Inheritance of Resistance to Race 5 of Powdery Mildew fungus Podosphaera xanthii in Melon and development of Race 5-Specific High Resolution Melting Markers

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Abstract

Powdery mildew (PM), caused by the biotrophic fungus *Podosphaera xanthii*, drastically reduces the yield and quality of melon (*Cucumis melo* L.). Knowledge on the genetic control and high throughput molecular markers linked with resistance against this disease are essential for breeding programs. The bioassay study of the F_1 and F_2 populations derived from the parents, 'PMR 5' (♀) and 'SCNU1154' (♂) revealed a monogenic dominant nature of resistance to the devastating race, race 5. Besides, we developed three SNP based high resolution melting markers, PMm-HRM-1, PMm-HRM-2, and PMm-HRM-3 based on the previously identified SNPs on chromosome 12 and validated those using 10 melon lines and 137 F_2 population. Among these, the SNP of marker PMm-HRM-1 causes a missense mutation (Lysine → Glutamic acid) in the LRR region of *MELO3C002393* and were able to distinguish the resistant vs susceptible genotypes from 10 diverse melon population and the segregating F_2 population with more than 90% genotyping efficiency. Other two markers were based on intergenic SNPs and had more than 80% genotyping efficiency F_2 population. These markers will be helpful to melon breeders to develop melon cultivars resistant to *P. xanthii* race 5 via marker assisted breeding programs.

Introduction

Melon (*Cucumis melo* L.) is a fruit crop of *Cucurbitaceae* family that is widely grown across the world in both temperate and tropical regions due to its high flavor, taste and nutritional value (Fernández-Trujillo et al. 2011). Melon fruit contains important nutritional compounds including sugar, vitamins, minerals, and antioxidants that play significant roles in human health (Lester 2008). The average production of melon is more than 33 million tons per year (FAOstat 2018). Every year, almost 25% of crops are lost owing to diseases caused by fungal, bacterial, viral, and insect pests (Agrios 1997).

Powdery mildew (PM) is one of the most devastating and widespread diseases of *Cucurbitaceae* vegetables, caused by two biotrophic fungi *Podosphaera xanthii* or *Golovinomyces cichoracearum* of which the former one has been reported in Korea (Lee et al. 2014; Kim et al. 2016a; Kim et al. 2016b). Among the twenty-eight reported races of *P. xanthii* that cause powdery mildew in melon (McCreight 2006), races 1, 2, and 3 are prevalent in America and races 0, 4, and 5 were identified in France (Bardin et al. 1999). Previous studies characterized races 1, N1, N2, and 5 that cause powdery mildew in melon (Hosoya et al. 1999) and later, seven different races (race 1, 2, 3, 4, 5, 6, and 7) has been reported (Kuzuya et al. 2004) followed by a new race, N5 in Japan (Kim et al. 2016b). Recently, we reported two new races, KN1 and KN2 that cause serious disease on melon in South Korea (Hong et al. 2018; Kim et al. 2016a; Kim et al. 2016b). Despite moderate success in controlling *P. xanthii* by agrochemicals, cost effectiveness, development of pathogenic resistance to fungicides due to long term use and ecological hazard is of great concern. This can be addressed by development of resistance cultivars. However, for race 5, molecular markers and elite varieties are yet to be reported.

Twelve major quantitative trait loci (QTLs) related to resistance to different races of powdery mildew have been identified in melon (Liu et al. 2010). Genes and QTLs conferring resistance to powdery mildew have

been reported in chromosomes 2, 5 and 12 (Ning et al. 2014). Among them, *Pm-R1-2* were against races 1 and *Pm-R5* on LG V was against race 5 in the TGR-1551 line (Yuste-Lisbona et al. 2010). Using simple sequence repeat (SSR) markers the QTL PmV.1 against races 1, 2, and 3 and QTL *PmXII.1* against races 1, 2, and 5 on LG XII was identified in PI 124112 (Perchepied et al. 2005).

