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ORIGINAL ARTICLE

Fine mapping of a dominantly inherited powdery mildew resistance major-effect QTL, *Pm1.1*, in cucumber identifies a 41.1 kb region containing two tandemly arrayed cysteine-rich receptor-like protein kinase genes

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Abstract

Key message **A dominantly inherited major-effect QTL for powdery mildew resistance in cucumber was fine mapped. Two tandemly arrayed cysteine-rich receptor-like protein kinase genes were identified as the most possible candidates.**

Abstract Powdery mildew (PM) is one of the most severe fungal diseases of cucumber (*Cucumis sativus* L.) and other cucurbit crops, but the molecular genetic mechanisms of powdery mildew resistance in cucurbits are still poorly understood. In this study, through marker-assisted backcrossing with an elite cucumber inbred line, D8 (PM susceptible), we developed a single-segment substitution line, SSSL0.7, carrying 95 kb fragment from PM resistance donor, Jin5-508, that was defined by two microsatellite markers, SSR16472 and SSR16881. A segregating population with 3600 F_2 plants was developed from the SSSL0.7 × D8 mating; segregation analysis confirmed a dominantly inherited major-effect QTL, *Pm1.1* in cucumber chromosome 1 underlying PM resistance in SSSL0.7. New molecular markers were developed through exploring the next generation resequenced genomes of Jin5-508 and D8. Linkage analysis and QTL mapping in a subset of the F_2 plants delimited the *Pm1.1* locus into a 41.1 kb region, in which eight genes were predicted. Comparative gene expression analysis revealed that two concatenated genes, *Csa1M064780* and *Csa1M064790* encoding the same function of a cysteine-rich receptor-like protein kinase, were the most likely candidate genes. GFP fusion protein-aided subcellular localization indicated that both candidate genes were located in the plasma membrane, but *Csa1M064780* was also found in the nucleus. This is the first report of dominantly inherited PM resistance in cucumber. Results of this study will provide new insights into understanding the phenotypic and genetic mechanisms of PM resistance in cucumber. This work should also facilitate marker-assisted selection in cucumber breeding for PM resistance.

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Xuewen Xu and Ting Yu have contributed equally to this work.

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Introduction

Cucumber, *Cucumis sativus* L., is an economically important vegetable crop, globally ranking 4th in quantity of world vegetable production (FAO STAT 2013, http://faostat3.fao.org). However, frequent occurrence of powdery mildew (PM) causes serious losses in yield and quality. PM is mainly caused by *Podosphaera fusca* (synonym *Podosphaera xanthii)* (Perez-Garcia et al. 2009), which occurs more frequently in subtropical and tropical areas, because of the optimum temperature for conidial germination (25–30 °C) and the availability of a wide range of hosts including a variety of cucurbit crops (Sitterly 1978; Zitter et al. 1996; Lebeda et al. 2011; He et al. 2013). At

present, in most parts of cucumber production areas aiming for fresh market consumption, fungicide application is the major method of disease control, which, however, is less effective under a disease outbreak since fruits are harvested almost daily (Fukino et al. 2013). In addition, long-term use of fungicides may cause the pathogen to develop resistance or insensitivity to them, rendering the chemicals ineffective. Thus, breeding cultivars resistant or tolerant to PM is a desirable strategy for disease control.

Marker-assisted selection (MAS) is more economic and more efficient than traditional breeding based on phenotypic selection (Lande and Thompson 1990). In recent years, due to the use of next generation sequencing technologies and the availability of genetic and genomics resources, a lot of PMR genes and quantitative trait loci (QTL) have been reported in cucumber. Sakata et al. (2006) identified six QTL on cucumber chromosomes 1, 5, 6 and 7 for powdery mildew resistance derived from PI 197088. Fukino et al. (2013) conducted further QTL mapping of PMR from this PI line with 111 recombinant inbred lines (RIL) and identified nine QTL for PM resistance that were distributed in six cucumber chromosomes. Hofstede et al. (2008) and de Ruiter et al. (2008) identified two major-effect QTL for PM resistance in the leaves (*pm-l*) and hypocotyls (*pm-h*), both of which were located in cucumber chromosome 5. Zhang et al. (2011) identified three QTL (*pm5.1*, *pm5.2* and *pm5.3*) in chromosome 5, and one (*pm6.1*) in chromosome 6 for PM resistance from a North China cucumber line K8. He et al. (2013) identified six QTL in chromosomes 1, 3, 4 and 5 for PM resistance in WI 2757 with major-effect QTL in chromosome 5. More recently, Nie et al. (2015) conducted fine genetic mapping of the major-effect QTL, *pm5.1* that was identified previously by de Ruiter et al. (2008) and He et al. (2013), and found a *MLO-like* gene as the most likely candidate for the *pm5.1* locus. The resistance conferred by *pm5.1* was due to insertion of a retrotransposon sequence into the exonic region of the *Mlo*-like gene (Nie et al. 2015). While these findings have provided insights into the genetic control of PM resistance in cucumber, it is far from satisfactory to understand the molecular mechanisms of host resistance against cucumber PM. In addition, most PMR genes or QTL in cucumber reported to date exhibit recessive inheritance, which is not convenient to use in cucumber breeding. Munger et al. (1979) and El-Jack and Munger (1983) found dominant inheritance of PMR in 'Spartan Salad 77-717' and PI 197088. Later, Morishita et al. (2003) suggested that PMR in197088-5, a selection from PI 197088, was controlled by two genes, one recessive and the other incompletely dominant. Expression of PMR in 197088-5 depends on interactions of the two genes and the temperature (Morishita et al. 2003).

