Journal of Genetics (2020)99:81
https://doi.org/10.1007/s12041-020-01242-6

RESEARCH ARTICLE

QTL identification for downy mildew resistance in cucumber using genetic linkage map based on SSR markers

PAWINEE INNARK[1], HADSAYA PANYANITIKOON[2], CHANULAK KHANOBDEE[3], SOMPID SAMIPAK[2,4] and CHATCHAWAN JANTASURIYARAT[2,4]*

[1]*Faculty of Science and Technology, Dhonburi Rajabhat University, Bangkok 10600, Thailand*
[2]*Department of Genetics, Faculty of Science, Kasetsart University, Bangkhen Campus, Bangkok 10900, Thailand*
[3]*Lampang Agricultural Research and Training Centre, Rajamangala University of Technology Lanna, Lampang 52000, Thailand*
[4]*Center for Advanced Studies in Tropical Natural Resources, National Research University-Kasetsart University (CASTNAR, NRU-KU), Kasetsart University, Bangkok 10900, Thailand*
*For correspondence. E-mail: fscicwj@ku.ac.th.

Received 25 May 2020; revised 17 July 2020; accepted 13 August 2020

Abstract. Fourteen cucumber lines were tested for genetic homozygosity and performed pairwise comparison to identify a pair with the highest DNA polymorphic level. Cucumber accessions CSL0067 and CSL0139 were selected to generate 315 F_2 populations. The genetic linkage map based on 66 polymorphic SSR markers was constructed. It composed of eight linkage groups (LGs) spanning 474.4 cM. Downy mildew disease reaction was evaluated in cotyledons, first and second true leaf on 7, 10, and 14 day after inoculation. The results showed that downy mildew resistance was controlled by multiple recessive genes. The susceptible to resistant ratio of F_2 progenies fit 9:7 susceptible/resistant segregation types corresponding to duplicate recessive epistasis. Fourteen QTLs were detected. The phenotypic variance ranged from 5.0 to 12.5%, while LOD values ranged from 3.538 to 9.165. Two major QTLs and two QTL hotspots were identified. Moreover, the additive effects data explained that these QTL reduced downy mildew susceptibility.

Keywords. cucumber; downy mildew; genetic mapping; quantitative trait loci; simple sequence repeat.

Introduction

Cucumber is scientifically known as *Cucumis sativus* L., belonging to the Cucurbitaceae family, which is the same botanical family as melons and squashes (Lower and Edwards 1986). Cucumber has a small chromosome complement with $2n=2x=14$ with a small haploid genome of 367 Mbp/genome (Arumuganathan and Earle 1991). Many cucumber varieties are traded in global market. In Thailand, cucumber is one of the important vegetable crops, which can grow throughout the year in all regions. Annual economic value of cucumber in Thailand market is more than US $10 million. However, there are many factors that influence the cucumber yield, e.g. pest, bacterial and fungal diseases. One of the most important fungal diseases in cucumber production is downy mildew, which has been responsible for annual yield losses.

Downy mildew caused by the fungus *Pseudoperonospora cubensis* (Berk. & Curt.) Rostow. (*P. cubensis*), is one of the most devastating diseases of cucurbits (Lebeda and Cohen 2011). It inflicts severe damages to cucumber in humid areas of production throughout the world including Thailand. The pathogen causes angular, chlorotic lesions on the foliage. These lesions appear angular because they are bound by leaf veins. During humid conditions, inspection of the underside of the leaf reveals grey brown to purplish-black lesions (Savory *et al*. 2011). Eventually, leaves turn necrotic and curl upwards. In addition, white-downy-spore masses can also be seen on the lower leaf surface (Lebeda and Cohen 2011). Normally, farmers spray fungicide to protect cucumber from getting the disease. Consequently, this method increases the cost of cucumber production and it is dangerous to farmers. Moreover, it also causes damage to the

Pawinee Innark and Hadsaya Panyanitikoon contributed equally to this work.

Published online: 30 October 2020

environment. One of the best solutions to solve this problem is to find downy mildew resistant cucumber cultivars.

Previously, many reports revealed the detection of downy mildew resistant QTLs in cucumber. Bai *et al*. (2008) mapped three QTLs controlling downy mildew on linkage groups 1 and 6 using a recombinant inbred line (RIL) population derived from a cross between S94 (North China type) and S06 (European type). Zhang *et al*. (2013) mapped five QTLs controlling downy mildew on chromosomes 1, 5 and 6 using an F_2 population derived from the cross K8×K18. Moreover, Pang *et al*. (2013) mapped three QTLs controlling downy mildew on chromosomes 5 and 6 using introgression line derived from interspecific hybridization between cucumber and wild *Cucumis* (*Cucumis hystrix*).

In the present study, F_2 mapping population composed of 315 lines was developed from a cross between two homozygous cucumber lines, CSL0067 and CSL0139. CSL0067 is resistance to downy mildew stain in field conditions in northern Thailand but CSL0139 is susceptible. Cucumber genetic linkage map was constructed using 66 SSR markers. Quantitative trait loci (QTL) accounting for resistance to downy mildew was identified. The information generated from this study will pave way for the development of cucumber downy mildew resistant varieties in future.