Resistant (*R*) genes play important roles in resistance to plant disease (Ellis et al. 2000; Harris et al. 2013). *R* genes have been reported in several plant species, including *Arabidopsis thaliana Oryza sativa* and *Cucumis sativus* (Meyers et al. 2003; Monosi et al. 2004; Wan et al. 2013). *R* genes are high-confidence candidate genes for conferring resistance to disease and can easily be turned into molecular markers suing SSRs, SNPs and insertion/deletions (InDels). Molecular markers have been instrumental in the selection of disease-resistant cultivars and accelerating breeding cycles. High throughput molecular markers against powdery mildew are scarce. We previously reported race 5 specific single nucleotide polymorphisms (SNPs) from whole-genome re-sequencing (WGR) on chromosomes 2, 5 and 12 (Natarajan et al. 2016; Howlader et al. 2020) and developed dCAPs markers based on three SNPs of chromosome 12 (Natarajan et al. 2016; Howlader et al. 2020). These markers needed to be converted to high resolution melting (HRM) to facilitate mass screening. Besides, the inheritance of resistance to *P. xanthii* race 5 has not been investigated yet. In this study, we therefore aimed to determine the inheritance pattern of resistance to *P. xanthii* race 5 in melon and develop high throughput HRM markers.

Materials And Methods

Plant materials and growth conditions

The melon parental lines, ‘PMR 5’ (♀) and ‘SCNU1154’ (♂), resistant and susceptible to *Podosphaera xanthii* race 5, respectively were used to develop F_1 and F_2 generation. Besides, the susceptible lines (susceptible to *P. xanthii* race 5) Edisto, WMR29, PMR 45 and NSL 74171; and resistant lines MR1, PI 414723, PI 124112 and NSL 86629 were used to validate the developed markers. All the seeds were obtained from Department of Horticulture, Sunchon National University, Korea (Kim et al. 2016a; Kim et al. 2016b). Plants were grown in a plant culture chamber at 25 ± 2°C under a long-day conditions (14h-light and 10h- dark cycle/80–120 μmol·m^{-2}·s^{-1} PPFD) with 60% relative humidity.

Powdery Mildew Culture And Inoculation

P. xanthii race 5 was obtained from the Department of Horticulture, Sunchon National University, Korea (Kim et al. 2015). *P. xanthii* race 5 were cultured on melon leaves in petri dishes on agar medium (Agar powder 6 g.L-1, Saccharose 10 g.L-1, D-Mannitol 20 g.L-1) and cultured leaves were maintained in a plant culture chamber. For *P. xanthii* race 5 inoculation, melon plants were inoculated with *P. xanthii* when the third true leaf was fully open by hand using a spray bottle with a spore concentration of 5 x 10^5 spores/ml, whereas control plants were sprayed with plain water. To maintain over 95% humidity, inoculated plants were covered with a plastic cover.

Assessment of P. xanthii race 5 resistance and statistical analysis

Inoculated leaves evaluated the disease symptoms at two weeks after inoculation (WAI). The plants were considered *P. xanthii* race 5-susceptible when the white spores of PM covered more than 10% of the overall melon leaves (Fig. 1). Goodness-of-fit of the segregating population were tested to determine the pattern of inheritance using PRISM 6 software (ver. 6.01, GraphPad Inc., San Diego, USA).