Among the types of segregated populations, the single-segment substitution line (SSSL) is a powerful tool for functional genomics, and molecular breeding and genetic dissection of QTLs, which has been used in many crops (Shen et al. 2014; Liu et al. 2008). With high level of uniformity of the genetic background except for a single substituted segment, all the phenotypic variations among SSSLs could be associated with the substituted segments in those lines. In our previous study (Lin et al. 2012), we developed a serious of SSSLs with PM resistance introgressed from Jin5-508 in the genetic background of PM susceptible D8 (PM susceptible, dwarf type plant architecture) which was facilitated by marker-assisted selection with simple sequence repeat (SSR) and sequence characterized amplified regions (SCAR) markers. At the BC_{11} generation, one such SSSL, namely SSSL0.7 exhibited high resistance to the powdery mildew pathogen. We developed a large segregating population with SSSL0.7. The objectives of the present study were to identify and fine map the major-effect QTL conferring resistance to PM in SSSL0.7. We developed a secondary F_2 segregating population from a cross between SSSL0.7 and the recurrent parent, D8. The analysis of recombination events in the PM region with SNP markers delimitated the PM locus to a 41.1-kb region. Candidate genes were predicted and analyzed by qRT-PCR. The markers linked to the substituted segment detected in our study might be useful for selection of PM resistance in different cucumber accessions.

Materials and methods

Plant materials

The PM-resistant single-segment substitution line SSSL0.7 was derived from marker-assisted backcrossing (Lin et al. 2012) with a North China type, indeterminate cucumber inbred line Jin5-508 as the donor of PM resistance and a American type, dwarf cucumber inbred line D8 as the recipient (recurrent parent, PM susceptible). Briefly, from 2004 to 2009, the F_1 of D8 × Jin5-508 was backcrossed with D8 for 11 generations (two generations per year). Starting from BC_2, at each generation, a SCAR marker linked with PMR in Jin5-508 was used to select a plant for backcrossing with D8 (Alfandi et al. 2009). At BC_{11}, 17 plants were self-pollinated to generate 17 families with a total of 449 plants. These chromosome segment introgression lines (CSILs) were near isogenic at the PMR loci as compared with the recurrent parent, D8. Microsatellite marker analysis of these CSIL identified a SSSL, SSSL0.7, that carried a chromosome fragment from Jin5-508 and was highly resistant to powdery mildew (Lin et al. 2012). SSSL0.7 was delimited by two SSR markers SSR16472 and SSR16881 in cucumber chromosome 1,

which were 0.7 cM apart on the genetic map developed by Ren et al. (2009). In this study, to clone the PM resistance locus carried by SSSL0.7, an F_2 population consisting of 3600 plants was developed by crossing SSSL0.7 with D8.

Powdery mildew screening

Powdery mildew resistance of parental lines Jin5-508, D8 and SSSL0.7 was repeatedly evaluated in three seasons (fall 2011 and 2012, spring 2013) in the greenhouses of Yangzhou University (Yangzhou, China). In 2014 spring greenhouse season, Jin5-508, D8, SSSL0.7 and (SSSL0.7 × D8) F_1 (50 plants each), and 3600 F_2 plants were screened for PMR. PM conidia were collected from naturally infected cucumber plants in the greenhouse. A spore suspension at 10^6 spores per ml was made by soaking heavily infected leaves in tap water (containing 0.01 % Tween-20 as surfactant). Inoculation was performed at the 3rd true-leaf stage by spraying the spore suspension evenly on the surface of the seedlings according to Morishita et al. (2003). After inoculation, the plants were maintained in a controlled growth chamber at 25 °C day/20 °C night with a 16-/8-h (light/dark) photoperiod. Fifteen days after inoculation, the percentage of infected area of each leaf of each sample plant was determined with the following visual rating scale: 0 = no symptom; 1 = infection area <30 %; 2 = 30–60 %; 3 = >60–80 %; 4 = >80 %; and 5 = leaf senesced. Disease index (DI) was calculated using the following equation: DI = ∑(Disease scale × number of leaves of that specific scale)/(number of leaves inoculated × the highest disease grade) × 100.

DNA extraction and SSR identification

Genomic DNA was extracted from young leaf tissues using the CTAB method. DNA concentration was estimated on a 0.8 % agarose gel. Each 25 µl PCR reaction contained 19.0 µl water, 2.5 µl 10 × buffer, 2 µl dNTPs (10 mM), 0.5 µl *Taq* DNA polymerase (10 U/µl), 0.25 µl each of left and right primers (50 ng/µl), and 0.5 µl DNA (10 ng/µl). The PCR amplifications were performed using a GeneAmp PCR system 9700 (Applied Biosystems Inc., Foster City, CA) with the following program: 94 °C for 4 min; 35 cycles of 94 °C for 15 s, 55 °C for 15 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min. Subsequently, 2 µl of the PCR product mixed with 1 µl of 6 × loading buffer was subjected to electrophoresis in a 6 % polyacrylamide gel following Sambrook and Russell (2001).

