A Sequence-amplified Characterized Region Marker for a Single, Dominant Gene in Melon PI 134198 that Confers Resistance to a Unique Race of *Podosphaera xanthii* in China

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Abstract. Melon production in China is threatened by cucurbit powdery mildew incited by *Podosphaera xanthii*, which can cause severe yield losses. Disease reactions of 10 melon *P. xanthii* race differentials indicated the presence of a unique race of *P. xanthii*, denoted pxCh1, in the Shanghai area. Resistance to *P. xanthii* race pxCh1 in melon PI 134198, which originated from India, was controlled by a single, dominant gene, designated *Pm-8*, in a cross with susceptible melon inbred S717. A search for a molecular marker linked to *Pm-8* was initiated using the same cross. Bulk segregant analysis of their F₂ progeny revealed one sequence-related amplified polymorphism (SRAP) marker of 148 bp linked to *Pm-8*, at 3.9 cM. The SRAP segment was recovered, cloned, and sequenced and converted into a sequence-amplified characterized region marker for marker-assisted selection of *Pm-8* in breeding programs to develop melon cultivars resistant to *P. xanthii* race pxCh1.

Cucurbit powdery mildew (CPM) is a common and serious disease of melon worldwide. CPM infection acts as a sink for photosynthates from the leaves, which leads to premature loss of foliage and, subsequently, to loss of fruit quality and yield (Sitterly, 1978). CPM is caused by two fungal species, *Podosphaera xanthii* (formerly known as *Sphaerotheca fuliginea* Schlech ex Fr. Poll.) and *Golovinomyces cichoracearum* (formerly known as *Erysiphe cichoracearum* DC ex Merat.). *P. xanthii* has been identified as the primary cause of CPM in China (Feng, 1996). Fungicides and resistant varieties are the major means of CPM control. Fungicides are, however, costly and can be rendered ineffective through emergence of insensitive races of the pathogen (McCreight, 2001). Resistant cultivars provide a more economical and environmentally safe control strategy but may select for resistance-breaking races (McCreight, 2006). Few melon cultivars in China are highly resistant to CPM, and development of CPM-

resistant cultivars is a high priority of many melon-breeding programs in China. There are many sources of genetic resistance to CPM on melon and many races of CPM have been reported on melon (McCreight, 2006; Pitrat, 2006).

Disease resistance breeding has traditionally been done by phenotypic selection. Efficiency of phenotypic selection is reduced by variability in the pathogen, infection, and disease development. Molecular markers tightly linked to CPM resistance genes can eliminate these sources of phenotypic variation (Tanksley et al., 1989) to enable more efficient breeding strategies in melon improvement (Fan et al., 2006). Development of molecular markers linked to the melon CPM-resistant genes is an essential step toward marker-assisted selection (MAS) and map-based gene cloning of CPM-resistant genes in melon.

Sequence-related amplified polymorphism (SRAP) is a simple marker detection method (Li and Quiros, 2001) that has been successfully applied in several species for different purposes (Ferriol et al., 2003; Mukhlesur et al., 2007; Mutlu et al., 2008; Sun et al., 2007). The SRAP technique provides preferential amplification of open reading frames (ORF) or ORF-related sequences. Selected SRAP polymerase chain reaction (PCR) products may be separated on polyacrylamide gels for further sequencing and analysis (Li and Quiros, 2001). SRAP markers are, however, expensive and technically demanding, which

makes them less useful for large-scale MAS. Simple and practical markers amenable to high throughput are preferred. Two such markers are sequence-amplified characterized region (SCAR) and cleavage amplification polymorphisms, both of which are highly repeatable and reliable because they are amplified by PCR under highly stringent conditions (Piao et al., 2004; Varshney et al., 2004).

We report inheritance of CPM resistance in Indian melon PI 134198 to a unique race of *P. xanthii* and a new SRAP marker linked to the dominant CPM resistance gene in this accession. The SRAP marker was converted into a SCAR marker, which will help breeders to develop new CPM-resistant melon by MAS.

Materials and Methods

Plant materials. PI 134198 originated from India. It is andromonoecious, resistant to CPM, and has thin, long ellipsoidal fruit that has reddish-colored flesh. S717 is an elite inbred typical of dessert melons grown in Eastern China: andromonoecious, susceptible to CPM, and round fruit with colored flesh. PI 134198 and S717, and their offspring, including F₁ (50 plants), F₂ (108 plants), and reciprocal backcross progenies formed by backcrossing with the resistant parent (BC_{PI 134198}; 61 plants) or by backcrossing with the susceptible parent (BC_{S717}; 77 plants), were used in this study.