Extraction Of Genomic Dna And High Resolution Melting Analysis

Genomic DNA (gDNA) was extracted from young leaves of ten melon plants using DNeasy Plant Mini Kit (QIAZEN, Hilden, Germany) according to the manufacturer's instructions. The concentration and quality of total gDNA were determined using an ND-1000 Micro-spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA High resolution melting (HRM) analysis combined with 3'-blocked and unlabeled oligonucleotide probe (HybProbe) specific to the SNP site was used to detect SNPs. The Primers used in this study were synthesized by Macrogen, Seoul, Korea (Table 1). The gDNA was then used for HRM analysis with a LightCycler 96 instrument (Roche, Mannheim, Germany). HRM was performed in 10 µL reaction mixtures containing 1 µL at 5ng/µL, 0.1 µL forward primer, 0.5 µL reverse primer, 0.5 µL probe (10 pmol), 0.3 µL SYTO 9 fluorescent dye (Invitrogen, Thermo Fisher Scientific, USA), 5 µL HS prime LP premix (GENETBIO, Daejeon, Korea), and 2.6 µl DDW. HRM conditions were as follows: an initial preincubation at 95°C for 300 s; followed by 40 cycle of 3 step amplification (95°C for 10 s, 64°C to 56°C for 15 s under touch down and 72°C for 15s); four readings per °C at the final step after 60 s at 95°C, 60 s at 40°C, and 1 s at 97°C. HRM data was performed using LightCycler 96 software at 100% discrimination for delta Tm and curve shape with a 0.2 positive/negative threshold level.

Table 1
Primer and probe sequences used for discriminating the resistance vs susceptible genotypes via High Resolution Melting (HRM) assay.

Marker name	SNP name	Primer (5'-3')		SNPs
		Direction	Sequence	
PMm-HRM-1	SNPR5-119	Forward	CAAATAAAGAACCTTCAGTTTGAATGG	
		Reverse	TGAGTTTAACAATTACCAAAATTCATGG	
		Probe	GTTTATCTTTTGCCTCGATGCGAAA	T > C
PMm-HRM-2	SNPR5-120	Forward	TATTAGATAAGGAATCAGCCCTTCG	
		Reverse	GTTAGCCAAATGCAGATCAAATCAG	
		Probe	TGGATCTGAATGGTTTCCTGAAAC	G > A
PMm–HRM-3	SNPR5-121	Forward	ATATTAGATAAGGAATCAGCCCTTCG	
		Reverse	CATATCATTTTACATATACCAACCTAGC	
		Probe	TTTCCTGAAACTGGGATTCCGTTTA	T > A
Bold and underlined letter indicate SNP position.				

Results

Inheritance of resistance to P. xanthii race 5 in melon

An F_2 population derived from a cross between the race 5-susceptbile melon line, SCNU1154 and -resistant line, PMR 5 to investigate the inheritance pattern of *P. xanthii* race 5 resistance in melon (Fig. S1). The susceptible parental line, SCNU1154 had more than 50% PM infected leaf area, while less than 10% of the leaf area were affected in resistant parental line, PMR 5 (Fig. 2) by *P. xanthii* race 5. Furthermore, bioassay results revealed that the F_1 (SCNU1154 x PMR 5) plants had less than 10% infected leaf area, indicating that resistance to *P. xanthii* race 5 is inherited as a dominant trait. Among the 137 *P. xanthii* race 5 inoculated F_2 plants, 104 plants showed resistance and 33 plants showed susceptible reactions. A chi-square (χ^2) test revealed that resistance to *P. xanthii* race 5 segregates at a 3:1 (resistant: susceptible) ratio, consistent with a single dominant gene conferring resistance. (Table 2).

Table 2
Inheritance of *P. xanthii* race 5 resistance in *Cucumis melo*.

Crosses	Generation	Number of Susceptible plants	Number of Resistant Plants	Expected ratio (S:R)	Chi-Square ($\chi 2$)	*p*
SCUN1154 (S)	P_1	12	0	-	-	-
PMR 5 (R)	P_2	0	12	-	-	-
PMR 5 x SCUN1154	F_1	0	12	-	-	-
PMR 5 x SCUN1154	F_2	33	104	1:3	0.06	0.805
Resistant and susceptible genotypes are assessed by > 10% and < 10% of PM affected leaf area, respectively.						