Genome resequencing and SNP marker development

The genomes of the two parental lines, Jin5-508 and D8 were resequenced with Illumina HiSeq 2500. In brief, 5 µg genomic DNAs were used for preparation of paired-end sequencing libraries with insert sizes of ~200 to 500 bp following manufacturer's instructions. A total of 54.9 and 61.7 million clean reads were obtained for Jin5-508, and D8 with an average sequencing depth 30× and 35×, respectively. Single nucleotide polymorphisms (SNPs) between the two lines were detected with the GATK software package (McKenna et al. 2010). Local realignment and base recalibration were performed to improve the accuracy of SNP genotyping. The 9930 cucumber draft genome [V2.0, http://www.icugi.org/cgi-bin/ICuGI/index.cgi)] was used as the reference. SNPs were filtered using the criteria SNP quality value >30 and base quality >30. All SNPs in the region delimited by SSR16472 and SSR16881 in chromosome 1 were considered for design of new markers. SNP-based dCAPS markers were designed with the dCAPS Finder 2.0 program (http://helix.wustl.edu/dcaps/dcaps.html), and Primer Premier 5.0 (http://www.premierbiosoft.com/). For genotyping with the dCAPS markers, following PCR reactions as described above, the appropriate restriction enzyme was added to the PCR reaction and incubated for 2 h at the temperature according to the manufacturer's instructions (New England Biolabs, Ipswich, MA, USA). Two microliters of digested PCR products were then mixed with 1 µl of 10 × loading buffer, and subjected to electrophoresis in a 6 % polyacrylamide gel following Sambrook and Russell (2001).

Linkage analysis

The phenotypic data of PM inoculation responses among the 3600 F_2 plants were subjected to quantitative and qualitative linkage analyses with molecular markers. From 3600 F_2 plants, 121 individuals were randomly selected for linkage analysis (PMR as a single gene) or QTL mapping (using DI data). All marker alleles derived from D8 were assigned as 'a' allele; those from SSSL0.7 as 'b' allele; heterozygous as 'h'. In linkage mapping, χ^2 test for goodness-of-fit was performed against the expected 1:2:1 segregation ratio for each marker. Linkage analysis was performed with the Kosambi mapping function using JoinMap 3.0 (Van Ooijen and Voorrips 2001) with the threshold LOD score of 2.5. QTL analysis was performed with WinQTL Cartographer (V2.5) using the CIM (composite interval mapping) model (Wang et al. 2012).

Gene prediction and sequence annotation

The genomic DNA region harboring the PMR locus was annotated with FGENESH (http://sunl.softberry.com/) and InterProScan (http://www.ebi.ac.uk/InterProScan). Genes in this region were predicted with BLASTx at the NCBI (National Center for Biotechnology Information) website (http://blast.ncbi.nlm.nih.gov).

Table 1 Primers used in the study for genetic mapping and gene expression analysis

Markers	Objectives	Size (bp)	Enzyme	9930 scaffold	Left primer (5′–3′)	Right primer (5′–3′)
SSR16472	Mapping	171		scaffold000059	CACCCACGTGCTGTAAAAAG	ACCAGTTAACACGTCAATA TTTTCT
SNP02	Mapping	224	*Hha I*	scaffold000059	CAATCACGCTCTTTCCCTTGCGCTG	GGAGGAGGTGGTGGAGGA
SNP09	Mapping	195	*Dde I*	scaffold000059	GACCATACAAATAACAGAATTCTAA	TTGCTTTTGTTTTCTTGTAC
SNP20	Mapping	215	*Mse I*	scaffold000059	GTATGTTCCATTAATTTCAAAGTTA	ACTTTTCCTTGATTTCCA
SSR16881	Mapping	127		scaffold000059	CCCTCTCAACATTTTCCACAA	CGAGGAGACTTGATGGGATG
Csa1M064720.1	qRT-PCR	94		scaffold000059	GGGTGATGGATACGATTG	ACCACTGTAGCACGAAGG
Csa1M064730.1	qRT-PCR	185		scaffold000059	CCTCCGCCACTTCTATTA	GCTTCTCCCCAAGTTCTC
Csa1M064740.1	qRT-PCR	150		scaffold000059	GGAAGGTATGGCGACGAT	GCTAACAGGGCAAACACG
Csa1M064750.1	qRT-PCR	195		scaffold000059	AGAATCCACCACAAATCC	TCTTGTTGAGACCCCACT
Csa1M064760.1	qRT-PCR	184		scaffold000059	CCCGAAACTCCAATCTCA	ATCCATCGTTGCCTATCA
Csa1M064770.1	qRT-PCR	170		scaffold000059	TCTCGCAACCACAAACTC	GCCCCAAATAAAATCCTG
Csa1M064780.1	qRT-PCR	155		scaffold000059	TGTGCTAATAAAGTTGGTGGGA	ATTGCTGAGGAATGAAGTGGTG
Csa1M064790.1	qRT-PCR	167		scaffold000059	AAACACGGAGTGCCAAGA	CGGATTCAGAAGGCGGTA
Actin	qRT-PCR	136		scaffold000035	TCGTGCTGGATTCTGGTG	GGCAGTGGTGGTGAACAT