Materials and methods

Plant materials

Fourteen cucumber lines, which have been self-pollinated for several generations were selected from germplasm collection at Rajamangkala University of Technology Lanna, Lampang, Thailand based on their downy mildew disease reaction in the field (table 1).

Table 1. Downy mildew disease reaction and percentage of homozygosity in 14 cucumber accessions.

Cucumber accessions	Downy mildew disease reaction	% Homozygosity
CSL0041	Susceptible	82.60
CSL0050	Susceptible	76.10
CSL0080	Susceptible	91.30
CSL0118 (S)	Susceptible	84.80
CSL0139	Susceptible	98.10
CSL0064	Susceptible	93.38
CSL0077	Susceptible	94.84
CSL0069 (5)	Susceptible	95.60
DMS	Susceptible	92.88
CSL0069 (2)	Resistance	91.30
CSL0118 (R)	Resistance	95.60
CSL0071	Resistance	99.46
CSL0069 (3)	Resistance	90.53
CSL0067	Resistance	88.24

Homozygosity test and polymorphic analysis for parental selection

Ten seeds from each cucumber lines were planted in planting tray filled with soil and grown in greenhouse for a week and then cotyledons were separately collected and stored at −20°C. Genomic DNA was extracted from cotyledon following the CTAB isolation method (Doyle and Doyle 1987). For homozygosity test, a total of 522 SSR markers from Ren *et al*. (2009) were screened. PCR amplification was carried out in 10 μL containing 50 ng of extracted genomic DNA, 10 μM specific forward primer, 10 μM specific reverse primer, 1 U *Taq* DNA polymerase enzyme, 1 mM dNTPs, 10x free $MgCl_2$ PCR buffer, 2.5 mM $MgCl_2$ solution and dH_2O. DNA was amplified under the following protocol: denaturation at 94°C for 5 min; then 35 cycles of denaturation at 94°C for 30 s, annealing at range 50–59°C for 30 s and extension at 72°C for 30 s, then final extension at 72°C for 5 min. Gel electrophoresis of 1% agarose gel in 0.5% TBE buffer was used and stained with EtBr to visualize DNA band under UV light. The detection of polymorphism from the PCR products was done on polyacrylamide gel electrophoresis. The data from homozygosity test and polymorphic DNA markers were used for the selection of two parents, based on the highest percentage of polymorphism between the two parental lines. The cross was made between resistant and susceptible lines and progenies were used for the QTL analysis.

Population development

The selected parental lines were crossed to produce F_1 plants. Further, a population of F_2 plants was created from self-pollination of F_1 seed. Three hundred and fifteen F_2 seeds, parental lines, F_1 and commercial checks were planted in the greenhouse at Chia Tai company, Chiang Mai, Thailand. Leaf of individual plants was collected to extract DNA using protocol from Doyle and Doyle (1987) for genetic map construction. Downy mildew evaluation was also performed for QTL detection.

Construction of SSR-based genetic linkage map

The selected SSR markers that showed clear polymorphism between parental lines were screened in F_2 population and scored as ‘A’, when allele was similar to resistant parent allele; ‘B’, when allele was similar to susceptible parent allele; and ‘H’, when both alleles were presented. Then, the data of SSR scoring were analysed by chi-square test for goodness of fit for segregation type of 1:2:1 (A/A: H: B/B) using IBM SPSS Statistics 19.0. Then, the linkage map was constructed using MAPMAKER/EXP 3.0. Marker data were assigned to linkage groups using a minimum logarithm of odds (LOD)-likelihood score of 3.0. The map function of

Kosambi was used to calculate the genetic distance between markers.

Downy mildew inoculum suspension preparation

Sporangia of *P. cubensis* were collected from cucumber leaves, showing downy mildew symptom. The leaf samples were soaked and brushed in sterile water to collect the sporangia. The concentration of sporangial suspension was measured by counting the number of sporangia using hemocytometer and adjusted to 1×10^4 sporangia/mL. Tween 20 was added to the sporangial suspension in the ratio 1μL : 2mL. The sporangial suspension was sprayed to the cucumber seedling within 1 h after preparation.

Downy mildew screening

F_2 population lines, parental lines, F_1 and commercial checks were planted in the greenhouse. After 7 days of planting, the seedling cotyledons were sprayed with inoculum suspension of *P. cubensis*. Following inoculation, seedlings were placed in dark with 100% relative humidity for 12 h. Then, individuals of F_2 plants were assessed for the level of downy mildew infection on cotyledons, the first true leaf, and the second true leaf on 7, 10, and 14 days after inoculation (DAI). The disease scores were classified to nine categories based on symptomatic leaf area: 0, absence of symptom; 1, 1–3%; 2, 3–6%; 3, 6–12%; 4, 12–25%; 5, 25–50%; 6, 50–75%; 7, 75–87%; 8, 87–99%; 9, 100%. Plants, which have score less than 3 were classified as highly resistant. The scores between 3 and 4 were classified as moderately resistance and the scores between 4 and 6 were classified as intermediate. The scores between 6 and 7 were classified as moderately susceptible and the scores between 7 and 9 were classified as highly susceptible (Call *et al*. 2012).