Cucurbit powdery mildew source, inoculation, and evaluation. CPM was collected at the QI Bao Campus, Shanghai Jiaotong University, China, and determined to be *P. xanthii* based on the presence of fibrosin bodies by microscopic observation. The CPM inoculum used in this test was obtained from a monospore culture and maintained on young plants of melon cv. Milong on isolated greenhouse benches. The CPM isolate was characterized before the genetic study based on the reactions of 10 commonly used race differentials (Table 1; McCreight, 2006). The differential genotypes were arranged in a randomized complete block design with three reps, 15 plants of each differential per rep.

CPM race characterization and genetic studies were done under the same experimental conditions in a growth chamber set at a 12-h photoperiod (photosynthetic photon flux = 250 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Seeds were sown in plastic pots (8 × 8 × 10 cm deep; one seed per pot). The soil media was an equal (volume basis) mixture of peat, cinders, and perlite. Several heavily infected leaves were soaked in a solution of Tween 20 at 0.02% (4 drops/L) in deionized water to obtain spores for inoculation. The suspension was filtered through two layers of cheesecloth, and the spore concentration was adjusted to 5 × 10⁴ spores/mL (Block and Reitsma, 2005). Seedlings were inoculated by spraying to runoff when the second true leaves were fully expanded. Humidity was maintained at 75 ± 10% relative humidity using a humidifier for 24 h.

Disease index (DI) of the first true leaf of every plant was evaluated 12 days post-inoculation in the genetic study. Percentage

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of the leaf area covered with CPM was estimated visually using a 0 (no obvious infection) to 10 (100% of the leaf covered with CPM) scale (Liu et al., 2008). DI of CPM race differentials were done on an individual plant basis and then averaged within reps. Disease phenotype was considered resistant when DI was 1.0 or less and susceptible when DI was greater than 1.0.

DNA extraction and bulk segregant analysis. Melon genomic DNA was extracted from young leaves using a CTAB method (Doyle and Doyle, 1990). Bulk segregant analysis (BSA) was used to identify SRAP markers linked to the powdery mildew resistance gene (Michelmore et al., 1991). For BSA, six DNA bulks were generated from the F₂ population and composed of five plants each; three bulks were comprised of resistant plants on which there was no sporulation on the first three true leaves; the other three bulks included susceptible plants that had an average of covered with mildew greater than 70% on the first three true leaves.

Sequence-related amplified polymorphism analysis. The PCR reaction was set up in a final volume of 20 µL containing 60 ng of DNA, 5.0 pmol of primer, 200 µM dNTPs, 1.5 mM MgCl₂, and 0.5U of *Taq* polymerase (Promega, Madison, WI) in 1× *Taq* buffer. The PCR program included an initial denaturing at 94 °C for 3 min followed by eight cycles of 94 °C for 30 s, 37 °C for 30 s, and at 72 °C for 90 s and then 35 cycles of 94 °C for 30 s, 48 °C for 30 s, and 72 °C for 90 s with a final extension of 72 °C for 7 min (Yuan et al., 2008). The products were separated on 4% denaturing polyacrylamide gels

and visualized using silver staining (Xu et al., 2002). The primer combinations that generated polymorphic bands between the bulks were tested on the bulked individuals to eliminate false-positive markers.

Sequence-amplified characterized region primer design and polymerase chain reaction analysis of sequences. Bands containing the targeted SRAP fragment were excised from sodium dodecyl sulphate–polyacrylamide gels and placed in 0.5-mL Eppendorf tubes. After adding 30 µL of ddH₂O, the gel slices were crushed with a pipette tip and held at 95 °C for 10 min. The tubes were centrifuged at a speed of 10,000 rpm for 1 min and 1 µL of the supernatant was used as template for selective amplification with the same primer combination. The reamplified PCR product was analyzed in a 1.5% agarose gel. A UNIQ-10 EZ Spin Column DNAGel Extraction Kit (Sangon, Shanghai, China) and a pGEM-T Easy Vector (Promega) was used to purify and clone the fragments into the *Escherichia coli* DH5α. Recombinant plasmid DNA was isolated and sequenced by Sangon Corp. (Shanghai, China). A pair of oligonucleotide primers was designed based on the sequence of the cloned fragment using Premier 5.0 software (Premier Biosoft Intl., Palo Alto, CA) and synthesized by Sangon Corp. PCR amplification with the SCAR primers was performed in a 10-µL reaction containing 20 ng template DNA, 5.0 pmol each SCAR primer, 100 µM dNTPs, 2.5 mM MgCl₂, and 0.2U of *Taq* polymerase (Promega) in 1× *Taq* buffer, 7.7 µL of ddH₂O. Samples were overlaid with mineral oil and the PCR protocol was: 94 °C for 4 min, 30 cycles: at 94 °C for 30 s, at 55 °C for 30 s, and at 72 °C for 60 s, final extension 72 °C for 5 min. Amplified products were separated in 2.5% agarose gels. Electrophoresis was performed at 100 V and maximum amperage for 1 h. Gels were stained with ethidium bromide and photographed with a digital, gel documentation system.