Quantitative reverse-transcription PCR (qRT-PCR) analysis of candidate gene

Total RNA was isolated from leave samples of Jin5-508, D8 and SSSL0.7 at 0, 12, 24, and 48 h after inoculation of powdery mildew pathogen using RNAiso Plus (Takara, China). The concentration of RNA samples was adjusted to 1000 μg/mL using Biophotometer Plus (Expander, Germany). RNA was reverse-transcribed using a Takara PrimeScript® RT reagent kit with gDNA eraser according to the manufacturer specifications. RT-PCR was performed using a RealMasterMix (SYBR Green) kit (TIANGEN, China) on an iQ™ 5 Multicolor real-time PCR detection system (Bio-RAD, USA) in 20 μL reactions. The PCR primers were designed with Primer Premier 5.0 (Premier Biosoft International, Palo Alto, CA, USA). The sequence information for all primers used in the present study is presented in Table 1. Three replications of each treatment and control were used for real-time RT-PCR. The relative mRNA expression was based on the $2^{-\triangle\triangle Ct}$ method. Each expression profile was independently verified in three technical replications under identical conditions.

Subcellular localization of Csa1M064780.1 and Csa1M064790.1 proteins

The coding region of two possible candidate genes, *Csa1M064780.1* and *Csa1M064790.1*, were amplified by PCR and subcloned into the PGEM-T Easy vector (Promega, USA). The DNA fragments were digested with *Xba* I and *Kpn* I, and then inserted into the pUC-GFP vector, which was verified by restriction and sequencing analysis. The verified recombinant plasmid was transformed into living onion epidermal cells by particle bombardment following Kinkema et al. (2000). The transformants were incubated on 1/2 MS medium for 24 h at 26 °C in dark. The fluorescence of GFP was visualized using a Leica TCS SP2/MP confocal laser scanning microscope (Leica Microsystems, Mannheim, Germany).

Results

Performance of powdery mildew resistance in SSSL0.7

Fifteen days after inoculation, no symptoms could be observed on the resistant parent Jin5-508 (DI = 0) in any of the four environments tested. The DI for D8 was 35.9, 36.7, 30.6 and 37.7 in 2011, 2012, 2013 and 2014, respectively. SSSL0.7 also showed consistent and high resistance to PM inoculation with DI of 6.3, 5.2, 3.7 and 3.3, respectively, in the 4 years. The level of resistance in SSSL0.7 was slightly

Fig. 1 Morphology and powdery mildew (PM) natural infestation responses of D8 (PM susceptible recurrent parent, *left*), Jin5-508 (PM resistance donor, *right*), and the derived single-segment substitution line SSSL0.7 (PM resistant, *middle*)

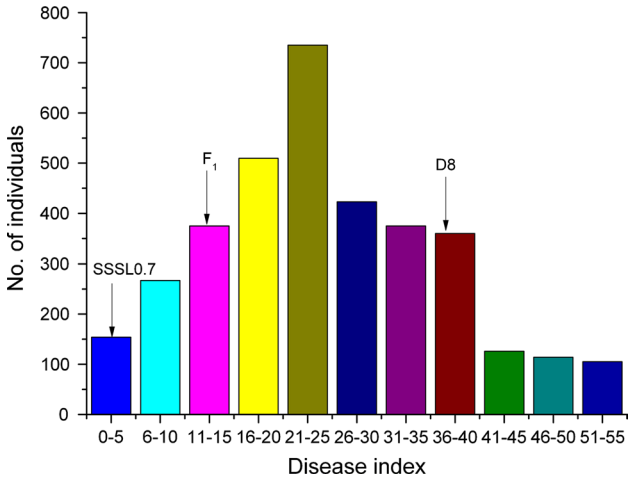

Fig. 2 Frequency distribution of disease index of powdery mildew inoculation responses among 3600 F_2 plants derived from the cross between D8 (PM susceptible recurrent parent) and SSSL0.7 (PM-resistant)

lower than its original donor Jin5-508, which was reasonable because we found some other introgression lines among the backcross derivatives that also exhibited low level PM resistance. These results suggested that a major-effect QTL from Jin5-508 was successfully introgressed to the D8 genetic background (Fig. 1).

In 2014 greenhouse screening tests, 3600 F_2 plants derived from D8 × SSSL0.7 were tested for responses to PM inoculation. Frequency distribution of the 3600 plants based on DI is shown in Fig. 2, which showed a largely normal distribution. The average DI for SSSL0.7, D8 and their F_1 was 3.3, 37.7 and 12.3, respectively. These results indicated that SSSL0.7 carried a dominant major-effect QTL for powdery mildew resistance, which, to our knowledge, was the first reported dominant gene to powdery mildew resistance in cucumber. We designated this locus as *Pm1.1*. The categorical data (R, S) of 121 selected individuals among the 3600 plants were used in linkage analysis to map *Pm1.1* (below).