Phenotypic analysis

Downy mildew disease scoring data was divided into two groups; susceptible group (highly and moderately susceptible) and resistant group (highly and moderately resistant)

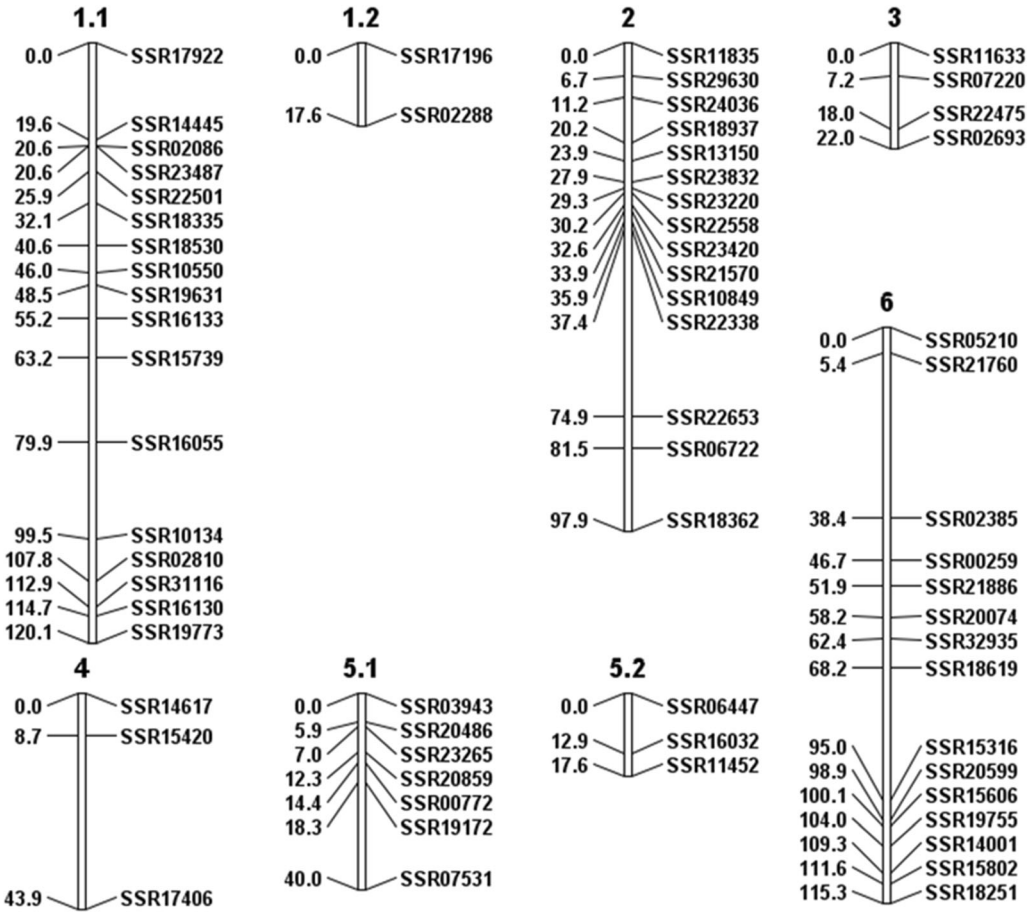

Figure 1. SSR linkage map of cucumber developed using 315 F_2 plants from the CSL0067 × CSL0139 cross. In each linkage, marker names are provided on the right side, while distances (in cM) are provided on the left.

Table 2. Distribution of SSR among eight linkage groups and corresponding chromosome (as reported by Ren *et al*. 2009) mapped with F_2 population from CSL0067 × CSL0139.

LG	Corresponding chromosome	SSR loci	Map length (cM)	Average distance (cM)*
1.1	1	17	120.1	7.5
1.2	1	2	17.6	17.6
2	2	15	97.9	7.0
3	3	4	22.0	7.3
4	4	3	43.9	22.0
5.1	5	7	40.0	6.7
5.2	5	3	17.6	8.8
6	6	15	115.3	8.2
Total		66	474.4	7.2
Average		8.25	59.3	

*Average genetic distances between adjacent markers.

and analysed by the chi-square test for goodness of fit for the segregation type of 3:1, 15:1 and 9:7.

QTL analysis

Genotypic data and phenotypic data were combined to analyse QTL mapping using the composite interval mapping (CIM) method by Zeng (1994), Qgene 4.3.10 software (Joehanes and Nelson 2008). Threshold was determined by means of 1000 permutation tests. The QTLs explaining more than 10% of the phenotypic effect were defined as major QTLs. Identified QTLs were named by the name of assay followed by the chromosome number, linkage group number and locus number.

