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ORIGINAL PAPER

QTL mapping of powdery mildew resistance in WI 2757 cucumber (*Cucumis sativus* L.)

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Abstract Powdery mildew (PM) is a very important disease of cucumber (*Cucumis sativus* L.). Resistant cultivars have been deployed in production for a long time, but the genetic mechanisms of PM resistance in cucumber are not well understood. A 3-year QTL mapping study of PM resistance was conducted with 132 $F_{2:3}$ families derived from two cucumber inbred lines WI 2757 (resistant) and True Lemon (susceptible). A genetic map covering 610.4 cM in seven linkage groups was developed with 240 SSR marker loci. Multiple QTL mapping analysis of molecular marker data and disease index of the hypocotyl, cotyledon and true leaf for responses to PM inoculation identified six genomic regions in four chromosomes harboring QTL for PM resistance in WI 2757. Among the six QTL, *pm1.1* and *pm1.2* in chromosome 1 conferred leaf resistance. Minor QTL *pm3.1* (chromosome 3) and *pm4.1* (chromosome 4) contributed to disease susceptibility. The two major QTL, *pm5.1* and *pm5.2* were located in an interval of ~40 cM in chromosome 5 with each explaining 21.0–74.5 % phenotypic variations. Data presented herein support two recessively inherited, linked major QTL in chromosome 5 plus minor QTL in other chromosomes that control the PM resistance in WI 2757. The QTL *pm5.2* for hypocotyl resistance plays the most important role in host resistance. Multiple observations in the same year revealed the importance of scoring time in the detection of PM resistance QTL. Results of this study provided new insights into phenotypic and genetic mechanisms of powdery mildew resistance in cucumber.

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Introduction

Powdery mildew (PM) caused mainly by *Podosphaera fusca* (Fr.) Braun & Shishkoff (formerly *Sphaerotheca*

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fuliginea Schlech ex Fr. Poll.) is probably the most common and conspicuous disease of cucumber (*Cucumis sativus* L.) and other cucurbit crops (Perez-Garcia et al. 2009). Its symptoms are characterized by the whitish, talcum-like, powdery fungal growth that develops on leaves, petioles and stems (Sitterly 1978; Zitter et al. 1996). The pathogen has a wide host range, may survive as conidia or mycelium on a variety of cucurbit crops and can easily develop in the cooler spring or fall seasons. The conidia are readily detached and borne by air currents. PM has especially been promoted by the fact that cucumbers are now grown year round in glass houses, or high tunnels, which are very favorable for the development of PM resulting in easy maintenance of the fungus.

Development of resistant cultivars is one of the most important components in integrated pest management of PM. Since 1940s, PM resistance (PMR) and its inheritance have been reported in a number of cucumber lines. Smith (1948) reported that PMR in the cucumber cultivar 'Puerto Rico 37', which was derived from 'Chinese Long' or an Indian accession PI 197087 (Kooistra 1968), was due to multiple recessive factors. PMR in PI 197087 may be controlled by 1–2 major and 1–2 minor genes (Barnes 1961). Fujieda and Akiya (1962) identified a single recessive gene underlying the PMR in 'Natsufushinari' (PI 279465 from Japan), whereas Kooistra (1968) proposed three recessive genes for PMR in cucumber: two from Natsufushinari and one from PI 200815 or PI 200818. Shanmugasundaram et al. (1971) was the first to differentiate hypocotyl and leaf PM resistances in cucumber, and they suggested a recessive gene *s* for hypocotyl resistance that played an important role in overall performance of PM resistance. Classical genetic analysis found that PMR in cucumber was linked with the *D* locus for dull fruit color (Vliet and Meijsing 1977; Fanourakis and Simon 1987; Walters et al. 2001) which has been mapped in cucumber chromosome 5 (Miao et al. 2011).

Since no single gene has been identified, the quantitative trait loci (QTL) mapping strategy was taken for molecular dissection of PMR in cucumber (Sakata et al. 2006; de Ruiter et al. 2008; Hofstede et al. 2008; Liu et al. 2008; Zhang et al. 2008, 2011). For example, using 97 recombinant inbred lines (RIL) and 154 markers, Sakata et al. (2006) identified six temperature-dependent resistance QTL in four linkage groups (LGs) underlying PMR in PI 197088-1 including one major QTL in LGII that confers resistance at both 20 and 26 °C. In the cucumber line NPI derived from a cross between Natsufushnari and PI 200815, Hofstede et al. (2008) and de Ruiter et al. (2008) identified two linked PMR QTL, *pm-l* for leaf resistance and *pm-h* for hypocotyl resistance. In yet another study with 130 $F_{2:3}$ lines in two environments, five QTL in three LGs were found to be responsible for PMR originated from a European greenhouse type cucumber line S06 (Liu et al. 2008). More recently, Zhang et al. (2011) identified three QTL, *pm5.1*, *pm5.2* and *pm5.3* in chromosome 5, and *pm6.1* in chromosome 6 that control PMR in a north China type cucumber line K8. While these studies have provided insights into the genetic control of PMR in cucumber, a clear picture is still lacking. The numbers and locations of QTL identified in these studies are inconsistent, which may be due to the sources of PM resistance used, the methods of bioassay, and environmental conditions used. In addition, molecular markers identified from these QTL mapping studies were not breeder friendly, or the resolution is not high enough for practical use in marker-assisted selection, not to mention fine mapping or cloning of the major-effect QTL.

The objectives of the present study were to investigate phenotypic mechanisms and identify molecular markers for major QTL of PMR in the cucumber inbred line WI 2757. Using 132 F_2 plants from WI 2757 × 'True Lemon', a microsatellite (SSR)-based linkage map with 240 marker loci was developed. Phenotypic responses in the hypocotyl, cotyledons and true leaves upon powdery mildew inoculation among F_3 families were collected in three greenhouse environments, which were then used in QTL mapping for PM resistance in WI 2757.

Materials and methods

Plant materials and powdery mildew bioassay

One hundred and thirty-two F_2 plants and F_2-derived F_3 families from the cross between two inbred cucumber lines, WI 2757 and True Lemon were used for QTL mapping. WI 2757 is a gynoecious inbred line with high resistance to powdery mildew (Peterson et al. 1982), and True Lemon is an andromonoecious heirloom that is highly susceptible to PM.

All phenotyping experiments were conducted in the Walnut Street Greenhouse of the University of Wisconsin at Madison. The temperature in the greenhouse was 23–32 °C during the day and 18–20 °C in the night with 12–14 h photoperiod and 50–80 % relative humidity. The PM pathogen (*S. fuliginea*) was isolated from diseased tissues of cucumber plants in the greenhouse. Its identity was verified by morphological observation under a microscope, which was conducted by the Plant Disease Diagnostics Laboratory of the University of Wisconsin, and its virulence was tested on WI 2757 and the susceptible cucumber line Straight 8.

Phenotypic data collection for plant responses to inoculation of the PM pathogen was performed in three experiments, EXPT1 (October 2010), EXPT2 (January

2011) and EXPT3 (May 2012). EXPT1 included two parental lines, their F_1, and 126 F_3 families with 18 plants per family in three replicates (6 plants per replicate). EXPT2 was the same as EXPT1 except that only 120 available F_3 families were used. Each test was a randomized complete block design. In EXPT3, eight plants from each of the 120 F_3 families were tested without replications. When the cotyledons were fully expanded, conidial water suspension with a concentration of 5×10^5 spores per ml was sprayed evenly on the cotyledons and the expanding first true leaf. Scoring of disease responses was conducted 10–14 days after inoculation depending on progress of symptoms. For each seedling, the hypocotyl, two cotyledons and first two true leaves (HY, CL, TL, respectively, hereinafter) were scored separately according to the level of sporulation of the fungus using a scale of 0–9, where 0 = absence of disease symptom or surface area of sporulation <10 %; 1 = 10–20 % surface areas covered with PM spores. Then, for each successive scale, the area covered with spores was increased by 10 %. Therefore, scale 9 indicated >90 % coverage of the surface or the tissue was dead or dry. Scoring of hypocotyl resistance was conducted only for EXPT2 and EXPT3. In EXPT3, the plants were scored three times at 4-day interval resulting in three data sets: EXPT3-1, EXPT3-2 and EXPT3-3 with the first scoring date being 10 days after inoculation. The CL data in EXPT3-3 were not collected because all cotyledons were fully covered with PM spores or dried when the third observation was conducted.