Molecular delimitation of substituted segment length in SSSL0.7

The introgressed fragment carrying *Pm1.1* in SSSL0.7 was delimited by two SSR markers SSR16472 and SSR16881, but the exact boundaries of breakpoints for this introgression were not known due to the limited number of available SSR markers. To gain a more precise estimation of the length of the introgressed fragment, 10 SNP-derived dCAPS markers were designed to further characterize the length of introgression in SSSL0.7. Among the 10 markers, five (SNP01 W to SNP05 W) were located in the region outside SSR16472, and five (SNP06 W to SNP10 W) were outside SSR16881. All 10 markers were polymorphic between D8 and Jin5-508, but monomorphic between SSSL0.7 and D8 indicating that the substituted segment in SSSL0.7 was correctly defined by SSR16472 and SSR16881. The physical distance of this Jin5-508-derived region was about 94.7 kb on scaffold000059 of 9930 (Huang et al. 2009).

Fine mapping of *Pm1.1* locus

Twenty SNP-derived dCAPS markers were designed within the 94.7 kb region to narrow down the substituted segment

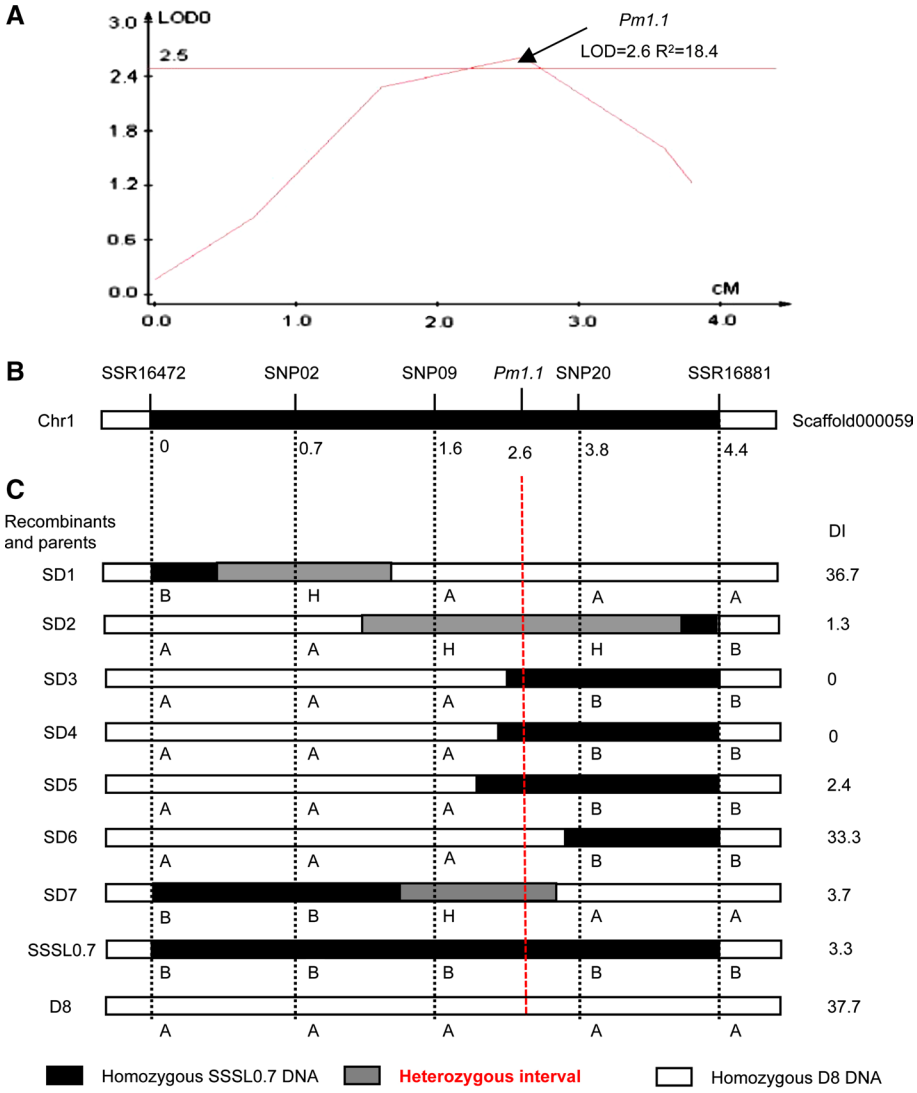

Fig. 3 Genetic and physical maps of powdery mildew resistance major-effect QTL *Pm1.1*. In **a** LOD curve in chromosome 1 from the QTL analysis based on 121 F_2 plants. **b** A linkage map for *Pm1.1* on chromosome 1 based on categorical data of disease inoculation responses among the 121 F_2 plants. *Numbers* below the chromosome are map locations in cM. **c** Analysis of the overlap mapping of the *Pm1.1* locus with 7 recombinants indicated the *Pm1.1* locus was residing in the 41.1-kb region flanked by SNP09 and SNP20. The *right* is the disease index (DI) of each recombinant and two parents. *Filled, gray* and *open bars* represent homozygous fragments from SSSL0.7, possible heterozygous interval and homozygous fragments from D8. Genotypes of five markers of each recombinant plant are also shown under each chromosome map

carrying the *Pm1.1* locus. Three of the markers, SNP02, SNP09, and SNP20, were polymorphic between Jin5-508 and D8. Primer sequence information of the three dCAPS markers together with the two flanking SSR markers SSR16472 and SSR16881 is presented in Table 1. QTL analysis was performed with the five markers and DI data from 121 randomly selected F_2 plants with the CIM model in WinQTL Cartographer 2.5. The result is shown in Fig. 3a. A QTL peak, *Pm1.1*, was present between two dCAPS markers SNP09 and SNP20 with a LOD score of 2.6. This QTL accounted for 18.4 % of the total phenotypic variations (Fig. 3a, b). Consistent with this, when categorical data were used in linkage analysis with the 121 plants, the *Pm1.1* locus was placed in between SNP09 and SNP20 suggesting *Pm1.1* was indeed located in the interval between SNP09 and SNP20.