Results and discussion

Homozygosity and parental lines selection

A total of 522 SSR markers were used to test DNA homozygosity in 14 cucumber lines (nine downy mildew susceptible lines and five downy mildew resistant lines). All 14 cucumber lines were confirmed for the downy mildew disease reaction in the field for several generations. The percentage of homozygosity in the susceptible lines ranged from 76.10% (CSL0050) to 98.10% (CSL0139), while the percentage of homozygosity in the resistant lines ranged from 88.24% (CSL0067) to 99.46% (CSL0071) (table 1). Based on the homozygosity and the disease reaction, four resistant and susceptible lines were selected, namely CSL0067 (R), CSL0071 (R), CSL0139 (S) and DMS (S). The percentage of the polymorphism between the pair of CSL0067 (R) and CSL0139 (S) was the highest (54.89%). These two lines were selected as the female parent and male parent, respectively, to develop the F_2 mapping population.

Table 3. The average of downy mildew scoring and Chi-square test for goodness of fit, comparisons between resistant and susceptible to downy mildew.

		Downy mildew scoring					No. of plants			Chi-square test for goodness of fit					
		Parental lines				Commercial checks				Expected ratio (S:R)					
										3:1		15:1		9:7	
Assays		CSL0067	CSL0139	F_1	F_2		Resistant (R)	Susceptible (S)	Total	χ^2 value*	*P* value	χ^2 value*	*P* value	χ^2 value*	*P* value
7 DAI	Cotyledons	4.0	7.0	7.0	7.6	9.0	0	282	282	–	–	–	–	–	–
	1st True leaf	3.0	5.0	5.0	4.2	6.0	156	80	236	212.633	0.000	1442.825	0.000	47.911	0.000
10 DAI	Cotyledons	5.0	9.0	9.0	8.4	9.0	1	307	308	100.017	0.000	18.455	0.000	236.013	0.000
	1st True leaf	4.0	6.0	7.0	5.3	7.0	78	132	210	16.514	0.000	342.046	0.000	3.725	0.054
14 DAI	Cotyledons	8.0	9.0	9.0	8.9	9.0	0	315	315	–	–	–	–	–	–
	1st True leaf	5.0	7.0	7.0	6.8	9.0	2	258	260	81.415	0.000	13.329	0.000	195.174	0.000
	2nd True leaf	3.0	4.0	4.0	4.0	6.0	193	27	220	461.673	0.000	2492.553	0.000	172.894	0.000