Development Of Snp Markers Using The Hrm Method

The identification of *P. xanthii* race 5-resistant genotypes are necessary for the development of *P. xanthii* race 5-resistant melon cultivars. Our research group recently reported three candidate SNPs in a *P. xanthii* race 5 resistance genes on chromosome 12 (Table 3) (Natarajan et al. 2016; Howlader et al. 2020). Using these three SNPs of chromosome 12, we designed primers and probes for high resolution melting (HRM) assay to detect polymorphism in PMR 5, SCNU1154 and F_1 generation (Fig. 3; Table 1 and Table S1).

Table 3
Genomic location of SNPs and their variation with the melon genome.

Chromosome	SNP name	SNP position		Gene	Ref*	SCNU1154	PMR 5
Chr12	SNPR5-119	23,192,595	Intragenic region (Exon)	MELO3C002393	T	C/C	T/T
Chr12	SNPR5-120	23,769,828	Intergenic region	MELO3C002316-MELO3C002317	G	A/A	G/G
Chr12	SNPR5-121	23,769,833	Intergenic region	MELO3C002316-MELO3C002317	T	A/A	T/T
**C. melo* genome database							

Validation Of The Hrm Markers

We used 10 diverse melon lines (Fig. 3 and Table S1) and 137 F_2 populations inoculated with *P. xanthii* race 5 to validate the association of three HRM markers, PMm-HRM-1, PMm-HRM-2, and PMm-HRM-3 with resistance to PM (Table S2). Among the 10 diverse melon lines, the HRM markers, PMm-HRM-2 and PMm-HRM-3 successfully distinguished resistance vs susceptible genotypes of all while the genotyping of the marker, PMm-HRM-1 was miss-matched for only two genotypes (PI 124112 and NSL 86629) (Fig. S2 and Table S1). However, the genotypes Edisto, WMR29, PMR45 and NSL 74171 showed different melting curve peaks for the marker PMm-HRM-3 (Fig. S3) which could be due to genetic differences of these two genotypes with other genotypes. HRM assay of the 137 F_2 individuals also indicated high detection (of resistance status) accuracy of 90.07%, 87.23% and 82.27% for the developed markers, PMm-HRM-1, PMm-HRM-2 and PMm-HRM-3, respectively. High correlations between the phenotypic and genotypic assays with the diverse melon genotypes and 137segregating F_2 individuals indicate the close linkage of these three SNPs, SNPR5-119, SNPR5-120 and SNPR5-121 with *P. xanthii* race 5 resistance in melon (Table S2).

Discussion

The development of PM resistance melon lines is essential to combat this devastating disease. We investigated the inheritance pattern of resistance to PM against a widespread Korean race, race 5 and developed high throughput molecular markers for selecting resistant lines. In our bioassay after inoculation with *P. xanthii* race 5, the F_1 populations from a cross between *P. xanthii* race 5-susceptbile (SCNU1154) and –resistant (PMR 5) lines were resistant, indicating the dominant nature of resistance to PM (Fig. 3 and Table 2). A phenotypic ratio of 3:1 (resistant: susceptible) was found in 137 F_2 plants, indicating a monogenic trait of resistance to *P. xanthii* race 5 (Table S2). Various patterns of inheritance of resistance to powdery mildew in melon were previously identified including polygenic against race N1 (Kim et al. 2016b), partial-dominant against KN1 (Nou, IS. unpublished data), co-dominant against race 1, 2 and 5 (Yuste-Lisbona et al. 2011) and dominant against race 1, pxCh1 and KN2 (Fukino et al. 2008; Liu et al. 2010; Nou, IS. unpublished data). Development of markers, especially the high throughput ones, is essential for mass screening and speed breeding these days (Wanga et al. 2021; Watson et al. 2018; Thomson 2014). As to the markers for selecting resistant vs susceptible lines, several SSR markers including CMBR150 and CMBR111 (Fukino et al. 2008), CMN1-38 (Fukino et al. 2007), CMBR120 (Kim et al. 2016b) and TJ29 (Gonzalo et al. 2005); SRAP marker Pm-8 (Liu et al. 2010) and CAPs markers, CAPS-Dde I (Zhang et al. 2013), and PM2-CAPS and PM5-CAPS (Yuste-Lisbona et al. 2011) were linked with linked to PM resistance against specific races. These markers were mainly used to developed linkage map and are of low throughput. SNPs, these days, can easily be identified and SNPs linked with specific phenotypic expressions can be used for developing high throughput genotyping upon validation in diverse natural population and in developed segregating populations (Mammadov et al. 2012; He et al. 2014; Thomson 2014; Yang et al. 2015)

