, G/A and G/T for c1p128, c1p230, c1p233, c1p211, c6p1146 and c6p1019, respectively, including the sequence of DArT clone in each significant marker are shown in Table 5. These SNPs could be good candidates for use in marker-assisted selection or gene cloning in the future.

Table 4 Candidate genes in or near area corresponding to significant markers

QTLs	Chr	Position in range of putative QTL (cM)	Resistant genes in or near area of significant markers*		
			Gene/protein	Position (cM)	Position (bp)
Q1A	1	68.9	No match		
Q1B	1	90.4–95.4	Cysteine-rich receptor-like protein kinase	92.4	92,495,639
			LRR receptor-like serine/threonine-protein kinase	94.4	69,580,840
Q6	6	46.3	Nematode resistance-like protein, TMV resistance protein N-like	26.1	205,519,960
Q12	12	104.2–113.7	No match		

QTLs = quantitative trait loci; Chr = chromosome location; LRR = leucine-rich repeats;
TMV = *tobacco mosaic virus*
* based on the results from NCBI Blast search.

Table 5 Single-nucleotide polymorphisms (SNPs) of parent, CA500 (S) and CA2106 (R), with sequences of DArTseq clone in each significant marker in *Q1B.1*, *Q1B.2* and *Q6* regions

QTLs	Marker	SNP between parent		Sequence
		CA500 (S)	CA2106 (R)	
Q1B.1	c1p128	AA	TT	TGCAGCTTGAACAGGATCTGGTGTAGTATATTCAAAAAAGGAAGTAATTTATATCAAA CCGATAGTTCT
Q1B.2	c1p230	GG	AA	TGCAGCATTTAGAAATTTCTCAATGTCTCAAAAGGAGAAGACTCTTGAGGGACT TGAGAAGGACCGCTG
	c1p233	CC	TT	TGCAGCAGTCTCATTTTTTGTCTAGCTGCTGCCAAGAAGGAAAACTTGCTTTTA CATCTATATGTATAC
	c1p211	CC	TT	TGCAGTTCAACAGCAGGATCTTCGGACGTGTGTGGCAGGGATTGTGATTGTAGGTAT GGATGACTGAGA
Q6	c6p1146	GG	AA	TGCAGATAGTCCAATAAAAAATTTGAGAGGAGGGAAAGGACCTCTCCTGGCT CGTGGATAGCATCTTGT
	c6p1019	GG	TT	TGCAGATTTCTTGATAGACACTTCCAATAATAGGCTACCAATTATGATGACCAAT CATCTTACAGATCG

QTLs = quantitative trait loci; R = resistant; S = susceptible

In conclusion, the genetic linkage map of the double haploid population derived from *C. annuum* 'CA500' and 'CA2106' was constructed using high-throughput DArTseq genotyping technology. Two novel QTLs (*Q1A* and *Q1B.2*) localized on chromosome 1 which confers resistance to the local strain of CMV in Thailand (CMV_{KPS10}) were identified. Additionally, candidate genes were identified to the QTL regions corresponding to disease-resistant genes and containing the kinase domain representing the R gene within the interval of *Q1B* (*Q1B.1* and *Q1B.2*). Resistant DH lines are a valuable source for improving CMV-resistant pepper using an identified marker associated with the detected QTLs.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This research was supported by the Center of Excellence on Agricultural Biotechnology, Office of the Permanent Secretary, Ministry of Higher Education, Science, Research and Innovation (AG-BIO/MHESI)**.** Dr Pitchayapa Mahasuk and Dr Anthimos Kampouridis provided English-editing assistance with an earlier draft. The Kasetsart University Research and Development Institute (KURDI), Bangkok, Thailand provided assistance with English editing.

References

Aznar-Fernández, T., Barilli, E., Cobos, M., Kilian, A., Carling, J., Rubiales, D. 2020. Identification of quantitative trait loci (QTL) controlling resistance to pea weevil (*Bruchus pisorum*) in a high-density integrated DArTseq SNP-based genetic map of pea. Sci. Rep. 10: 33. doi.org/10.1038/s41598-019-56987-7

Barilli, E., Cobos, M.J., Carrillo, E., Kilian, A., Carling, J., Rubiales, D. 2018. A high-density integrated DArTseq SNP-based genetic map of *Pisum fulvum* and identification of QTLs controlling rust resistance. Front. Plant Sci. 9: 167. doi.org/10.3389/fpls.2018.00167