For each F_3 family, PM resistance was evaluated using a disease index (DI) following Zhang et al. (2011) in which $DI = \sum[(s \times n)/(S \times N)] \times 100$, where n is the number of plants with each disease rating, s the disease rating scale, N the total number of plants under investigation, and S is the highest disease rating scale (9 in this study). Within a plant, disease symptoms started from the hypocotyl and two cotyledons, and progressed to new leaves; expanding leaves in general showed no infection. Plants in some F_3 families exhibited retarded or late growth, which may have low disease scores. Those plants were excluded in calculation of DI to avoid any confounding effects. Thus, DI means of 13 traits were obtained for QTL analysis: two for EXPT1 (CL2010 and TL 2010), three for EXPT2 (HY2011, CL2011, and TL2011), and eight for EXPT3 (HY2012.1, HY2012.2, HY2012.3, CL2012.1, CL2012.2, TL2012.1, TL2012.2, and TL2012.3).

Statistical analysis of phenotypic data

Statistical analysis of phenotypic data was performed using SAS v9.3 (SAS Institute Inc., Cary, NC, USA). Pearson's correlation coefficients among the recorded traits (HY, CL and TL) were estimated with the PROC CORR function based on DI means of each F_3 family across three replications in 2011 EXPT2. To estimate the effects of experiment, F_3 family, and family-by-experiment interactions, analysis of variance (ANOVA) was conducted using the PROC GLM procedure in SAS with the model of $Y_{ij} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \varepsilon_{ij}$, where Y_{ij} is the DI mean of jth F_3 family of the ith experiment, μ the DI mean of the F_3 population, τ_i the effect of ith experiment (environment), β_j the effect of the jth family, $(\tau\beta)_{ij}$ the interaction of the ith environment with jth family, and finally, ε_{ij} is the random error.

Genotyping and linkage map development

Polymorphic cucumber or melon SSR markers (Ren et al. 2009; Cavagnaro et al. 2010) between WI 2757 and True Lemon were used to genotype 132 F_2 plants. DNA extraction, PCR amplification of molecular markers and gel electrophoreses followed Li et al. (2011). For each marker, χ^2 test for goodness-of-fit was performed against the expected 1:2:1 segregation ratio. Linkage analysis was carried out using JoinMap 3.0 software. Linkage groups were determined with a minimum LOD score of 4.0 and a recombination fraction of 0.3. Genetic distance was calculated with Kosambi mapping function. We used R/qtl software for QTL mapping (see below). A refitting of the genetic map developed with JoinMap 3.0 in R/qtl expanded the map from ~610 cM to over 1,000 cM, which did not agree with other published results, so we kept the JoinMap 3.0 version of the genetic map in QTL analysis.

The Gy14 cucumber genome has been sequenced and assembled (Yang et al. 2012). The physical locations in the Gy14 scaffold and whole genome assemblies of all mapped markers were used to verify their genetic map locations. Inference of chromosomal locations of molecular markers on the map was performed with BLASTn or in silico PCR according to Cavagnaro et al. (2010). Chromosome assignment of linkage groups followed Yang et al. (2012).

QTL analysis

Preliminary analysis of phenotypic data suggested that PM resistance in HY, CL and TL may be controlled by linked loci. Since the composite interval mapping (CIM) algorithm has some limitations in estimating the joint contribution to the genetic variance of multiple linked QTL (Zeng et al. 1999), Multiple QTL mapping (MQM) procedure (Broman et al. 2003; Arends et al. 2010) was employed, which is part of the R/qtl software package (http://www.rqtl.org/). The DI means of each F_3 family of each experiment were used for QTL analysis. Each trait was mapped separately first with simple interval mapping and then with MQM to refine QTL map positions. Genome

wide LOD threshold values ($P < 0.05$) for declaring the presence of QTL were estimated from 10,000 permutations of each trait. Since the LOD thresholds among the traits varied between 3.21 and 3.50, a single LOD of 3.50 was used as the threshold for all traits. For each QTL detected, in addition to chromosome location and LOD support value, the MQM analysis also reported the phenotypic variation (R^2 %) explained by this QTL, as well as the additive and dominance effects. Since the dominance effects were calculated based on $F_{2:3}$ family means, which are expected to be reduced by half relative to their F_2 parents, the estimated dominance effects were adjusted by multiplications of two (Mather and Jinks 1971). Gene action was determined by the absolute value of the estimated dominance-to-additive effect ratio (|d/a|) following Stuber et al. (1987) (additive 0–0.20, partial dominance 0.21–0.80, dominance 0.81–1.20, and over-dominance >1.20). For each QTL, a 2-LOD support interval was calculated and defined by left and right markers. The flanking markers could be real markers on the genetic map or pseudo markers assigned by the R/qtl procedure (Arends et al. 2010).

The QTL were named according to the chromosome locations and tissue source of resistance (cl = cotyledon, tl = true leaf, h = hypocotyl) prefix with pm (lower case due to the recessive nature of PM resistance). For example, *pm-tl5.1*, *pm-hy5.1* and *pm-hy5.2* designated the first QTL for true leaf, the first and second QTL for hypocotyl resistance in cucumber chromosome 5, respectively. A QTL that explained more than 15 % observed phenotypic variations was considered a major-effect QTL.

Results

Phenotypic data of powdery mildew resistance

Disease index (DI) was calculated for hypocotyl (HY), cotyledons (CL) and true leaves (TL) of each plant and family means in each experiment were used for ANOVA analysis. The means of DI for the two parental lines, F_1 plants, and F_3 families of each experiment, as well as F values in ANOVA are presented in Table 1. Since the DI means among F_3 families, experiments, and experiment × family interactions were all significantly different ($P < 0.001$), subsequent analyses were based on DI means of families in different experiments. The frequency distribution of DI of HY, CL and TL among F_3 families in EXPT2 and EXPT3-2 is exemplified in Fig. 1 (data for CL in 2011 EXPT3 were from EXPT3-1), and that for all three experiments is provided in supplementary Fig. S1 (online materials).

In all experiments, the DI means of HY, CL and TL in F_1 were similar to those of the susceptible parent True Lemon (Table 1) indicating the recessive nature of resistance in HY, CL and TL to powdery mildew in WI 2757.

Table 1 Statistics of disease index (DI) means in parental lines, F_1 and F_3 families across experiments

Traits	EXPT	Parents and F_1			F_3 Family		F values		
		WI2757	TL	F_1	Mean ± SD	Range	Family	EXPT	Family*EXPT
HY	EXPT1	–	–	–	–	–			
	EXPT2	19.1	62.0	63.4	47.9 ± 27.3	0.0–100.0			
	EXPT3-1	0.0	54.8	57.1	9.8 ± 19.3	0.0–100.0			
	EXPT3-2	1.4	98.5	75.7	39.7 ± 40.8	0.0–100.0			
	EXPT3-3	0.0	99.3	95.8	51.4 ± 45.4	0.0–100.0			
	ANOVA test						35.4***	95.1***	8.5***
CL	EXPT1	62.5	94.7	92.1	90.1 ± 19.8	5.6–100.0			
	EXPT2	23.6	63.0	66.7	58.1 ± 22.3	0.0–94.4			
	EXPT3-1	45.8	95.1	93.4	82.4 ± 23.2	0.0–100.0			
	EXPT3-2	96.5	100.0	100.0	94.8 ± 15.0	11.1–100.0			
	EXPT3-3	–	–	–	–	–			
	ANOVA test						20.3***	2708.4***	6.9***
TL	EXPT1	20.8	41.5	39.6	34.4 ± 24.9	0.0–100.0			
	EXPT2	22.5	62.2	62.1	51.1 ± 21.7	0.0–100.0			
	EXPT3-1	0.0	6.3	4.0	4.6 ± 3.9	0.0–22.2			
	EXPT3-2	21.9	54.9	50.0	52.3 ± 23.3	11.0–100.0			
	EXPT3-3	39.9	100.0	94.4	84.6 ± 23.3	0.0–100.0			
	ANOVA test						10.4***	415.2***	3.3***