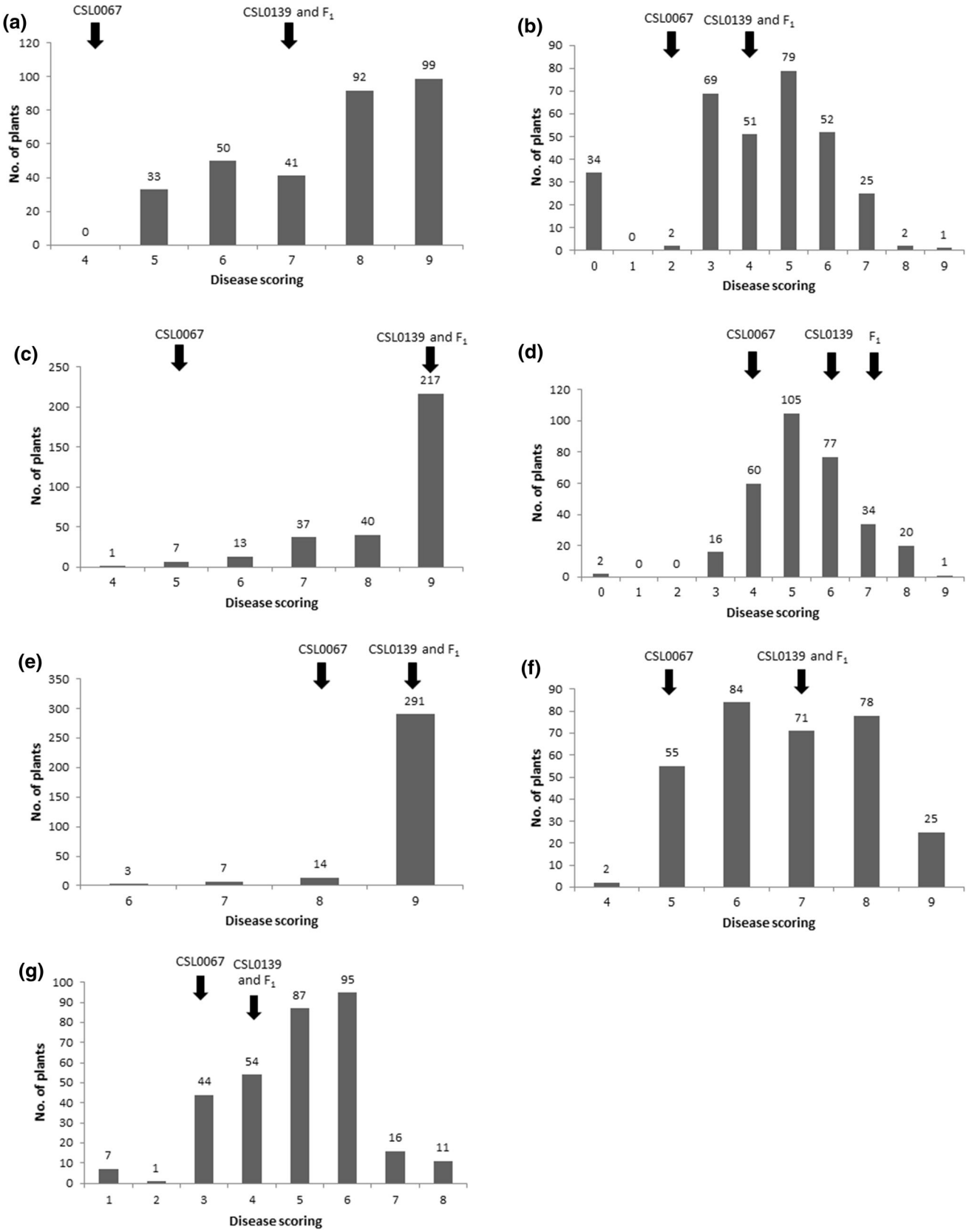

Figure 2. Frequency distribution of disease scoring in F_2 population in (a) cotyledons at 7 DAI, (b) first true leaf at 7 DAI, (c) cotyledons at 10 DAI, (d) first true leaf at 10 DAI, (e) cotyledons at 14 DAI, (f) first true leaf at 14 DAI, (g) second true leaf at 14 DAI.

Construction of SSR-based genetic linkage map

Sixty-six SSR markers, which showed clear polymorphism between the parental lines were used for the genetic linkage map construction. Linkage analysis and map construction were done using the MapMaker program. The resulting cucumber genetic map had eight linkage groups spanning 474.4 cM and covered six chromosomes. The average numbers of the SSR markers in each linkage group was 8.25 markers and the average length of the linkage group was 59.3 cM. The average range between the SSR markers was 7.2 cM (figure 1; table 2). All linkage groups were assigned according to the reported SSR markers from previous study (Ren *et al*. 2009). Our genetic linkage map missed the linkage group 7 (Arumuganathan and Earle 1991) because no polymorphic marker was identified on this chromosome. Additional markers are needed to generate the complete linkage map for the cucumber genome. The average marker interval ranged from 6.7–22.0 cM with the mean of 7.2 cM. This result was similar to the average interval of SSR markers in the cucumber genetic linkage map using inter simple sequence repeats and sequence-related amplified polymorphism markers by Alfandi *et al*. (2010).

Phenotypic distribution of downy mildew resistance

The downy mildew disease reaction of the parental lines (CSL0067 and CSL0139), the F_1 plants, the F_2 population, and the commercial checks were recorded for the level of the downy mildew infection on cotyledons, first true leaf, and second true leaf after 7, 10 and 14 DAI (table 3). The frequency distribution of the disease scoring of cotyledons, first true leaf and second true leaf at 7, 10 and 14 DAI among F_2 population is shown in figure 2. The frequency distribution of the disease score on cotyledons at 7, 10 and 14 DAI was skewed toward the susceptibility, which revealed susceptible dominant for downy mildew genes (figure 2, a, c&e). On the other hand, the disease score distribution showed normal distribution in the first true leaf and second true leaf at 7, 10 and 14 DAI (figure 2, b, d, f&g). These results presented the segregation for the downy mildew resistant genes in F_2 population. In this study, the resistant parental line (CSL0067) and the susceptible parental line (CSL0139) can be distinguished by cotyledon at 7 and 10 DAI but cannot be distinguished at 14 DAI. However, all the true leaves at 7, 10 and 14 DAI could be used to distinguish the disease reaction between the parental lines. The explanation for the separation limitation in cotyledons at 14 DAI could be due to the age restriction in cotyledon. Cucumber plants received food and nutrient from the cotyledon until true leaves were revealed (Bisognin *et al.* 2005). In general, cotyledon would dry out after 10 DAI, and hence, the disease scoring is not reliable after that.

Chi-square test in downy mildew disease scoring

The chi-square test for the goodness of fit information showed that downy mildew disease reactions fit 9:7 susceptible/resistant segregation ratio (χ^2=3.725, P=0.054) in first true leaf at 10 DAI. This ratio corresponds to the duplicate recessive epistasis (table 3). The chi-square test for the goodness of fit data in cotyledons at 7, 10 and 14 DAI, in first true leaf at 7 and 14 DAI, and in second true leaf at 14 DAI were different from the expected ratio of 3:1 and 15:1 (susceptible:resistant) (table 3). Therefore, the downy mildew resistant genes were not coinciding with the single

Table 4. QTL for downy mildew resistance detected in 315 F_2 population derived from CSL0067 × CSL0139 cross by using composite interval mapping.