Based on the whole Genome Re-Sequencing of PM susceptible genotype, SCNU1154 and resistant genotypes, Edisto47, MR-1, and PMR5, we have identified the genome-wide SNPs, of which 112 SNPs and 12 InDels were observed in powdery mildew responsive chromosomes, chromosomes 2, 5 and 12 (Natarajan et al. 2016; Howlader et al. 2020). Among these, three polymorphic SNPs namely, SNPR5-119,

SNPR5-120 and SNPR5-121 demonstrated race 5-specific genetic variation in melon (Kim et al. 2016a; Kim et al. 2016b). Among these, three SNPs, namely, SNPR5_119, SNPR5_120, and SNPR5_121 on chromosome 12 (closely located to the SSR marker CMBR150) were later identified to be associated with the phenotypic variation against race 5-specific susceptible (SCNU1154, PMR45, WMR29, and Edisto47) and resistant (PI414723, PMR5, and MR1) lines in *C. melo* via derived cleaved amplified polymorphic sequence (dCAPS) assay, indicating their linkage with resistance against PM race-5 (Howlader et al. 2020). Here, we designed HRM markers and validated their genotyping efficacy in wide range of diverse natural population and the developed F_2 segregating generations. Among these three markers, PMm-HRM-1 showed more than 90% accuracy in genotyping the resistance status of both natural and F_2 segregating populations, opposed to only ~80% accuracy of the other two markers, PMm-HRM-2 and PMm-HRM-3 in F_2 population (Table S1). The SNPs, SNPR5-120 and SNPR5-121 are located in the intergenic region between the genes, MELO3C002316 and *MELO3C002317.* Whereas, SNPR5-119 is located in the intragenic region of the intron-less leucine-rich repeat (LRR) receptor-like protein kinase family gene, *MELO3C002393* on chromosome 12 (Table 3). Plant *R* genes are mainly encoded nucleotide-binding site leucine-rich repeat (NBS-LRRs), LRR, receptor-like protein kinases (RLKs) and LRR-RLKs domains, all of which are involved in plant defense against pathogens (Ellis et al. 2000; Harris et al. 2013; Kourelis and Van Der Hoorn 2018; Brotman et al. 2013; Marone et al. 2013). The LRR domain provides pathogen recognition specificity (DeYoung and Innes 2006; Eitas and Dangl 2010). Moreover, the NBS-LRR domain in plant immune receptors relied on the LRR domain for perform many important roles (Padmanabhan et al. 2009), and mutations in the LRR domain may result in a susceptible phenotype. SNPR5-119 results in a missense mutation (Lysine → Glutamic acid) in the LRR region of *MELO3C002393* indicating its potential role in eventual protein product and thereby, influencing the ability to confer resistance against race 5 (Fig. S4). Taken together, these findings suggest that the SNP maker, SNPR5-119 may play potential roles and can effectively be used in HRM assay to detect *P. xanthii* race 5 resistant and susceptible melon genotypes and hence, can be used as high throughput molecular markers in MAS Based breeding programs.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The raw sequence data from this study have been deposited in the publicly accessible National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/) database as PRJNA804585. The datasets supporting the conclusions of this article are included within the article and its additional files.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

I.-S.N. and H.-J.J. conceptualized the work, J.-E.H. conducted all experiments and J.-E.H. & M.R.H. prepared the data, interpreted the results, and wrote the manuscript. All authors read and approved the final manuscript.