TL (in row 2) True Lemon, *HY* hypocotyl, *CL* cotyledon, *TL* (in column 1) true leaf

*** $P < 0.001$

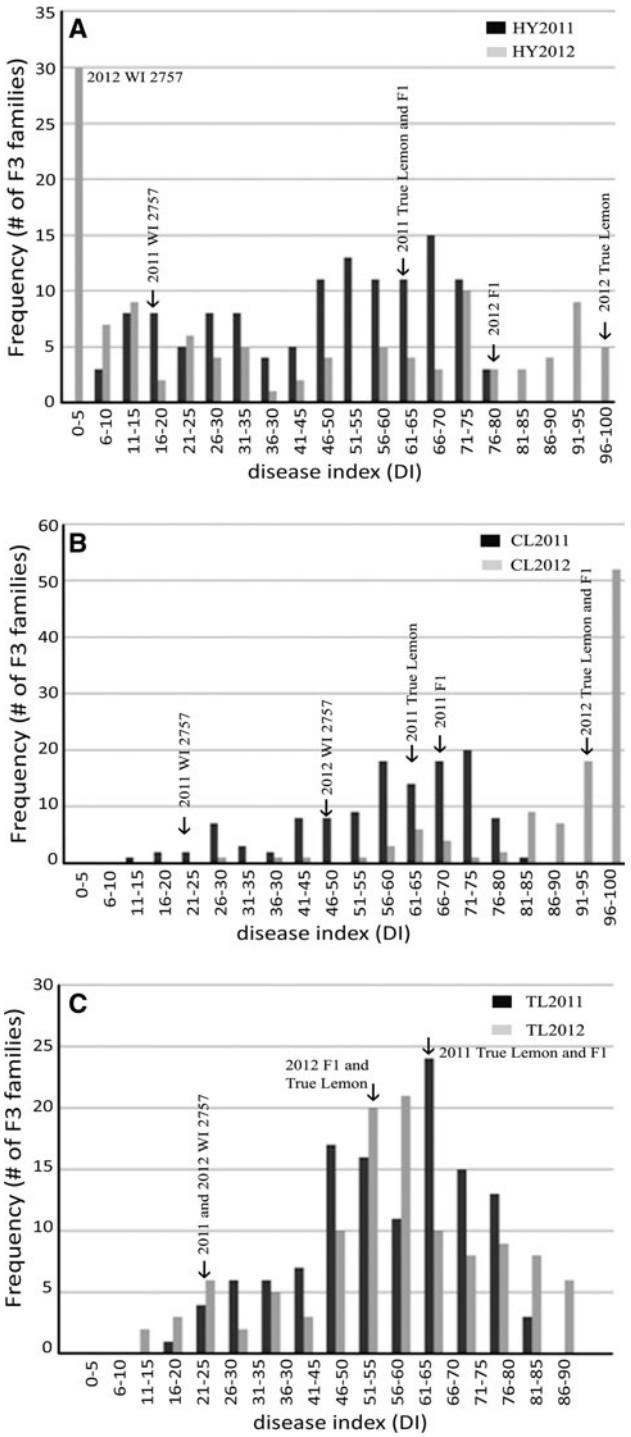


Fig. 1 Frequency distribution of disease index (*DI*) of hypocotyl (*HY*, **a**), cotyledons (*CL*, **b**) and true leaf (*TL*, **c**) among F_3 families of WI 2757 × True Lemon in responses to powdery mildew inoculation. Only 2011 EXPT2 and 2012 EXPT3-2 data are shown (second observation of EXPT3, but CL data from EXPT3-1)

However, the responses to inoculation of PM pathogen varied significantly among the three experiments. As seen from Fig. 1, the frequency distribution of DI means of HY in EXPT3-2 was more skewed toward the resistant parent (WI 2757) than that in EXPT2, and the two parents, WI 2757 and True Lemon belonged to the two most extreme groups, respectively, in EXPT3-2 (Fig. 1a).

The seemingly bimodal frequency distribution of HY2011 data (Fig. 1a) may suggest a single recessive gene *pm-h* (Walters et al. 2001) controlling hypocotyl resistance in WI 2757. Using the DI of the two parents and their F_1 as references (Table 1), segregation of F_3 families for HY resistance was analyzed and each corresponding F_2 plant in EXPT2 and EXPT3-2 was classified into one of the three categories: homozygous resistant (*pm-h/pm-h*, lower DI means), homozygous susceptible (*Pm-h/Pm-h*, high DI means), and heterozygous (*Pm-h/pm-h*, intermediate DI means). As a result, segregation among F_2 plants was 30:62:28, and 33:48:32, respectively, from EXPT2 and EXPT3-2 data, which were consistent with a single recessive gene underlying hypocotyl resistance in WI 2757 ($P = 0.9048$ in χ^2 test against 1:2:1 expected ratio for EXPT2 and $P = 0.2759$ for EXPT3-2). The categorical data for hypocotyl PM resistance from EXPT2 (2011) was used in linkage analysis to place *pm-h* onto the genetic map (see below).

In EXPT1 and EXPT3, the disease symptoms developed much faster on CL than on HY or TL, and the distribution of DI means skewed heavily toward the susceptible parent (Fig. 1; Fig. S1), which could also be seen from the very high DI means but low standard derivation of CL across F_3 families in EXPT1 (90.1 ± 19.8) and EXPT3-2 (94.8 ± 15.0) (Table 1). In contrast, DI distribution in EXPT2 was relatively more symmetrical. Symptom development on the TL lagged behind as compared with HY and CL, and progressed from older to younger leaves. The DI means of TL in all experiments showed largely normal distribution (Fig. S1).

Shanmugasundaram et al. (1971) suggested that the major recessive gene for HY resistance (*pm-h*) is also essential for leaf (complete) resistance. We examined the correlation of hypocotyl and leaf resistances in WI 2757 using EXPT2 data. We first calculated Pearson's correlation coefficients among DI means of F_3 families for the HY, CL and TL, which were 0.4635 (HY vs. CL), 0.3455 (HY vs. TL) and 0.3376 (CL vs. TL). All these correlation coefficients, although not high, were statistically significant ($P < 0.001$) suggesting that PM resistance in HY, CL and TL was positively related. We further examined the correlation of powdery mildew resistance among HY, CL and TL with categorical data from EXPT2. Among the 30 F_2 plants with high-level HY resistance (presumably had *pm-h/pm-h* genotype), 28 (93 %) showed high (19) or intermediate (9) CL resistances and only 2 were susceptible. Of the 30 plants with high HY resistance, 27 had high (16) or intermediate (11) TL resistance and 3 were susceptible. Similar observation was obtained with EXPT3-2 data (data

not shown) suggesting that hypocotyl resistance may indeed correlate with that in CL or TL.

In EXPT3, three sets of data (EXPT3-1, EXPT3-2 and EXPT3-3) were collected at 4-day intervals. The DI means of parental lines, F_1 and F_3 family plants are listed in Table 1. The frequency distributions of DI means for HY, CL and TL are presented in supplementary Fig. S2 (online materials). While the overall patterns of frequency distributions of HY and CL were relatively consistent among the three observations, the distribution pattern of TL showed some significant shift. These dynamic changes had obvious consequences in QTL detection (below).