Assays		Chromosome*	LG	QTL name	Position (cM)	LOD peak**	Flanking markers	R^2 (%)	Additive effect
Cotyledons	7 DAI (cot7)	5	5.1	*Cot7_5.1_1*	12	6.407	SSR03943-SSR19172	8.9	–0.568
		5	5.1	*Cot7_5.1_2*	38	7.884	SSR19172-SSR07531	10.9	–0.674
	10 DAI (cot10)	5	5.1	*Cot10_5.1*	4	9.165	SSR03943-SSR20859	12.5	–0.539
	14 DAI (cot14)	5	5.1	*Cot14_5.1*	2	4.421	SSR03943-SSR20486	6.3	–0.147
1st True leaf	7 DAI (first7)	1	1.1	*First7_1.1*	34	3.538	SSR22501-SSR18530	5.0	0.705
		6	6	*First7_6*	78	5.140	SSR18619-SSR15316	7.2	–0.904
	10 DAI (first10)	6	6	*First10_6*	64	7.121	SSR20074-SSR15316	9.9	–0.575
	14 DAI (first14)	5	5.1	*First14_5.1_1*	4	6.641	SSR03943-SSR20486	9.3	–0.552
		5	5.1	*First14_5.1_2*	12	5.750	SSR23265-SSR19172	8.1	–0.495
		6	6	*First14_6*	64	6.507	SSR20074-SSR15316	9.1	–0.532
2nd True leaf	10 DAI (second10)	6	6	*Second10_6*	64	4.544	SSR20074-SSR18619	6.4	–0.344
		2	2	*Second14_2*	0	5.435	SSR11835-SSR29630	7.6	–0.527
	14 DAI (second14)	5	5.1	*Second14_5.1*	8	5.399	SSR03943-SSR20859	7.6	–0.560
		6	6	*Second14_6*	38	5.954	SSR21760-SSR21886	8.3	–0.563

*According to Ren *et al.* (2009).
**According to permutation function in Qgene, using a probability level of 0.05.

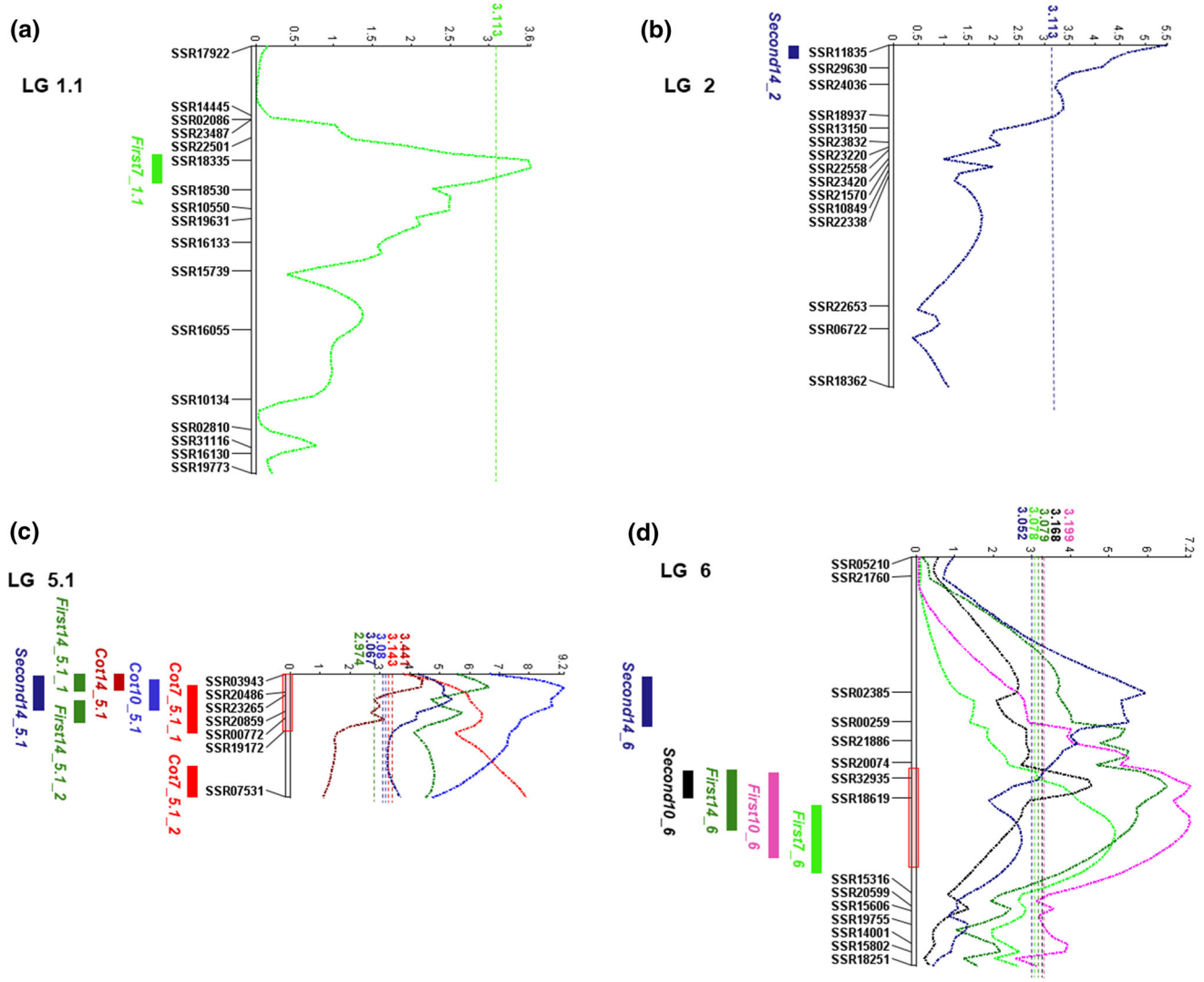

Figure 3. QTL detection for downy mildew resistance in (a) linkage group 1.1, (b) linkage group 2, (c) linkage group 5.1, and (d) linkage group 6. Dotted line=LOD threshold with a probability level of 0.05. QTL hotspots are shown as red blocks on linkage group.

dominant gene or the duplicate dominant epistasis interaction.