To further verify the position of *Pm1.1*, all 3600 secondary F_2 plants were genotyped with SSR16472 and SSR1688, from which seven recombinants (SD1 to SD7) were identified. Then, the seven plants were genotyped with three dCAPS markers. Genotypic data of five markers in the seven recombinant plants, and the four intervals defined by these markers are illustrated in Fig. 3c. Two of the seven recombinants, SD01 and SD06 were highly susceptible to PM, and the rest were highly resistant. Comparison of the marker-defined intervals and the PMR of the seven plants led to the conclusion that *Pm1.1* must reside in the 41.1 kb region flanked by SNP09 and SNP20.

Annotation and genes prediction in the 41.1 kb region

Annotation of the 41.1 kb genomic region delimited by SNP09 and SNP20 predicted 8 genes in this region. The predicted functions and associated information of the eight genes are presented in Table 2. Two of the genes (*Csa1M064720.1* and *Csa1M064750.1*) encoded proteins with unknown functions. *Csa1M064730.1* was predicted

Table 2 Predicted genes in 43.2 kb region harboring the *Pm1.1* major-effect PM resistance QTL. 9930 V2.0 is the 9930 draft genome assembly version 2.0

No.	Genes	Predicated functions	*E* value	9930 V2.0 start	9930 V2.0 end
1	*Csa1M064720.1*	DNAJ heat shock N-terminal domain-containing protein	1.00E−122	6,818,031	6,818,303
2	*Csa1M064730.1*	Gibberellin 2-oxidase (*Nicotiana sylvestris*)	2.00E−115	6,826,512	6,828,846
3	*Csa1M064740.1*	Pyruvate orthophosphate dikinase regulatory protein (*Glycine max*)	6.00E−141	6,829,924	6,831,950
4	*Csa1M064750.1*	Predicted protein (*Populus trichocarpa*)	5.00E−36	6,832,326	6,835,124
5	*Csa1M064760.1*	Late embryogenesis abundant hydroxyproline-rich glycoprotein (*Arabidopsis thaliana*)	9.00E−69	6,842,192	6,843,022
6	*Csa1M064770.1*	Harpin inducing protein 1-like (*Nicotiana tabacum*)	3.00E−06	6,844,829	6,845,425
7	*Csa1M064780.1*	Cysteine-rich receptor-like protein kinase	4.00E−62	6,847,780	6,850,456
8	*Csa1M064790.1*	Cysteine-rich receptor-like protein kinase	1.00E−45	6,854,657	6,856,632

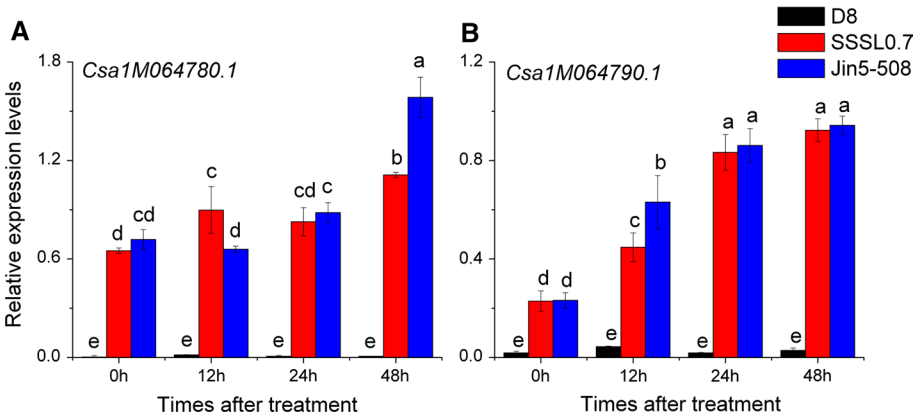

Fig. 4 Expression level of *Pm1.1* candidate genes *Csa1M064780.1* and *Csa1M064790.1* in D8 (susceptible, *black*), Jin5-508 (resistant, *blue*) and SSS0.7 (resistant, *red*) parental lines. Data are the means of three replicates (±SD). The cucumber *β-actin* gene was used as an internal control. Each value denotes the mean relative level of expression of three replicates. Means with the same *lowercase letter* do not significantly differ by the least significant difference (LSD) test at $p \leq 0.05$ with a completely randomized design (color figure online)

to encode a protein with gibberellin 2-oxidase activity that acts specifically on C-20 gibberellins. *Csa1M064740.1* was predicted to encode a pyruvate orthophosphate dikinase (PPDK) regulatory protein that has both kinase and phosphatase activities towards PPDK. *Csa1M064760.1* and *Csa1M064770.1* were predicted to encode proteins belong to late embryogenesis abundant (LEA) family. *Csa1M064780.1* and *Csa1M064790.1* were both predicted to encode a cysteine-rich receptor-like protein kinase (Table 1).