Linkage map construction

Among 3,200 SSR markers screened between the two parental lines, 245 (6.4 %) were polymorphic and 240 were successfully mapped. The resulting genetic map is graphically presented in supplementary Fig. S3 (online materials). This map contained 240 SSR markers and the *pm-h* locus, which was mapped at 83.3 cM location in chromosome 5 using the HY categorical data from EXPT2. When the HY segregation data from EXPT3-2 were used, *pm-h* was located at 85.1 cM of the same chromosome, which was probably due to inconsistent genotypes in several F_2 plants between the two bioassays. Therefore, the *pm-h* map location in Fig. S3 was tentative, and refinement is needed in future studies. Major statistics of this genetic map are presented in supplemental Table S1 (online materials). Since many new markers were mapped, the genetic map presented herein should be a useful resource for the cucurbit research community. As such, more details (marker names, map locations, scaffold locations, primer sequences) of mapped markers are provided in supplemental Table S2 (online materials).

This genetic map covered 610 cM in seven linkage groups, which was shorter than the expected 700–750 cM for the cucumber genome, but had adequate coverage when compared with the high-resolution map of cucumber by Yang et al. (2012). The marker orders were highly consistent with their physical location in the Gy14 scaffolds (Table S2). The mean marker interval of this map was 2.5 cM with few gaps larger than 10 cM in chromosomes 2, 3, and 6. Therefore, this high-quality genetic map was suitable for subsequent QTL mapping.

QTL analysis

QTL for PM resistance in HY, CL and TL across experiments over 3 years

The F_3 family DI means of 13 traits from three experiments (environments) were used in QTL mapping with the MQM procedure in R/qtl. For each trait, the LOD threshold to declare significant QTL was obtained through permutation tests with 10,000 repetitions. Since the LOD scores among the 13 traits varied from 3.21 to 3.50, a single LOD score of 3.5 was employed as the threshold for all traits. From DI data of EXPT1 (CL2010, TL2010), EXPT2 (HY2011, CL2011 and TL2011), and EXPT3-2 (HY2012-2, CL2012-2 and TL2012-2), 10 QTL were identified in six genomic regions of in three chromosomes. A global view of map locations of these 10 QTL across seven chromosomes is shown in Fig. 2a, and LOD profiles of the 6 QTL in chromosome 5 are presented in Fig. 2b. The details of all QTL (chromosome and map locations, QTL peak location,

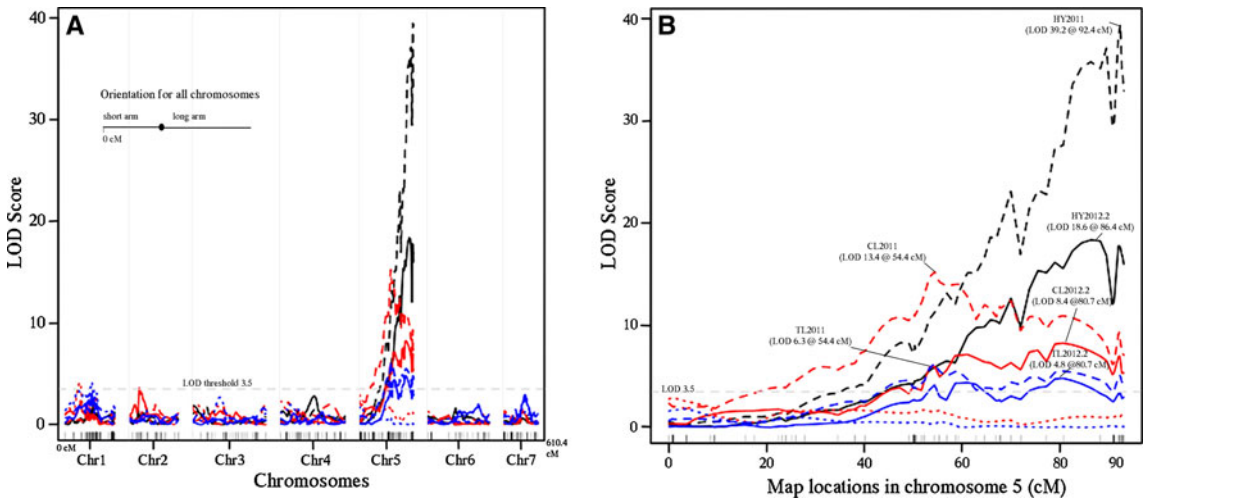


Fig. 2 QTL mapping of PM resistance in hypocotyl (*HY*), cotyledons (*CL*) and true leaves (*TL*) based on phenotypic data in 3 years (2010, 2011 and second observation of 2012). **a** A global view of map locations of 10 QTL is presented. **b** LOD curves of the six QTL in chromosome 5. LOD profiles were based on simple interval mapping, which differed slightly from MQM profiles shown in Table 2 for some QTL. The *dashed line* is LOD threshold (LOD 3.5) based on 10,000 permuted samples

supporting LOD score, 2-LOD support interval, R^2 value, additive and dominance effects, as well as gene actions, arranged by trait) are provided in Table 2. Note that in Table 2, the 2-LOD support interval of a QTL may be defined by pseudo markers assigned by the MQM program. For example, the left marker for QTL *pm-cl1.1* was c1.loc7 (16.8 cM), which was a pseudo marker (locus 7) at 16.8 cM in chromosome 1.

In EXPT1, *pm-cl1.1* at 26.7 cM (LOD 3.9, $R^2 =$ 15.7 %) and *pm-tl1.1* at 46.2 cM (LOD 4.2, $R^2 = 16.9$ %) of chromosome 1 were the only two QTL detected (Table 2). In EXPT2, the QTL *pm-hy5.1* at 92.4 cM (LOD 39.2) of chromosome 5 contributed to hypocotyl resistance with a very high LOD score and explained 49.1 % phenotypic variations. The HY2011 data also detected a QTL peak at 55.4 cM, which was probably just a local maximum, rather than a true QTL, and therefore was not counted. The 2011 CL data detected two QTL for cotyledon resistance: *pm-cl4.1* at 72.5 cM of chromosome 4 (LOD 3.9, $R^2 = 6.1$ %) and *pm-cl5.1* at 54.4 cM of chromosome 5 (LOD 13.4, $R^2 = 25.6$ %). *pm-cl4.1* seemed to contribute to disease susceptibility. For true leaf resistance, only one QTL, *pm-tl5.1*, was detected by TL2011 data, which was co-localized with *pm-cl5.1* in chromosome 5 (LOD 6.3, $R^2 = 21.4$ %).

From EXPT3-2 data, four QTL were detected including *pm-cl1.1* (LOD 4.3, $R^2 = 9.5$ %) in chromosome 1, *pm-cl5.2*, *pm-hy5.1*, and *pm-tl5.2* in chromosome 5 (Table 2). Since the 2-LOD intervals of the three QTL in chromosome 5 overlapped, it was difficult to determine their exact locations (see "Discussion" below).

Based on the dominance-to-additive effect ratios (|d/a| value), the gene action of a QTL could be additive (ratio 0–0.20), partial dominance (ratio 0.21–0.80), dominance (ratio 0.81–1.20), or overdominance (ratio >1.20) (Stuber et al. 1987). The |d/a| values of all detected QTL in this study are listed in Table 2. Among the 10 QTL detected from EXPT1, EXPT2 and EXPT3-2, three showed overdominance (*pm-cl1.1* by CL2010 and CL2012.1, *pm-cl5.2* by CL2012.2) and seven had partial dominance or

Table 2 QTL for powdery mildew resistance in cucumber inbred line WI 2757 based on data from EXPT1 (2010), EXPT2 (2011) and three observations of EXPT3 (2012, EXPT3-1, EXPT3-2 and EXPT3-3)