Previous reports showed that the inheritance pattern for the downy mildew resistance in cucumber was both qualitative resistance controlled by a single gene and a quantitative resistance controlled by multiple genes (Van Vliet and Meysing 1976; Fanourakis and Simon 1987; Doruchowski and Lakowska-Ryk 1992; Zhang *et al*. 2007). The inheritance of the downy mildew resistance depends on the different identification and evaluation methods, mapping population, types of the resistant gene, and the pathotypes of *P. cubensis*. In this study, we found that the resistance to downy mildew was controlled by the recessive genes, since F_1 plants were susceptible same as the susceptible parent (CSL0139). The chi-square test also indicated that the downy mildew disease reactions fit with 9:7 susceptible/resistant segregation ratio, corresponding to the duplicate recessive epistasis. This result was consistent with previous studies that the inheritance of resistance in cucumber was controlled by a recessive gene (Fanourakis and Simon 1987; Doruchowski and Lakowska-Ryk 1992). Moreover, Badr and Mohamed (1998) and El-Hafaz *et al*. (1990) reported that the resistance in downy mildew was the result of an epistatic interaction between a dominant susceptible gene and a recessive resistance gene.

QTL analysis in F_2 population

A total of 14 QTL positions for the downy mildew resistance in the CSL0067 × CSL0139 F_2 population were identified. The phenotypic variance ranged from 5.0% to 12.5%, while the LOD value ranged from 3.538 to 9.165 (table 4). Two major QTLs, which showed more than 10% of phenotypic variance were located on LG5.1 were detected using the phenotypic data of cotyledon at 7 and 10 DAI (QTL named

Cot7_5.1_2 and *Cot10_5.1*). Major QTL *Cot7_5.1_2* was located between SSR19172 and SSR07531 markers and explained 10.9% of phenotypic variation with the LOD of 7.884. While the other major QTL *Cot10_5.1* was located between SSR03943 and SSR20859 SSR markers and explained 12.5% of the phenotypic variation with the LOD of 9.165 (table 4). Moreover, there were two QTL hotspots, which can detect QTL with several phenotypic datasets including QTL on LG5.1 and on LG6 (figure 3, c&d). The additive effects of these nine QTLs were all negative indicating that the majority of QTL reduced downy mildew susceptibility.

The results showed that all seven phenotypic datasets were positively correlated with each other at the 0.01 level of confidence except cotyledon at 14 DAI. Several QTLs were detected in the same region as referred to as QTL hotspots. This result suggested that the same resistance genes were expressed in every stage of plant from cotyledon stage to second true leaf stage. The disease scoring of all three cotyledon stages detected a hotspot QTL on LG5.1 located between SSR03943 and SSR19172 markers (figure 3c), while the disease scoring of all leaf stages showed QTLs on the remaining linkage groups. Our results coincided with previous studies by Fukino *et al.* (2008) and He *et al*. (2013). The effect of these QTLs varied depending on strain and plant tissue, and developmental stage used in the analysis (Cohen 1993; Boiteux *et al*. 1995; McCreight 2003). The QTLs detected in this study were located on cucumber chromosomes 1, 2, 5 and 6, which coincided with the QTLs detected in previous studies by Bai *et al*. (2008), who mapped three QTLs controlling downy mildew resistance on the linkage groups 1 and 6; and Zhang *et al*. (2013) mapped five QTLs on chromosomes 1, 5 and 6; as well as Pang *et al.* (2013) mapped three QTLs on chromosomes 5 and 6. There was a novel QTL identified in this study on chromosome 2. It could be used as a new source of downy mildew resistant in cucumber breeding programme in the future.

Acknowledgements

This research was funded by National Center for Genetic Engineering and Biotechnology (BIOTEC) and the Faculty of Science, Kasetsart University. The greenhouse for phenotypic analysis was screened at Chia Tai Company Limited, Chiang Mai, Thailand.

References

Alfandi M., Ji Y., Shen L., Qi X., Xu Q. and Chen X. 2010 Construction of genetic linkage map and localization of QTLs for powdery mildew resistance in cucumber (*Cucumis sativus* L.). *Acta Hort. (ISHS)* **871**, 33–41.

Arumuganathan K. and Earle E. D. 1991 Nuclear DNA content of some important plant species. *Plant Mol. Biol. Rep*. **9**, 208–218.

Badr L. A. A. and Mohamed F. G. 1998 Inheritance and nature of resistance to downy mildew disease in cucumber (*Cucumis sativus* L.). *Ann. Agric. Sci.* **36**, 2517–2544.