Expression analysis of *Pm1.1* candidate genes in response to PM inoculation

Expression dynamics of all eight genes in D8, SSSL0.7 and Jin5-508 at 0, 12, 24 and 48 h after inoculation of PM pathogen were investigated to identify possible candidate gene(s) for *Pm1.1* in this 41.1 kb interval. The results are presented in Fig. 4 and supplemental Fig. S1. Two of the genes, *Csa1M064780.1* and *Csa1M064790.1*, exhibited consistent patterns in the two PMR lines (Jin5-508 and SSL0.7) and the susceptible D8. That is, the relative expressions of both genes were low and stable in D8 before and after inoculation; both were up-regulated in SSSL0.7 and Jin5-508 12 h after inoculation. The expression level *Csa1M064790.1* kept increasing after inoculation at all three time points, whereas that of *Csa1M064780.1* did not show any significant increase 12 h after inoculation (Fig. 4). On the other hand, no consistent trends in expression levels were found among the three cucumber lines for the remaining six genes (Fig. S1) suggesting that both *Csa1M064780.1* and *Csa1M064790.1* were good candidates for the *Pm1.1* locus in cucumber.

Subcellular localization of *Csa1M064780.1* and *Csa1M064790.1* proteins

The cellular location of *Csa1M064780.1* and *Csa1M064790.1* proteins was investigated in onion

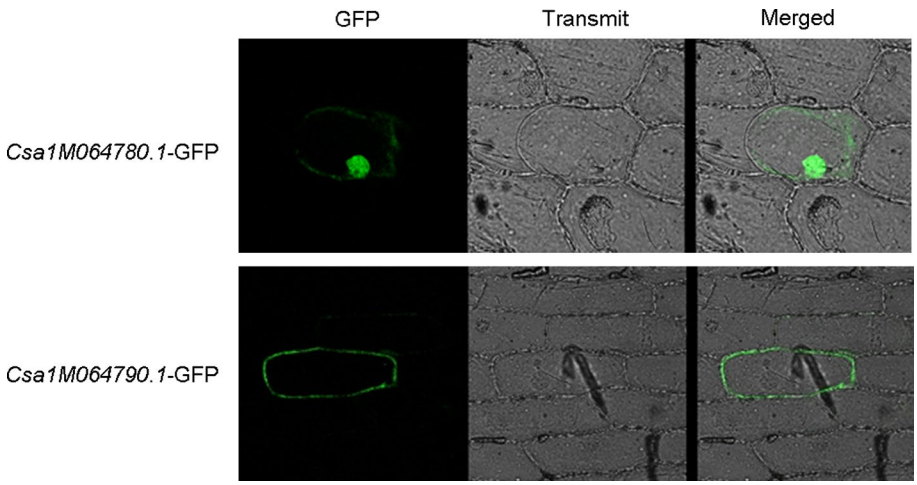

Fig. 5 Membrane localization of the*Csa1M064780.1* and *Csa1M064790.1*-GFP fusion protein. Full coding sequences of *Csa1M064780.1* and *Csa1M064790.1* were fused with green fluorescent protein (GFP) gene. The recombinant plasmids of *Csa1M064780.1*-GFP and *Csa1M064790.1*-GFP were then transformed into onion epidermal cells by particle bombardment, and viewed by confocal microscopy

epidermal cells transiently expressing gene fusions to the green fluorescent protein (GFP) by particle bombardment to provide further evidence for the potential roles of *Csa1M064780.1* and *Csa1M064790.1* in transcriptional regulation. Csa1M064780.1-GFP fluorescence was observed in plasma membrane and nucleus, but Csa1M064790.1-GFP fluorescence was observed exclusively in the plasma membrane (Fig. 5).

Discussion

We used a single-segment substitution line (SSSL0.7) for fine genetic mapping of a major-effect QTL, *Pm1.1,* for powdery mildew resistance in cucumber. Several types of mapping populations have been used in previous QTL mapping studies of PM resistance in cucumber (Sakata et al. 2006; Liu et al. 2008; Zhang et al. 2011; He et al. 2013; Fukino et al. 2013; Nie et al. 2015) including F_2, $F_{2:3}$ or RIL populations. A limitation of these populations is the genetic background noises that sometimes may mask the true QTL effects, especially for minor-effect QTLs (Wei et al. 2015). SSSL0.7 possessed a small fragment (~94.7 kb) from the donor of PM resistance (Jin5-508) that had a simple genetic background, which allowed more precise mapping of the target QTL (Qi et al. 2013). In screening tests over 4 years, the powdery mildew resistance of SSSL0.7 was highly consistent and stable, indicating the 94.7-kb region truly carried a PMR locus. On the other hand, the DI of SSSL0.7 was slightly higher than the donor line Jin5-508 (less resistant) indicating SSSL0.7 carried a major-effect QTL (*Pm1.1*) and additional QTL may exist in Jin5-508 for complete resistance to powdery mildew. This was consistent with our observations that other SSSL carrying different Jin5-508 chromosome fragments were also resistance to PM although the performance of SSSL0.7 was the best among all SSSLs we developed from the Jin5-508 × D8 cross (Lin et al. 2012). Characterization of other powdery mildew-resistant SSSL will also allow us to have a complete picture of the genetic structure of powdery mildew resistance in Jin5-508.