Traits	EXPT	QTL	Chr	Peak (cM)	LOD	R^2	Add	Dom	|d/a|	2-LOD support interval[a]	
										Left	Right
Cotyledon											
CL2010	EXPT1	*pm-cl1.1*	1	26.7	3.9	15.7	−7.8	10.8	1.4	cl.loc7 (16.8)	UW084490 (55.2)
CL2012.2	EXPT3-2	*pm-cl1.1*	1	24.7	4.3	9.5	−5.3	10.6	2.0	SSR00160 (0.0)	c1.loc11 (24.7)
CL2011	EXPT2	*pm-cl4.1*	4	72.5	3.9	6.1	5.4	6.4	1.2	UW084382 (34.3)	c4.loc38 (98.7)
CL2011	EXPT2	*pm-cl5.1*	5	54.4	13.4	25.6	−13.2	5.8	0.4	SSR32717 (51.7)	SSR10725 (55.4)
CL2012.1	EXPT3-1	*pm-cl5.1*	5	58.6	13.1	41.0	−16.4	15.6	1.0	UW084461 (52.3)	UW001619 (64.6)
CL2012.2	EXPT3-2	*pm-cl5.2*	5	80.7	8.5	20.8	−6.8	9.2	1.4	SSR07531 (56.8)	UW084533 (88.3)
Hypocotyl											
HY2012.1	EXPT3-1	*pm-hy3.1*	3	89.8	3.5	8.5	1.4	−14.2	10.2	c3.loc28 (66.3)	SSR30236 (104.9)
HY2012.1	EXPT3-1	*pm-hy4.1*	4	54.2	4.5	11.3	6.6	−0.2	0.0	SSR06225 (45.9)	c4.loc24 (67.1)
HY2012.2	EXPT3-2	*pm-hy5.1*	5	86.4	18.6	55.1	−33.4	27.0	0.8	UW013256 (80.7)	SSR13237 (93.1)
HY2011	EXPT2	*pm-hy5.1*	5	92.4	39.2	49.1	−18.7	20.4	1.0	SSR10911 (92.2)	UW084353 (92.7)
HY2012.1	EXPT3-1	*pm-hy5.1*	5	92.4	8.9	24.7	−8.0	5.2	0.6	UW013295 (77.3)	SSR13237 (93.1)
HY2012.3	EXPT3-3	*pm-hy5.1*	5	92.4	40.2	74.5	−41.4	37.4	1.0	c5.loc25 (84.5)	UW084353 (92.7)
True leaf											
TL2010	EXPT1	*pm-tl1.1*	1	46.2	4.2	16.9	−8.9	−3.4	0.4	SSR04304 (13.3)	c1.loc15 (57.2)
TL2012.1	EXPT3-1	*pm-tl1.2*	1	83.7	3.8	14.6	−1.6	1.2	0.8	c1.loc18 (68.0)	SSR05817 (85.2)
TL2011	EXPT2	*pm-tl5.1*	5	54.4	6.3	21.4	−7.9	6.4	0.8	c5.loc16 (45.5)	SSR13237 (93.1)
TL2012.3	EXPT3-3	*pm-tl5.1*	5	54.4	7.6	27.1	−11.0	12.4	1.2	UW084461 (52.3)	UW084533 (88.3)
TL2012.2	EXPT3-2	*pm-tl5.2*	5	80.7	4.9	18.3	−9.0	6.0	0.6	SSR32717 (51.7)	SSR13237 (93.1)

CL cotyledon, *TL* true leaf, *HY* hypocotyl, *LOD* logarithm of odds score, R^2 percentages of the phenotypic variance explained by individual QTL, *Add* additive effect of resistance allele, *Dom* dominance effects, |*d/a*| is the absolute value dominance-to-additive effect ratio indicating gene actions (additive 0–0.20; partial dominance 0.21–0.80; dominance 0.81–1.20; and overdominance >1.20) (Stuber et al. (1987)

[a] The 2-LOD support interval is the interval in which the LOD score is within 2.0 units of its maximum, which was delimited by left and right markers. Numbers in parentheses after each marker are map locations (in cM) of this marker. A marker without a specific name is pseudo marker assigned by R/qtl

dominance gene actions, suggesting that dominance may play an important role in PM resistance in WI 2757.

Effects of scoring time on detection of PM resistance QTL

To investigate the effects of scoring time on QTL detection, three datasets were collected at 4-day interval in EXPT3, namely, EXPT3-1, EXPT3-2 and EXPT3-3 (Table 1; Fig. S2). MQM analysis was conducted on DI means of F_3 families from eight traits (HY2012.1, HY2012.2, HY2012.3, CL2012.1, CL2012.2, TL2012.1, TL2012.2, and TL2012.3). Information on QTL detected from the three observations is summarized in Table 2, and LOD curves of mapped QTL are graphically presented in supplementary Figs. S4A (all QTL) and S4B (QTL mapped in chromosome 5) (online materials). Note that the LOD profiles in Fig. S4A were drawn based on simple interval mapping, which differed slightly from MQM profiles shown in Table 2 for some QTL.

For hypocotyl resistance, a major QTL, *pm-hy5.1* at 92.4 cM of chromosome 5 was detected by HY2012.1 and HY2012.3 datasets with strong LOD score support (LOD 8.9, $R^2 = 24.7$ %, and 40.2, $R^2 = 74.5$ %, respectively). The hypocotyl resistance QTL at 86.4 cM detected with HY2012.2 data (LOD 18.6, $R^2 = 55.1$ %) were presumably the same QTL as *pm-hy5.1* (see "Discussion" below). The HY2012.1 data also detected two minor QTL, *pm-hy3.1* in chromosome 3 (LOD 3.5, $R^2 = 8.5$ %), and *pm-hy4.1* in chromosome 4 (LOD 4.5, $R^2 = 11.3$ %). Both QTL seemed to promote susceptibility to PM inoculation (with positive additive effect, higher DI values were associated with more severe disease symptoms).

As shown in Table 2, three QTL were detected by cotyledon data of CL2012.1 and CL2012.2: one (*pm-cl5.1*) by CL2012.1 at 58.6 cM of chromosome 5 (LOD 13.1, $R^2 = 41.0$ %), and two by CL2012.2 (*pm-cl1.1*, LOD 4.3, $R^2 = 9.5$ %; *pm-cl5.2*, LOD 8.5, $R^2 = 20.8$ %). The true leaf data also detected three QTL: *pm-tl1.1* by TL2012.1 (chromosome 1 at 83.7 cM, LOD 3.8, $R^2 = 14.6$ %), *pm-tl5.1* by TL2012.3 (chromosome 5 at 54.4 cM, LOD 7.6, $R^2 = 27.1$ %), and *pm-tl5.2* by TL2012.2 (chromosome 5 at 80.7 cM, LOD 4.9, $R^2 = 18.3$ %). The mode of gene action of each QTL based on the |d/a| ratio was largely consistent across the three observations of EXPT3 (Table 2). Similar to that found in EXPT1 and EXPT2, dominance gene action seemed to play an important role in PM resistance in WI 2757.

The results of MQM analysis on the three datasets from EXPT3 revealed two important facts on QTL mapping of PM resistance in WI 2757 cucumber. First, the time of disease scoring significantly affected the detection of PMR QTL in both QTL locations and magnitudes of effects, which was especially true for QTL with small effects.