Bai Z. L., Yuan X. J., Cai R., Liu L. Z., He H. L., Zhou H. F. *et al*. 2008 QTL mapping of resistance gene to downy mildew in cucumber. *Prog. Nat. Sci*. **18**, 706–710.

Bisognin D. A., Velasquez L. and Widders I. 2005 Cucumber seedling dependence on cotyledonary leaves for early growth. *Pesq. Agropec. Bras., Brasília* **40**, 531–539.

Boiteux L. S., Reifschneider F. J. B. and Pessoa H. B. S. V. 1995 Phenotypic expression of quantitative and qualitative components of partial resistance to powdery mildew (*Sphaerotheca fuliginea* race 1) in melon (*Cucumis melo*) germplasm. *Plant Breed*. **114**, 185–187.

Call A. D., Criswell A. D., Wehner T. C., Klosinska U. and Kozik E. U. 2012 Screening cucumber for resistance to downy mildew caused by *Pseudoperonospora cubensis* (Berk. and Curt) Rostov. *Crop Sci*. **52**, 577–592.

Cohen R. 1993 A leaf disk assay for detection of resistance of melons to *Sphaerotheca fuliginea* race 1. *Plant Dis*. **77**, 513–517.

Doruchowski R. W. and Łąkowska-Ryk E. 1992 Inheritance of resistance to downy mildew (*Pseudoperonospora cubensis* Berk & Curt) in *Cucumis sativus*. In *Proceedings of the V Eucarpia Cucurbitaceae symposium*, pp. 132–138. Skierniewice-Warszawa, Poland.

Doyle J. J. and Doyle J. L. 1987 A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* **19**, 11–15.

El-Hafaz A., El-Din B., El-Doweny H. H. and Awad M. M. W. 1990 Inheritance of downy mildew resistance and its nature of resistance in cucumber. *Ann. Agric. Sci*. **28**, 1681–1697.

Fanourakis N. E. and Simon P. W. 1987 Analysis of genetic linkage in the cucumber. *J. Hered*. **78**, 238–242.

Fukino N., Ohara T., Monforte A. J., Sugiyama M., Sakata Y., Kunihisa M. *et al*. 2008 Identification of QTLs for resistance to powdery mildew and SSR markers diagnostic for powdery mildew resistance genes in melon (*Cucumis melo* L.). *Theor. Appl. Genet*. **118**, 165–175.

He X., Li Y., Pandey S., Yandell B. S., Pathak M. and Weng Y. 2013 QTL mapping of powdery mildew resistance in WI 2757 cucumber (*Cucumis sativus* L.). *Theor. Appl. Genet*. **126**, 2149–2161.

Joehanes R. and Nelson J. C. 2008 QGene 4.0, an extensible Java QTL-analysis platform. *Bioinforma* **24**, 2788–2789.

Lebeda A. and Cohen Y. 2011 Cucurbit downy mildew (*Pseudoperonospora cubensis*)—biology, ecology, epidemiology, host-pathogen interaction and control. *Eur. J. Plant Pathol*. **129**, 157–192.

Lower R. L. and Edwards M. D. 1986 Cucumber breeding In Breeding vegetable crops (ed. M. J. Bassett), p. xii + 584pp. AVI Publishing, Westport.

McCreight J. D. 2003 Genes for resistance to powdery mildew races 1 and 2US in melon PI 313970. *Hort. Sci.* **38**, 591–594.

Pang X., Zhou X., Wan H. and Chen J. 2013 QTL mapping of downy mildew resistance in an introgression line derived from interspecific hybrization between cucumber and *Cucumis hystrix*. *J. Phytopathol*. **161**, 536–543.

Ren Y., Zhang Z., Liu J., Staub J. E., Han Y., Cheng Z *et al*. 2009 An integrated genetic and cytogenetic map of the cucumber genome. *PLoS One* **4**, e5795.

Savory E. A., Granke L. L., Quesada-Ocampo L. M., Varbanova M., Hausbeck M. K. and Day B. 2011 The cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. *Mol. Plant Pathol.* **12**, 217–226.

Van Vliet G. J. A. and Meysing W. D. 1976 Relation in the inheritance of resistance to *Pseudoperonospora cubensis* Rost and *Sphaerotheca fuliginea* Poll. in cucumber (*Cucumis sativus* L.). *Euphytica* **26**, 793–796.

Zeng Z. B. 1994 Precision mapping of quantitative trait loci. *Genetics* **136**, 1457–1468.

Zhang S. P., Liu M. M., Miao H., Zhang S. Q., Yang Y. H., Xie B. Y. *et al*. 2013 Chromosomal mapping and QTL analysis of resistance to downy mildew in *Cucumis sativus*. *Plant Dis.* **97**, 245–251.

Zhang S. Q., Gu X. F., Zhang S. P. and Zou Z. R. 2007 Genetic analysis of downy mildew resistance in cucumber. *Acta Bot. Boreal.-Occid. Sin.* **27**, 2416–2420.

Corresponding editor: H. A. Ranganath