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Statement

All our experiments complied with local and national regulations.

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Figures

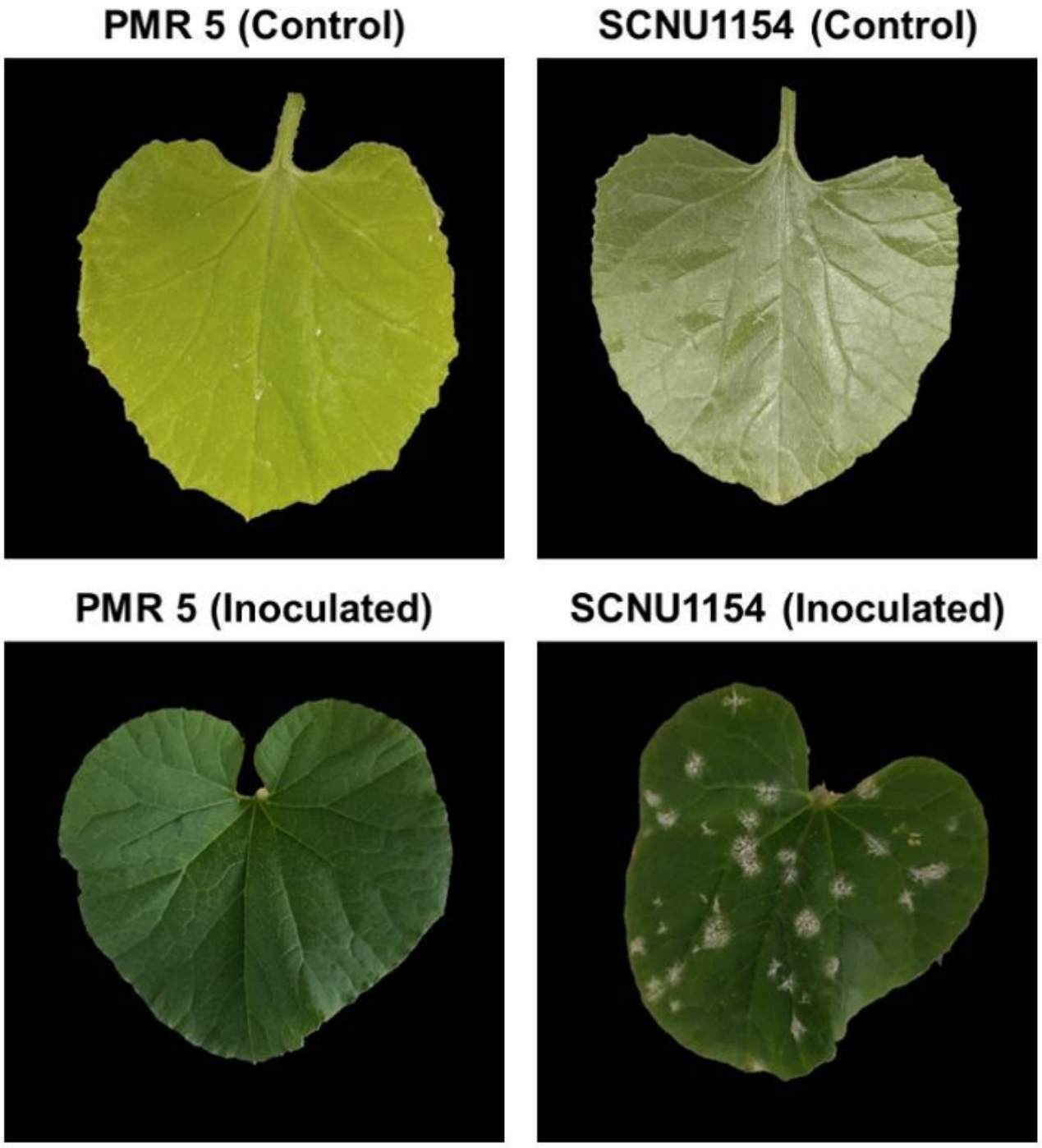

Figure 1

Phenotypes of melon lines PMR 5 (resistant) and SCNU1154 (susceptible) after inoculation with *P. xanthii* race 5.