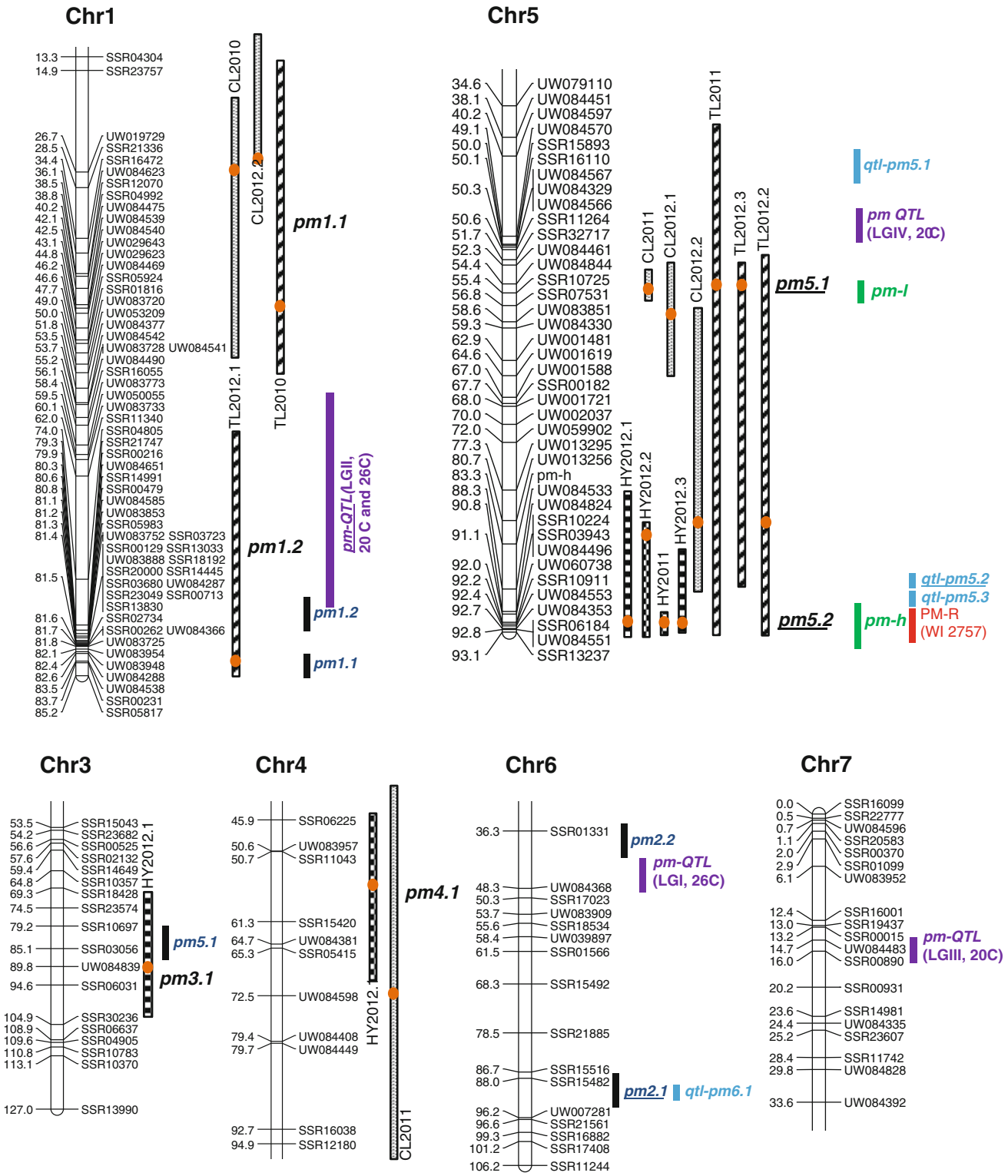
Fig. 3 Summary of powdery mildew resistance QTL detected in the present and other studies. The cucumber genetic map was developed from the present study, but only chromosome segments harboring PMR QTL are shown. *Vertical bars* (*hatched* TL, *dotted* CL, *checked* HY) represent 2-LOD support interval and *orange filled circles* are QTL peak locations. LOD support intervals of QTL in all other studies are not available; thus the *lengths of vertical bars* for these QTL do not represent the confidence intervals. The trait name detected QTL is listed alongside each *vertical bar*. *Underlined* QTL are major-effect QTL that explained more than 20 % genotypic variations in each study where available. QTL symbols from original publications were used. Map locations of PM QTL from other publications were inferred from in silico PCR or BLASTn using primer sequences of QTL-associated molecular markers, and are approximations

Interestingly, with the progression of the PM disease, the number of detected QTL decreased from five in four chromosomes in EXPT3-1 to two in one chromosome in EXPT3-3 (Table 2), suggesting that EXPT3-1 might be the right time for PMR QTL detection. Second, while minor- or moderate-effect QTL were detected in chromosomes 1, 3 and 4, chromosome 5 harbored major QTL of PM resistance (Table 2). The QTL at 92.4 cM region conferred primarily hypocotyl resistance, whereas the QTL near 54.4 cM region accounted mainly for cotyledon resistance.

QTL for powdery mildew resistance in WI2757

From the 3 experiments, 17 QTL (6 for CL, 6 for HY and 5 for TL) were detected by 11 traits (Table 2). For better visualization, the map locations and 2-LOD support intervals of these QTL are graphically presented in Fig. 3 along with powdery mildew resistance QTL detected in previous studies. From the present study, six genomic regions in four chromosomes harboring QTL for PM resistance could be clearly recognized including one each in chromosomes 3 and 4 and two each in chromosomes 1 and 5. In four of the six regions, more than one QTL was mapped in nearby but distinct peak locations of the same chromosomal region (Fig. 3).

The co-localization of multiple QTL may suggest that they are actually the same or tightly linked QTL. For example, for hypocotyl resistance, the data supported one QTL, *pm-hy5.1* on chromosome 5 at 92.4 cM for HY2011, HY2012.1 and HY2012.3 (Table 2) with 2-LOD support interval of (77.3, 93.1) (Table 2; Fig. 3). Although the QTL peak detected by HY2012.2 data was at 86.4 cM, this QTL had a 2-LOD support interval of (80.7, 93.1). This was most likely an artifact due to wide spacing of markers, modest sample size or missing data; in other words, this trait may be consistent with the 92.4 cM QTL. For the same reasoning, both QTL, *pm-cl5.2* of CL2012.2 and *pm-tl5.2* of TL2012.2 at 80.7 cM in chromosome 5 may be consistent with *pm-hy5.1* at 92.4 cM (by HY2011); the