To fine map the *Pm1.1* locus in SSSL0.7, we developed a secondary, large F_2 mapping population from the cross between the near isogenic SSSL0.7 and susceptible parent, D8, that segregated only at the *Pm1.1* locus. The high level of resistance exhibited by the F_1 indicated the dominant nature of PMR in SSSL0.7 (Fig. 1). Despite of the fact that only one gene (*Pm1.1*) was segregating in the F_2 population, the frequency distribution of plants based on DI was still largely normal (Fig. 2) suggesting effects of environmental factors in expression of the resistance. Nevertheless, both QTL mapping and linkage analysis with 121 F_2 plants placed *Pm1.1* into the 41.1 kb interval defined by the two markers SNP09 and SNP20 (Fig. 3). The LOD support score (2.6) and the phenotypic variations explained by this QTL ($R^2 = 18.4$ %) (Fig. 3) were unexpectedly low. A similar phenomenon was found during map-based cloning of the PMR QTL *pm5.1* (Nie et al. 2015). The reasons for this were probably due to the relatively small population and few markers employed in linkage analysis.

Through fine genetic mapping, we delimited the *Pm1.1* locus into a 41.1 kb region in which eight genes were predicted (Table 2). Through comparative expression analysis, two tandem arrayed CRK genes, *Csa1M064780.1* and *Csa1M064790.1* were shown to be the candidate genes for *Pm1.1* (Fig. 4, supplemental Fig. S1). While their expression remained low in the susceptible D8, both genes were up-regulated upon PM pathogen inoculation in the resistance SSSL0.7 and Jin5-508 (Fig. 4). Both of *Csa1M064780.1* and *Csa1M064790.1* proteins were expressed in the plasma membrane, but Csa1M064780.1-GFP fluorescence was also observed in the nucleus (Fig. 5) suggesting possible different roles of *Csa1M064780.1* and *Csa1M064790.1* in PM inoculation responses. At

this time, it is not known if the PM resistance conferred by *Pm1.1* was the result of functions of *Csa1M064780.1* or *Csa1M064790.1*, or both. Functional analysis of *Csa1M064780.1* and *Csa1M064790.1* by complementation tests and other strategies is underway.

In plants, the receptor-like kinases (RLK) play fundamental roles in perceiving external stimuli, activating downstream signaling pathways, and regulating cellular behavior in response to pathogen infection. RLK contain an extracellular signal-sensing domain connected by a single transmembrane domain to an intracellular protein kinase domain (Shiu and Bleecker 2003; Osakabe et al. 2013). In the cucumber Gy14 draft genome, Wang et al. (2014) identified 192 homologs of the leucine-rich repeat (LRR) type RLK. One of the largest RLK subfamilies is the CRK which are transmembrane proteins characterized by the presence of two domains of unknown function 26 (DUF26) in their ectodomain. In the *Arabidopsis thaliana* genome, among the ~600 RLK, there are 44 CRK (Wrzaczek et al. 2010). The biological functions of CRK are largely uncharacterized. Recently, several studies have showed that CRK control important aspects of plant development and stress adaptation in response to biotic and abiotic stimuli in a non-redundant fashion, especially in reactive oxygen species/redox signaling and sensing (e.g., Czernic et al. 1999; Niina et al. 2014; Bourdais et al. 2015). In barley, a cysteine-rich receptor-like protein kinase gene *HvCRK1* was observed to accumulate transiently following powdery mildew pathogen inoculation in barley (Rayapiram et al. 2012). In the present study, we provided evidence that one of the two cysteine-rich receptor-like protein kinase genes or both are the best possible candidate gene(s) for the dominantly inherited major-effect QTL for PMR in cucumber. This work added a new horizon for CRK in host resistance against fungal pathogens. Our work also provided the starting point in elucidating the regulatory mechanisms and enzyme kinetics underlying the CRK gene in the PMR pathway in cucumber.

In this study, for the first time, we identified a dominantly inherited major-effect QTL, *Pm1.1*, for PM resistance in cucumber. Previous QTL mapping studies identified recessively inherited loci for PM resistance (e.g., Kooistra 1968; Fujieda and Akiya 1962; Shanmugasundaram et al. 1971; He et al. 2013) with major-effect QTLs located, in most studies, in chromosome 5. Nie et al. (2015) cloned a candidate gene, *pm5.1*, for the major QTL in cucumber chromosome 5. The resistance conferred by *pm5.1* was due to an insertion of retrotransposon into *MLO-like* gene (Nie et al. 2015). The relationship of *Pm1.1* and *pm5.1* in contributing to PMR in cucumber merits further investigation. Several PMR QTL were identified in the cucumber line WI2757 (He et al. 2013). While the major-effect QTL in WI2757 was consistent with the location of *pm5.1* cloned by Nie et al. (2015), He et al. (2013) also identified QTL in chromosome 1 which seems to be co-localized with *Pm1.1* identified in the present study (based on a common marker SSR16472). It will be interesting to see whether or not the powdery mildew-resistant line Jin5-508 possesses *pm5.1* (retrotransposon insertion). If so, it may facilitate the understanding of interactions of different QTL in contributing to the host resistance to the powdery mildew pathogen in cucumber.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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