TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2022) 46: 123-133
© TÜBİTAK
doi:10.3906/bot-2111-17

Mapping quantitative trait loci and developing first molecular marker for race 5 of *Podosphera xanthii* resistance in melon (*Cucumis melo* L.)

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Received: 08.11.2021 • **Accepted/Published Online:** 09.03.2022 • **Final Version:** 29.03.2022

Abstract: Powdery mildew of melon caused by the *Podosphaera xanthii* factor is a disease that causes serious yield losses economically in all areas where melon is grown from Asia to Europe and America. The use of resistant varieties for the control of the disease is the most environmentally friendly method. However, traditional methods of melon breeding have made a noteworthy input to resistance varieties improvement, but they have been slow and incompetent in targeting complex powdery mildew disease resistance traits. To overcome these problems and to facilitate breeding programs, molecular markers closely linked to the *Pm-R5* gene are needed for marker-assisted selection (MAS). The present study aimed to map quantitative trait locus for powdery mildew resistance and identify the molecular markers linked with the *Pm-R* gene which provides resistance to race 5 of *P. xanthii* for MAS breeding in melons. A total of 310 primers/combinations belonging to six marker systems including SRAP, SSR, ISSR, TRAP, CAPS, SCAR in the RIL population consisting of 136 genotypes, developed from crosses PMR-6 and susceptible inbred line were used for QTL mapping. Quantitative trait loci analysis determined one major QTL (*Pm-R*) on linkage group I for resistance to races 5 of powdery mildew. The SSR12202 marker is linked to the *Pm-R* gene QTL at 2.12 cM genetic distance, and the CMCT170b and CMMS30-3 markers are determined in the support interval of this QTL. The co-dominant markers, together with the map information reported here could be used for genotyping selection of resistance to race 5 of *P. xanthii* in melon breeding.

Key words: Disease resistance, MAS breeding, *Podosphaera xanthii*, PMR6, race 5, QTL mapping

1. Introduction

Melon (*Cucumis melo* L.) is one of the most important vegetable species on nutrition with its nutrients and minerals (Meena et al., 2018), and is widely cultivated for its edible fruit from the warm temperate zone to tropical areas (Pech et al., 2007). Also, it is one of the most polymorphic species that has many diverse species, and several intraspecific classifications (Robinson and Decker-Walters, 1997; Stepansky et al., 1999; Nuñez-Palenius et al., 2008; Szamosi et al., 2010; Guliyev et al., 2018). Turkey is the secondary center of diversity for melon (Sari et al., 2008) and therefore has valuable genetic resources (Sari et al., 2007; Sensoy et al., 2007; Solmaz et al., 2010; Kacar et al., 2012; Frary et al., 2013) and has valuable genes that can be utilized in breeding programs (Kacar et al., 2012). *Cucumis melo* var. *cantalupensis* and var. *inodorus* are the most economically significant melons grown in the European, Mediterranean, and Asian countries (McCreight et al., 1993).

Powdery mildew (PM) is probably the most destructive disease affecting cucurbitaceous plants worldwide. It is caused by *Podosphaera xanthii* (Castagne) U. Braun and N. Shishkoff (*Sphaerotheca fuliginea* Schlech previously ex Fr.Poll.) and *Golovinomyces orontii* (Castagne) (*Golovinomyces cichoracearum* or *Erysiphe cichoracearum* DC ex Merate) (Heluta, 1988; Bardin et al., 1999; Braun and Takamutsu, 2000; Lebeda and Sedláková, 2010; Pirondi et al., 2015). *Podosphaera xanthii* is a major problem in many countries of the world among the diseases affecting melon crops (Robinson and Decker-Walters, 1997; Kuzuya et al., 2006; Wang et al., 2011). In Turkey, *P. xanthii* is the main causal fungus of PM in melon cultivation (Křístková et al., 2009; Ünlü et al., 2010). Disease symptoms are white powder-like spots on the vegetative parts and seldom seen in the fruit (Zitter et al., 1996), and in the next period causes the death of the leaves, reduces yield, and strongly decreases the quantity and quality of crop yields (Robinson and Decker-Walters, 1997).

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Twenty-two races of *P. xanthii* have been reported on melon (McCreight, 2006). Although races of PM populations depend on the melon cultivar, cultivate season, and ecological conditions (Hosoya et al., 1999), especially five races (race 1, 2, 3, 4, and 5) were the most common and destructive in the worldwide (Bardin et al., 1999). For example, the most common races in Southern European regions are races 1, 2, and 5 (Cohen and Eyal, 1988; Bardin et al., 1997; Del Pino et al., 2002). Races 1 and 2 are the major races in China (Ma et al. 2011), Brazil (Reifschneider et al., 1985, Kobori et al., 2004; Rabelo et al., 2017), Spain and Israel (Cohen and Eyal, 1988; Kenigsbuch and Cohen, 1992), United States (McCreight et al., 1987; McCreight and Coffey, 2011), Greece (Vakalounakis and Klironomou, 1995), race 1 and 5 in Japan (Hosoya et al., 1999) and race 3 in Israel (Cohen et al., 1996). Similarly, races 4 and 5 were named for strains that broke the resistance of other resistant varieties in France (Bardin et al., 1997). In Turkey, the presence of race 5 has been reported (Ünlü et al., 2010).