Chr1
13.3 SSR04304
14.9 SSR23757
26.7 UW019729
28.5 SSR21336
34.4 SSR16472
36.1 UW084623
38.5 SSR12070
38.8 SSR04992
40.2 UW084475
42.1 UW084539
42.5 UW084540
43.1 UW029643
44.8 UW029623
46.2 UW084469
46.6 SSR05924
47.7 SSR01816
49.0 UW083720
50.0 UW053209
51.8 UW084377
53.5 UW084542
53.7 UW083728 UW084541
55.2 UW084490
56.1 SSR16055
58.4 UW083773
59.5 UW050055
60.1 UW083733
62.0 SSR11340
74.0 SSR04805
79.3 SSR21747
79.9 SSR00216
80.3 UW084651
80.6 SSR14991
80.8 SSR00479
81.1 UW084585
81.2 UW083853
81.3 SSR05983
81.4 UW083752 SSR03723
SSR00129 SSR13033
UW083888 SSR18192
SSR20000 SSR14445
81.5 SSR03680 UW084287
SSR23049 SSR00713
SSR13830
81.6 SSR02734
81.7 SSR00262 UW084366
81.8 UW083725
82.1 UW083954
82.4 UW083948
82.6 UW084288
83.5 UW084538
83.7 SSR00231
85.2 SSR05817
CL2010
CL2012.2
TL2012.1
TL2010
pm1.1
pm1.2
pm-QTL(LGII, 20 C and 26C)
pm1.2
pm1.1
Chr5
34.6 UW079110
38.1 UW084451
40.2 UW084597
49.1 UW084570
50.0 SSR15893
50.1 SSR16110
UW084567
50.3 UW084329
UW084566
50.6 SSR11264
51.7 SSR32717
52.3 UW084461
54.4 UW084844
55.4 SSR10725
56.8 SSR07531
58.6 UW083851
59.3 UW084330
62.9 UW001481
64.6 UW001619
67.0 UW001588
67.7 SSR00182
68.0 UW001721
70.0 UW002037
72.0 UW059902
77.3 UW013295
80.7 UW013256
83.3 pm-h
88.3 UW084533
90.8 UW084824
SSR10224
91.1 SSR03943
UW084496
92.0 UW060738
92.2 SSR10911
92.4 UW084553
92.7 UW084353
92.8 SSR06184
UW084551
93.1 SSR13237
HY2012.1
HY2012.2
HY2011
HY2012.3
CL2011
CL2012.1
CL2012.2
TL2011
TL2012.3
TL2012.2
pm5.1
pm5.2
qtl-pm5.1
pm QTL (LGIV, 20C)
pm-l
qtl-pm5.2
qtl-pm5.3
pm-h
PM-R (WI 2757)
Chr3
53.5 SSR15043
54.2 SSR23682
56.6 SSR00525
57.6 SSR02132
59.4 SSR14649
64.8 SSR10357
69.3 SSR18428
74.5 SSR23574
79.2 SSR10697
85.1 SSR03056
89.8 UW084839
94.6 SSR06031
104.9 SSR30236
108.9 SSR06637
109.6 SSR04905
110.8 SSR10783
113.1 SSR10370
127.0 SSR13990
HY2012.1
pm5.1
pm3.1
Chr4
45.9 SSR06225
50.6 UW083957
50.7 SSR11043
61.3 SSR15420
64.7 UW084381
65.3 SSR05415
72.5 UW084598
79.4 UW084408
79.7 UW084449
92.7 SSR16038
94.9 SSR12180
HY2012.1
CL2011
pm4.1
Chr6
36.3 SSR01331
48.3 UW084368
50.3 SSR17023
53.7 UW083909
55.6 SSR18534
58.4 UW039897
61.5 SSR01566
68.3 SSR15492
78.5 SSR21885
86.7 SSR15516
88.0 SSR15482
96.2 UW007281
96.6 SSR21561
99.3 SSR16882
101.2 SSR17408
106.2 SSR11244
pm2.2
pm-QTL (LGI, 26C)
pm2.1
qtl-pm6.1
Chr7
0.0 SSR16099
0.5 SSR22777
0.7 UW084596
1.1 SSR20583
2.0 SSR00370
2.9 SSR01099
6.1 UW083952
12.4 SSR16001
13.0 SSR19437
13.2 SSR00015
14.7 UW084483
16.0 SSR00890
20.2 SSR00931
23.6 SSR14981
24.4 UW084335
25.2 SSR23607
28.4 SSR11742
29.8 UW084828
33.6 UW084392
pm-QTL (LGIII, 20C)
References:
This study, hypocotyl resistance
This study, cotyledon resistance
This study, true leaf resistance
Sakata et al et al. (2006)
Zhang et al. (2008)
Liu et al. (2008)
de Ruiter et al. (2008)
Zhang et al. (2011)

QTL detected by TL2010 data at 46.2 cM were probably the same as the QTL at 26.7 cM (by CL2010) or the one at 24.7 cM (by CL2012.2) in chromosome 1. Therefore, for the QTL detected from CL2011, CL2012.1, TL2011, and TL2012.3 data in chromosome 5 peaked around the 54.4 cM location, a single QTL, *pm5.1*, could be assigned at this location to reconcile all the QTL from different experiments and tissue origins. Similarly, *pm5.2* could be designated for a QTL peaked around 92.4 cM in chromosome 5 for hypocotyl resistance detected by HY2011, HY2012.1, HY2012.2 and HY2012.3. By synthesizing information from Table 2, six such QTL could be recognized which included *pm1.1* and *pm1.2* in chromosome 1, *pm3.1* in chromosome 3, *pm4.1* in chromosome 4, as well as *pm5.1* and *pm5.2* (*pm-h*) in chromosome 5. Details of the six-consensus QTL for powdery mildew resistance in WI 2757 cucumber are summarized in Table 3 and graphically presented in Fig. 3. Among them, the major-effect QTL, *pm5.1* at 54.4 cM was responsible mainly for leaf resistance; *pm5.2* at 92.4 cM was the major QTL for hypocotyl resistance, which was largely consistent with the map location of *pm-h* at 83.3 cM based on categorical data. The recessive *pm1.1* and *pm1.2* in chromosome 1 are QTL for leaf resistance; meanwhile, both *pm3.1* (chromosomes 3) and *pm4.1* (chromosome 4) contributed to leaf or hypocotyl susceptibility.

Discussion

Effects of environmental factors and scoring time on QTL detection

Data of 3-year experiments reveal that phenotypic responses in host plants to inoculation of PM pathogen were easily influenced by environmental conditions. This can be seen from the value and distribution of DI means of parental lines, F_1 and F_3 families (Table 1; Fig. 1). EXPT2 was conducted in January 2011, which is the coolest month of the year with low light intensity and short day length. While the seedlings did not grow as fast as in other seasons, these conditions promoted the development of PM symptoms in the greenhouse allowing for revealing maximum genetic variations within the population (low DI means with large standard derivation among F_3 families, Table 1). Thus, the frequency distribution of DI was more symmetric (less skewed) in EXPT2. On the other hand, disease symptoms progressed more quickly in EXPT1. By the time of scoring the plants, most cotyledons were fully covered with PM spores (high DI means with small standard derivation among F_3 families: 90.1 ± 19.8) (Table 1) making it difficult to differentiate among test plants (small genetic variations in the population). Only two QTL were detected in EXPT1 with relatively low LOD support. In contrast, results from EXPT2 and EXPT3 were more consistent in QTL detection (Table 2).

A closer look into the data in Tables 1 and 2 provided us important clues about the effects of scoring time on QTL detection for powdery mildew resistance in WI 2757. Two and four QTL were detected in EXPT1 (2010) and EXPT2 (2011), respectively. In EXPT3 (2012), 11 QTL were identified in three observations with 5, 4 and 2 QTL identified from EXPT3-1, EXPT3-2 and EXPT3-3, respectively (Table 2). It seems that more QTL with stronger LOD support and narrower LOD support interval were detected in a scoring time point when more genetic variations in the population could be captured. For example, in QTL analysis of HY data, while the major QTL (*pm5.2*) was detected in all observations (HY2011, HY2012.1, HY2012.2 and HY2012.3), the HY2012.1 data identified two (*pm3.1* and *pm4.*1) additional minor QTL (Table 2; Fig. 3) suggesting that the scoring time of EXPT3-1 (2012) might be preferable time points and environments for detection of hypocotyl resistance QTL. For cotyledon resistance, data from CL2011, CL2012.1 and CL2012.2 each detected one QTL at the *pm5.1* location, whereas CL2010 data detected a minor-effect QTL in chromosome 1 (*pm1.1*) (Fig. 3). The failure for CL2010

Table 3 Summary of QTL for powdery mildew resistance in WI 2757 based on QTL mapping in hypocotyl, cotyledons and true leaves over 3 years' greenhouse experiments

QTL	Chr	Putative map location (cM)	Traits that detected QTL at this location[a]	Notes
pm1.1	1	24.7	CL2010, TL2010, CL2012.2	Leaf resistance
pm1.2	1	83.7	TL2012.1	Leaf resistance
pm3.1	3	89.8	HY2012.1	Hypocotyl resistance
pm4.1	4	54.2	HY2012.1, CL2011	Leaf and hypocotyl susceptibility
pm5.1	5	54.4	CL2011, CL2012.1,	Leaf resistance
pm5.2 (pm-h)	5	92.4	HY2011, HY2012.1, HY2012.2, HY2012.3	Hypocotyl resistance

[a] Only traits with clear 2-LOD support intervals were listed

data to detect a QTL at *pm5.1* location was probably due to the fast development of disease symptoms resulting in high DI means among F_3 families with low standard derivation (90.1 ± 19.8) (Table 1), which left little genetic variations to explore in this population for QTL mapping. These results pointed out the importance of identifying right scoring time in phenotyping PM resistance to increase the power of QTL detection.