Caranta, C., Palloix, A., Lefebvre, V., Daubeze, A.M. 1997. QTLs for a component of partial resistance to cucumber mosaic virus in pepper: Restriction of virus installation in host-cells. Theor. Appl. Genet. 94: 431–438. doi.org/10.1007/s001220050433

Caranta, C., Pflieger, S., Lefebvre, V., Daubèze, A.M., Thabuis, A., Palloix, A. 2002. QTLs involved in the restriction of cucumber mosaic virus (CMV) long-distance movement in pepper. Theor. Appl. Genet. 104: 586–591. doi.org/10.1007/s001220100753

Chaim, A., Grube, R., Lapidot, M., Jahn, M., Paran, I. 2001. Identification of quantitative trait loci associated with resistance to *cucumber mosaic virus* in *Capsicum annuum*. Theor. Appl. Genet. 102: 1213–1220. doi.org/10.1007/s001220100581

Chellappan, P., Vanitharani, R., Ogbe, F., Fauquet, C.M. 2005. Effect of temperature on geminivirus-induced RNA silencing in plants. Plant Physio. 138: 1828–1841. doi.org/10.1104/pp.105.066563

Choi, S., Lee, J.H., Kang, W.H., et al. 2018. Identification of *cucumber mosaic resistance 2* (*cmr2*) that confers resistance to a new *cucumber mosaic virus* isolate P1 (CMV-P1) in pepper (*Capsicum* spp.). Front. Plant Sci. 9: 1106. doi.org/10.3389/fpls.2018.01106

Chung, B.N., Choi, K.S., Ahn, J.J., Joa, J.H., Do, K.S., Park, K.S. 2015. Effects of temperature on systemic infection and symptom expression of turnip mosaic virus in Chinese cabbage (*Brassica campestris*). Plant Pathol. J. 31: 363–370. doi.org/10.5423/PPJ.NT.06.2015.0107

Clark, M.F., Adams, A.N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34: 475–483. doi.org/10.1099/0022-1317-34-3-475

Collard, B.C.Y., Jahufer, M.Z.Z., Brouwer, J.B., Pang, E.C.K. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 142: 169–196. doi.org/10.1007/s10681-005-1681-5

Feil, H., Purcell, A.H. 2001. Temperature-dependent growth and survival of *Xylella fastidiosa in vitro* and in potted grapevines. Plant Dis. 85: 1230–1234. doi.org/10.1094/PDIS.2001.85.12.1230

Fulton, T.M., Chunwongse, J., Tanksley, S.D. 1995. Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Mol. Biol. Rep.13: 207–209. doi.org/10.1007/BF02670897

Grube, R.C., Zhang, Y., Murphy, J.F., Loaiza-Figueroa, F., Lackney, V.K., Provvidenti, R., Jahn, M.K. 2000. New source of resistance to *cucumber mosaic virus* in *Capsicum frutescens*. Plant Dis. 84: 885–891. doi.org/10.1094/PDIS.2000.84.8.885

Jones, R.W., Jackson, A.O., Morris, T.J. 1990. Defective-interfering RNAs and elevated temperatures inhibit replication of tomato bushy stunt virus in inoculated protoplasts. Virology 176: 539–545. doi.org/10.1016/0042-6822(90)90024-L

Kang, W.H., Hoang, N.H., Yang, H.B., et al. 2010. Molecular mapping and characterization of a single dominant gene controlling CMV resistance in peppers (*Capsicum annuum* L.). Theor. Appl. Genet. 120: 1587–1596. doi.org/10.1007/s00122-010-1278-9

Kapoor, S., Sharma, A., Handa, A. 2018. Correlation between symptoms and ELISA for the detection of cucumber mosaic virus in bell pepper. Int. J. Curr. Microbiol. Appl. Sci. 7: 400–406. doi.org/10.20546/ijcmas.2018.706.045

Kosambi, D.D. 1944. The estimation of map distances from recombination values. Ann. Eugen. 12: 172–175. doi.org/10.1111/j.1469-1809.1943.tb02321.x

Lapidot, M., Paran, I., Ben-Joseph, R., Ben-Harush, S., Pilowsky, M., Cohen, S., Shifriss, C. 1997. Tolerance to cucumber mosaic virus in pepper: Development of advanced breeding lines and evaluation of virus level. Plant Dis. 81: 185–188. doi.org/10.1094/PDIS.1997.81.2.185

Li, N., Yin, Y., Wang, F., Yao, M. 2018. Construction of a high-density genetic map and identification of QTLs for cucumber mosaic virus resistance in pepper (*Capsicum annuum* L.) using specific length amplified fragment sequencing (SLAF-seq). Breed Sci. 68: 233–241. doi.org/10.1270/jsbbs.17063