Segregation data were subjected to χ^2 analysis to ascertain the goodness of fit between the expected and observed segregation ratios for a single dominant gene and for the phenotypic data, analysis between the SCAR markers, and the CPM resistance locus.

The SCAR marker was tested on 74 resistant and 34 susceptible F₂ plants. Linkage of PM resistance locus was performed with the software package MAPMAKER/EXP, Version 3.0 (Lander et al., 1987). The recombinant frequencies between the CPM-resistant locus and molecular markers were calculated through two-point tests and link-

age map was constructed by three-point or multiple-point tests with a minimum limit of detection threshold of 3.0. The recombination values were converted into centiMorgans (cM) using the Kosambi mapping function. For mapping the marker, the F₂ population was also analyzed with the microsatellite markers (CMBR8, CMBR120, TJ29, CMBR111, and CMBR150) previously described by Fukino et al. (2008) as linked to powdery mildew resistance were also evaluated. PCR amplification was carried out following the same protocol of the SCAR primer, and the genetic linkage map was drawn using MapChart 2.2 software (Voorrips, 2002).

Results

Cucurbit powdery mildew race evaluation. The isolate of CPM used in this research was unique based on the reaction pattern of the 10 differentials (Table 1) and was denoted as pxCh1. The key difference between this isolate and previously reported isolates was the resistant reaction of Iran H in contrast to the susceptible reactions of ‘Védrañtais’ and ‘Fuyu 3’ (Table 1). This reaction pattern was distinct from any patterns of the known races (McCreight, 2006).

Genetic analysis for powdery mildew resistance. The F₁ was resistant, suggesting that CPM resistance of PI 134198 was controlled by a single, dominant gene (Table 2). The F₂ populations segregated 74 resistant to 34 susceptible in a good fit to a 3:1 ratio (Table 2) expected for a single dominant resistance gene (Table 2). The BC_{PI 134198} plants were resistant with three exceptions, whereas the BC_{S717} segregated in a good fit to the expected 1:1 ratio (Table 2). This CPM resistance gene will be referred to henceforth as *Pm-8*.

Identification of a sequence-related amplified polymorphism marker linked to the *Pm-8* gene. The 700 primer combinations used to screen the parental DNA in SRAP analysis amplified 8146 discrete genomic fragments (average 10 to 12 products per primer pair). Polymorphisms were amplified with 55 primer pairs (7.8%) resulting in 246 polymorphic bands (3.0%) between the resistant and susceptible parental genotypes. All primer pairs that generated polymorphic bands were tested on the resistant and susceptible bulks. SRAP bands present in one pool and absent in the other were regarded as candidate markers linked to *Pm-8*. One primer combination (E-05/M-03) produced a 148-bp DNA fragment that was found only in the resistant parent, resistant bulks, and resistant F₂ plants.

Sequence analysis. The SRAP marker E-05/M-03 linked to *Pm-8* was cloned with the objective of converting it into a simple PCR-based marker. The polymorphic amplified DNA band corresponding to the SRAP marker was reamplified and a single band with the expected size was observed on an agarose gel. The band was then purified and sequenced and found to be 148 bp long and did not show significant homology to any other known gene.

Table 1. Disease ratings of melon cucurbit powdery mildew race differentials in a controlled inoculation test.

Differential	Disease	
	Mean rating ^a	Phenotype
Iran H	0.8 b	Resistant
PMR 5	1.2 c	Susceptible
PMR 45	0.0 a	Resistant
PMR 6	0.0 a	Resistant
WMR 29	0.7 b	Resistant
MR-1	0.0 a	Resistant
PI 124112	1.4 c	Susceptible
Védrañtais	3.2 d	Susceptible
Fuyu 3	4.2 e	Susceptible
Harukei 3	3.9 de	Susceptible

^aRated on a 0 (0% of the first true leaf area covered with mildew) to 10 (100% of the first true leaf area covered with mildew) scale. Mean of 45 plants for each differential. Means separation using Student's *t* test; those followed by different letters are significantly different, *P*_{0.05}.

Table 2. Disease reactions of PI 134198, S717, and their F₁ and segregating generations to *Podosphaera xanthii* race pxCh1.

Line or population	Resistant	Susceptible	Expected ratio	χ^2	<i>P</i>
PI 134198	30		All resistant		
S717		30	All resistant		
F ₁	46	4	All resistant		
F ₂	74	34	3:1	2.41	0.18
BC _{PI 134198}	58	3	All resistant		
BC _{S717}	36	41	1:1	0.22	0.68

Sequence-amplified characterized region analysis and linkage analysis. Based on the sequence of the cloned fragment, a pair of oligonucleotide primers was designed with Primer 5.0 software as follows: forward primer, 5'-CCCTATGCTCATTAACCTTCG-3'; reverse primer, 5'-GCTGCATAATGCC-TAGCTA-3'.