Although the two major QTL (*pm5.1* and *pm5.2*) were consistently identified in EXPT2 and EXPT3, the overlapping 2-LOD support intervals for several QTL (Fig. 3) as well as the multiple peaks in the LOD curves of QTL in chromosome 5 (Fig. 2b; Fig. S4B) may suggest noises in the phenotypic data, which may be due to several reasons. First, the large 2-LOD support interval of CL2012.2 may be the result of rapid symptom development on the cotyledons as reflected by the high F_3 family DI means and low standard derivation within the population (94.8 ± 15.0, Table 1). Second, the limited number of recombinants in the moderate-sized mapping population (120–126 F_3 families) used for phenotyping in this study may prevent separation of linked QTL (Fig. 3). To improve the precision of QTL mapping, more accurate phenotyping could be achieved with a larger $F_{2:3}$ population and higher marker density in the target regions. Development of an advanced intercross population segregating only in this region (Darvasi and Soller 1995) will also be beneficial. Lastly, all QTL for true leaf resistance from this study had large 2-LOD support intervals (Fig. 3). For example, the 2-LOD intervals of QTL detected by TL2012.1, TL2012.2 and TL2012.3 spanned the *pm5.1* and *pm5.2* genomic regions in chromosome 5 making it difficult to decide their locations in this chromosome. While the population size may be a reason, more likely, the time for scoring true leaf resistance in the present study might be too early to reveal the global picture of true leaf resistance. We only scored the first two true leaves at the seedling stage. Examining leaf resistance in later stages of adult plants may be helpful for more accurate detection of QTL for true leaf resistance.

Powdery mildew resistance QTL in WI 2757 and other cucumber sources

Genes for cucumber epidermal features, like the *u* for uniform immature fruit color and *D* for dull fruit color form a cluster in chromosome 5 (Fanourakis and Simon 1987; Miao et al. 2011). With classical genetic analysis, Kooistra (1971) found that the gene *D* is closely linked with one of the PMR genes derived from PI 200815 or Natsufushinari. The PM resistance gene in WI 2757 was linked with the *D*/*u* genes with a genetic distance from 14.2 to 35.7 cM (Fanourakis and Simon 1987; Vakalounakis 1992; Walters et al. 2001). Based on scaffold information of SSR markers flanking the *u*/*D* gene cluster (Miao et al. 2011), the approximate location of this cluster on the genetic map developed herein is between UW001481 (62.9 cM) and UW001616 (64.6 cM) (Table S2), which will be approximately 9 and 29 cM away from the two QTL, *pm5.1* (54.4 cM) and *pm5.2* (92.4 cM), respectively. Thus, the PM resistance gene identified by Vakalounakis (1992) or Walters et al. (2001) probably corresponds to *pm5.2* of this study that controls the hypocotyl resistance in WI 2757 (Table 3; Fig. 3).

Several studies have been carried out in cucumber for QTL mapping of PM resistance. For convenience of discussion, the QTL detected from previous and the present studies are summarized in Fig. 3. These QTL were distributed in six of the seven cucumber chromosomes (1, 3, 4, 5, 6 and 7) with major QTL ($R^2 > 20$ %) mapped in chromosome 1 by Sakata et al. (2006), chromosome 5 by Zhang et al. (2011), de Ruiter et al. (2008) and the present study (Table 2). The two QTL, *pm-l* (leaf resistance) and *pm-h* (hypocotyl resistance) reported in Hofstede et al. (2008) and de Ruiter et al. (2008) are highly consistent in map locations with *pm5.1* and *pm5.2* identified herein, respectively (Fig. 3). Using bulked segregant analysis (BSA), Zhang et al. (2008) identified a PM resistance locus in WI 2757 (PM-R) that was linked with two markers located in the *pm5.2* region of chromosome 5. Meanwhile, Zhang et al. (2011) detected three QTL, *qtl-pm5.1*, *qtl-pm5.2*, and *qtl-pm5.3* in chromosome 5, all of which are presumably controlling leaf resistance because hypocotyl resistance was not screened in this study. While *qtl-pm5.2* and *qtl-pm5.3* were largely co-localized with *pm5.2*, the location of *qtl-pm5.1* in Zhang et al. (2011) was probably the same QTL as *pm5.1* in our study (Fig. 3). In addition, *pm1.2* from the present study seemed to be consistent with a major QTL in PI 197088-1 identified by Sakata et al. (2006) and two QTL (*pm1.1* and *pm1.2*) by Liu et al. (2008) in chromosome 1; *pm3.1* in chromosome 3 was at a similar location with *pm5.1* (chromosome 3) by Liu et al. (2008). The QTL *pm-4.1* for hypocotyl and cotyledon susceptibility in chromosome 4 was not detected in any previous studies. On the other hand, PMR QTL were detected by Sakata et al. (2006), Liu et al. (2008) (a major QTL *pm2.1*) and Zhang et al. (2011) in chromosomes 6 and 7, but no significant QTL were detected in either chromosome in the present study (Fig. 3).

While environmental conditions may contribute to the differences in QTL locations and magnitudes of effects on PM resistance among these studies, some other factors may also cause the discrepancies in QTL detection. First, different PM resistance sources were used for QTL mapping in these studies. WI 2757 used in the present study and Zhang et al. (2008) has a complicated pedigree with its PM resistance source from the India germplasm line PI 197087

(Peterson et al. 1982). PI 197088-1, a selection from PI 197088 also from India was the PM resistant source used by Sakata et al. (2006). The sources of resistance for *pm-l* and *pm-h* in de Ruiter et al. (2008) and Hofstede et al. (2008) were NPI, which was derived from Natsufushnari and PI 200815. Zhang et al. (2011) used K08 as the PM resistant parent, which is a north China fresh market type cucumber. In Liu et al. (2008), the resistant parent S06 belongs to European greenhouse market type.

The methods and timing of phenotyping PM resistance and molecular mapping strategies employed by these studies may have different consequences in QTL mapping. For example, BSA was used by Zhang et al. (2008, 2011) to identify molecular markers associated with PM resistance. de Ruiter et al. (2008) used natural infection in the greenhouse. Sakata et al. (2006) conducted PM screening in two temperature regimes (20 and 26 °C) and detected temperature-dependent QTL. In addition to inoculation on live plants, Liu et al. (2008) also used leaf disk assay, and inoculation was conducted at three full-leaf stage. Finally, hypocotyl resistance was screened by only de Ruiter et al. (2008) and the present study.

Inheritance of powdery mildew resistance in WI 2757 cucumber

Data presented herein support two recessively inherited, linked QTL in chromosome 5 (*pm5.1* and *pm5.2*) that were the major resistance factors for powdery mildew resistance in WI 2757. Dominance gene action seems to be important for host resistance. Meanwhile, *pm1.1* and *pm1.2* had moderate contributions ($R^2 \sim 15$ %) to host resistance, and *pm3.1* and *pm4.1* promoted susceptibility. The *pm5.1* was a major QTL for cotyledon resistance (R^2 20.8–41.0 %, Table 2). While *pm4.1* and *pm5.2* contributed to hypocotyl resistance, the major QTL *pm5.2* explained up to 74.5 % phenotypic variations (Table 2). This is largely consistent with the previous notion of Shanmugasundaram et al. (1971) and Walters et al. (2001) that there may be a single recessive gene (*pm-h*) for hypocotyl resistance which is also essential for leaf resistance. However, the hypocotyl resistance conferred by *pm5.2* in this study is co-localized with PM resistance QTL (Fig. 3) that detected by Zhang et al. (2008) (PM-R from WI 2757) and Zhang et al. (2011) in which hypocotyl resistance was not evaluated. In addition, it is difficult to decide the exact locations in chromosome 5 of QTL for true leaf resistance due to overlapping 2-LOD intervals with *pm5.1* and *pm5.2* (Fig. 3). Therefore, the mechanisms of interactions among cotyledon, true leaf and hypocotyl resistance QTLs (pleiotropic, epistatic or linkage) are not known, which merit further investigation. Nevertheless, results from the present study provided new insights into the phenotypic mechanisms and genetic basis of PM resistance in cucumber. The molecular markers flanking the two major QTL in chromosome 5 could be a useful tool in marker-assisted selection in cucumber breeding for the recessively inherited powdery mildew disease. This study also provides a good starting point for fine mapping and cloning of PM resistance major QTL in WI 2757 in the near future.

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