Min, W., Rye, J., Ahn, S. 2014. Developmental changes of recessive genes-mediated cucumber mosaic virus (CMV) resistance in peppers (*Capsicum annuum* L.). Korean J. Hortic. Sci. Technol. 32: 235–240. doi.org/10.7235/hort.2014.13092

Monma, S., Sakata, Y. 1977. Screening of *Capsicum* accessions for resistance to cucumber mosaic virus. J. Japan. Soc. Hort. Sci. 65: 769–776. doi.org/10.2503/jjshs.65.769

Palukaitis, P., García-Arenal, F. 2003. Cucumoviruses. Adv. Virus Res. 62: 241–323. doi.org/10.1016/S0065-3527(03)62005-1

Palukaitis, P., Roossinck, M.J., Dietzgen, R.G., Francki, R.I.B. 1992. Cucumber mosaic virus. Adv. Virus Res. 41: 281–348. doi.org/10.1016/S0065-3527(08)60039-1

Patarapuwadol, S., Sompratoom, W., Sitadhani, K., Wasee, S. 2010. New source of resistance to Thai isolate of cucumber mosaic virus and chilli veinal mottle virus in *Capsicum* germplasm collection. In: Proceedings of the 14th EUCARPIA Meeting on Genetics and Breeding of Capsicum & Eggplant. Valencia, Spain, pp. 191–197.

Raman, H., Raman, R., Kilian, A., et al. 2014. Genome-wide delineation of natural variation for pod shatter resistance in *Brassica napus*. PLoS ONE 9: e101673. doi.org/10.1371/journal.pone.0101673

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org/, 15 July 2021.

R Studio Team. 2015. R Studio: Integrated development for R. RStudio, Inc., Boston, MA (Computer Software v0.98.1074). http://www.rstudio.com/, 15 July 2021.

Soler, S., Díez, M.J., Nuez, F. 1998. Effect of temperature regime and growth stage interaction on pattern of virus presence in TSWV-resistant accessions of *Capsicum chinense*. Plant Dis. 82: 1199–1204. doi.org/10.1094/PDIS.1998.82.11.1199

Suzuki, K., Kuroda, T., Miura, Y., Murai, J. 2003. Screening and field trials of virus resistant sources in *Capsicum* spp. Plant Dis. 87: 779–783. doi.org/10.1094/PDIS.2003.87.7.779

Szittya, G., Silhavy, D., Molnár, A., Havelda, Z., Lovas, A., Lakatos, L., Bánfalvi, Z., Burgyán, J. 2003. Low temperature inhibits RNA silencing-mediated defence by the control of siRNA generation. EMBO J. 22: 633–640. doi.org/10.1093/emboj/cdg74

Tamada, T., Harrison, B.D. 1981. Quantitative studies on the uptake and retention of potato leafroll virus by aphids in laboratory and field conditions. Ann. Appl. Biol. 98: 261–276. doi.org/10.1111/j.1744-7348.1981.tb00759.x

Van Ooijen, J.W. 2006. JoinMap® 4.0, Software for the Calculation of Genetic Linkage Maps in Experimental Populations. Kyazma B.V. Wageningen. the Netherlands.

Van Ooijen, J.W., Boer, M.P., Jansen, R.C., Maliepaard, C. 2002. MapQTL®4.0, Software for the Calculation of QTL Positions on Genetic Maps. Plant Research International. Wageningen, the Netherlands.

Voorrips, R.E. 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. J. Hered. 93: 77–78. doi.org/10.1093/jhered/93.1.77

Yao, M., Li, N., Wang, F., Ye, Z. 2013. Genetic analysis and identification of QTLs for resistance to *cucumber mosaic virus* in chili pepper (*Capsicum annuum* L.). Euphytica 193: 135–145. doi.org/10.1007/s10681-013-0953-8

Zhang, X., Zhang, X., Singh, J., Li, D., Qu, F. 2012. Temperature-dependent survival of Turnip crinkle virus-infected Arabidopsis plants relies on an RNA silencing-based defense that requires dcl2, AGO2, and HEN1. J. Virol. 86: 6847–6854. doi.org/10.1128/JVI.00497-12

Zitter, T.A., Murphy, J.F. 2009. Cucumber mosaic. The Plant Health Instructor. St. Paul, MN, USA.https://www.apsnet.org/edcenter/disandpath/viral/pdlessons/Pages/Cucumbermosaic.aspx,15 July 2021. doi:10.1094/PHI-I-2009-0518-01