These SCAR primers amplified a single, 124-bp band named SCAR0305 in the CPM-resistant parent and F₂ plants (Fig. 1). This band was absent in S717, the susceptible parent. SCAR0305 was present in 73 of the 74 CPM-resistant plants and absent in all 34 susceptible F₂ plants.

Mapping of the Pm-8 linked markers. The analysis of the segregation data from the two markers (SCAR0305 and CMBR150) on the 108 F₂ individuals and the resistance trait allowed us to assign the position of these markers in the melon genomic region of Linkage Group VII (Fukino et al., 2008) surrounding the *Pm-8* locus. Four other markers (CMBR8, CMBR120, TJ29, and CMBR111) were not linked with *Pm-8*. The genetic linkage distances of *Pm-8* with SCAR0305 and CMBR150 were 3.90 and 28.60 cM, respectively.

Discussion

Powdery mildew has been a serious disease of cucurbit plants since ancient times (Ballantyne, 1975). Melon was first cultivated in China 2500 years ago (Wang et al., 2005), but there are no previous scientific reports of CPM races on melon from China, a contrast to the United States and France (McCreight, 2006; McCreight et al., 1987). A unique race of *P. xanthii*, pxCh1, was identified in China based on the reaction pattern of 10 commonly used melon race differentials (McCreight, 2006). High-level resistance to pxCh1 was observed on Iran H, which is generally susceptible to cucurbit CPM (McCreight, 2006; Pitrat, 1998). Kristková et al. (2004) reported incomplete resistance in Iran H against two strains of *P. xanthii* and complete resistance to one strain of *G. cichoracearum*. Based on our results and those of Kristková et al. (2004), we concluded that *P. xanthii* isolate pxCh1 is a new race and is the first reported race of *P. xanthii* on melon in China.

Seventeen dominant, CPM resistance genes were included in the latest melon gene list (Pitrat, 2006). This list does not include

the six or so recessive and codominant CPM resistance genes reported in melon (McCreight, 2003; McCreight and Coffey, 2007; Pitrat and Besombes, 2008). A QTL for CPM resistance to *P. xanthii* race 1 and N1 was recently reported in melon breeding line AR 5 (Fukino et al., 2008). Fukino et al. (2008) reported microsatellite markers CMBR8, CMBR120, TJ29, CMBR111, and CMBR150 linked to the CPM QTL locus in AR 5 in response to *P. xanthii* races 1 and N1. Only CMBR150 was linked with *Pm-8* locus in PI 134198 in linkage group VII of the Fukino et al. (2008) map. These results from distinctly different resistance sources and CPM races indicate that there is a resistance gene cluster in linkage group VII or the allelic genes for *Pm-8* can be found on the linkage group VII of the map (Fukino et al., 2008).

SRAP markers have become widely used in developing genetic maps, for MAS breeding programs (Mukhlesur et al., 2007; Sun et al., 2007; Yuan et al., 2008), and for saturating genomic regions of interest with markers for the ultimate goal of map-based cloning of target genes. Selection efficiency can, however, be improved when a SRAP marker is transformed into a SCAR marker (Mukhlesur et al., 2007).

The result from the present research, in which a 124-bp band was present in 73 of the 74 resistant F₂ plants, but absent in all of the 34 susceptible F₂ plants, confirms the tight linkage of the SCAR marker (SCAR0305) to *Pm-8* and indicates that this marker may be useful for MAS. This marker is expected to facilitate development of CPM-resistant melon cultivars carrying the relevant gene for the resistant trait and will be useful for map-based cloning of CPM resistance in melon.

The study presented here focused mainly on the development of molecular markers tightly linked to the *Pm-8* locus. We are now examining these markers in a larger population to estimate the genetic distance more accurately and to determine their relative position from the *Pm-8* gene. If one or more markers are found linked with the *Pm-8* locus more closely in the larger population, then they will provide a basis for eventual positional cloning of the *Pm-8* gene. The whole genome sequence of melon will soon be available, and we believe it will facilitate discovery of markers more closely linked to the *Pm-8* locus and to the cloning of more genes in melon by position cloning.

Fig. 1. Electrophoresis patterns of polymerase chain reaction-amplified with genomic DNA of 10 melon samples, which was used as a template DNA to test candidate SCAR primer pairs (SCAR0305): M, molecular marker, 100-bp ladder; lane 1, CPM-resistant parent (PI 134198); lane 2, CPM-susceptible parent (S717); lanes 3 to 6, CPM-resistant F₂ plants; lanes 7 to 10, CPM-susceptible F₂ plants. SCAR, sequence-amplified characterized region; CPM, cucurbit powdery mildew.

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