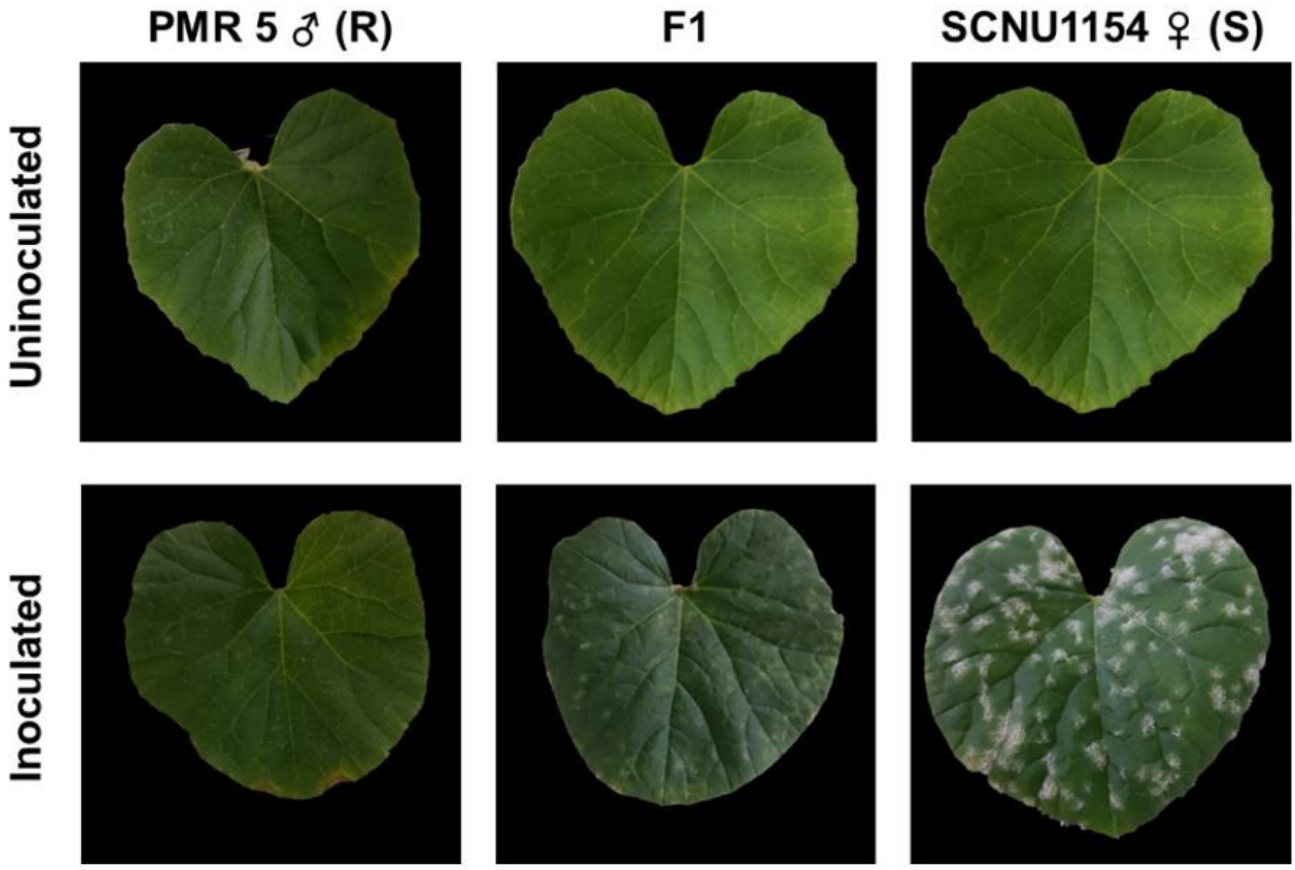

Figure 2

Phenotypes of the resistant and susceptible, and their F_1 generation two-weeks after inoculation with *P. xanthii* race 5. R. Resistance; S. Susceptible

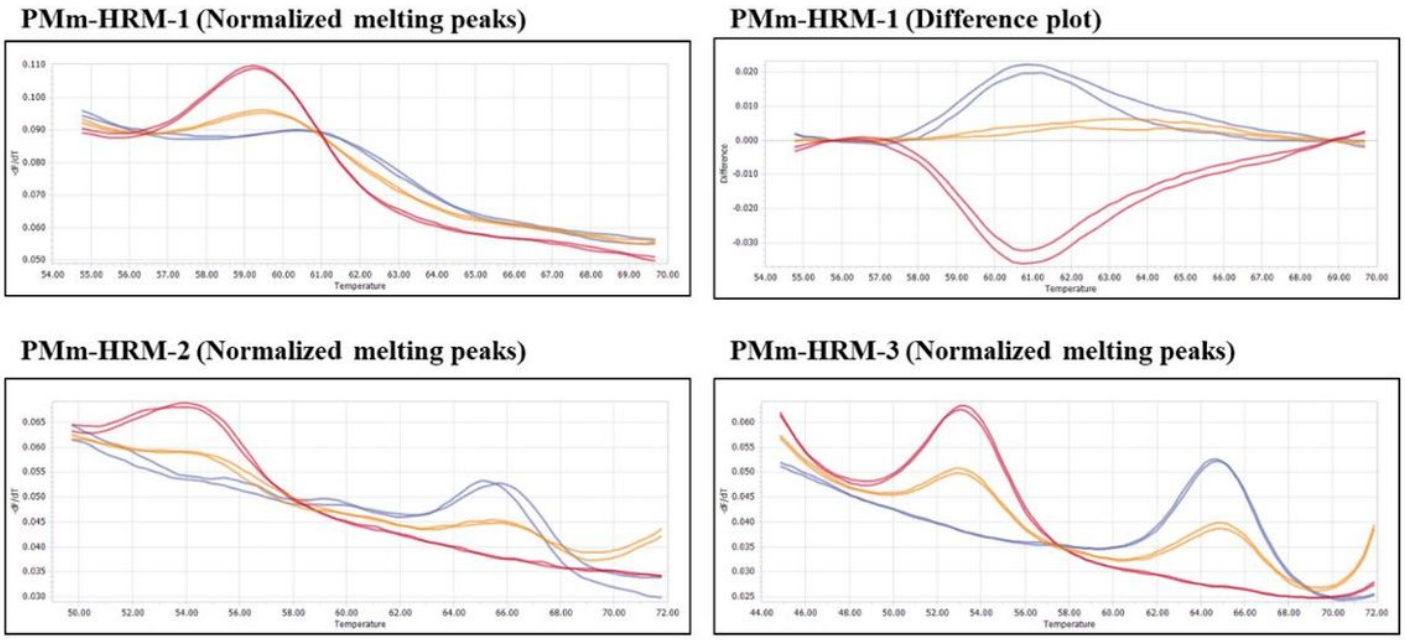

Figure 3

Melting curves of three HRM markers, PMm-HRM-1, PMm-HRM-2 and PMm-HRM-3, developed and validated in this study. Blue curves, Plants of 'PMR 5' genotype (resistant); red curves, Plants of 'SCNU1154' genotype (susceptible); Orange curves, heterozygous plants.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigures.pdf
- SupplementaryTables.xlsx