However, several sources of resistance against *P. xanthii* have been identified to the races (Cohen and Eyal, 1988; Bertrand and Pitrat, 1989; Bardin et al., 1999; McCreight, 2003; McCreight, 2006). Besides, resistance plant varieties of reactions against *P. xanthii* could show significant differences (Perchepied et al., 2005). Its inheritance is still confusing, except for 'PMR 45' which has only one dominant gene (Epinat et al., 1993; Kuzuya et al., 2006; McCreight, 2006; Pitrat and Besombes, 2008; Hong et al., 2018). Seventeen PM resistance genes were identified by many researchers (Ning et al., 2014). Therefore, sources of new PM resistance genes are always of great interest to melon breeders and growers (McCreight, 2003). PMR6 has similar parentage as PMR5 (Harwood and Markarian, 1968), and is resistant to the *P. xanthii* race 1, 2, and 5 (Cohen and Eyal, 1988; Cohen et al., 1990; Ünlü et al., 2010; Zhu et al., 2016).

Synthetic fungicides, particularly multisite fungicides, are often used for controlling the fungus. However, unconsciously and overconsumption of pesticides to combat pathogenic microorganisms may lead to a decrease in sensitivity and causes resistance (Fatmawati and Daryono, 2016). Therefore, it is important to improve resistant melon varieties. Most plants cultivated under greenhouse conditions comprise hybrid varieties. According to Pitrat and Besombes (2008) first breeding powdery mildew resistant melon cultivars started in the 1930s in California with the release of 'PMR 45'. In fact, although a wide phenotypic variability for resistance parent has been reported as a result of the domestication process (McCreight, 2003; McCreight, 2006; Pitrat and Besombes, 2008, Wang et al., 2011), the new world PM resistance melon varieties are narrow due to the difficulties of breeding to be obligate parasite (Ünlü et al., 2010), complex inheritance and resistance mechanisms (Kuzuya et al., 2006). However, inbreeding studies for resistance to biotic and abiotic stresses, selection based on DNA markers provides substantial advantages over traditional phenotypic screening alone (Tanksley et al., 1989). In recent years, the improvement of DNA markers has been providing the genetic structure of plants and the construction of linkage maps could provide valuable knowledge about the location and relationships among genes or QTL associated with important agricultural traits such as disease resistance (Tanksley et al., 1989; Yuste-Lisbona et al., 2011b; Kim et al., 2015). On the other hand, the determination of specific DNA markers closely linked to the related genes or QTL could contribute a useful tool for marker-assisted selection in breeding programmes (Perchepied et al., 2005; Yuste-Lisbona et al., 2008; Choi et al., 2020).

During the last decades, several QTL genetic maps to powdery mildew have been constructed using the accessions WMR9 (Pitrat, 1991), PI 124112 (Perchepied et al., 2005), PI134198 (Liu et al., 2010), TGR-1551 (Yuste-Lisbona et al., 2011a; Yuste-Lisbona et al., 2011b; Beraldo-Hoischen et al., 2012), K7-1 (Zhang et al., 2013), PI 414723 (Fazza et al., 2013), Edisto47 (Ning et al., 2014), PMR5, and PMR45 (Kim et al., 2016) as resistant donor lines in melon. Also, the identification of specific DNA markers closely linked to the resistance genes or QTL using PI134198 which provides resistance to races pxCh1 of *P. xanthii* (Liu et al., 2010), TGR-1551 to races 1, 2, and 5 (Yuste-Lisbona et al., 2011a), K7-1 to race 2F (Zhang et al., 2013) and newly designed InDel PCR marker used in this study can be used to discriminately introduce an MR-1 PM resistance gene for the development of PM-resistant melon varieties (Choi et al., 2020) for MAS in breeding programmes.

Here we report the QTL mapping of resistance genes carrying to races 5 of *Podosphera xhanthii* in PMR6 using the RIL population and identify markers closely linked to genes of interest for MAS breeding purposes were aimed.

2. Materials and methods

2.1. Population development

A RIL population consisting of 136 F6 lines was constructed by single-seed descent from an F2 population generated from the cross of two lines belonging to the Batı Akdeniz Agricultural Research Institute gene pool, PMR6 (♂), and TKÜ3 (♀). PMR6 genotype is resistant to the PM pathogen *P. xanthii* race 1, 2, and 5 (Hao et al., 2015), whereas TKÜ3 is susceptible pure line of cantaloupe type melon.

2.2. Isolate and scoring of resistance to *P. xanthii*

The *P. xanthii* isolate used in this experiment to race 5 was based on the reactions of a set of differential melon

lines [1 susceptible standard variety (Ananas, BATEM) and 1 resistant genotype (PMR 6)] to isolate inoculated on the second true leaf of cotyledons under controlled conditions, using an eyelash brush according to the protocol of Ünlü and Ünlü (2012). PM inoculations were carried out in a greenhouse at 20–28°C with a 14 h:10 h light:dark photoperiod for about 15 days. All population were evaluated according to the level of sporulation of the fungus on a scale of 1–4 of Yuste-Lisbona et al. (2010).

2.3. DNA extraction and molecular analyses

Genomic DNA was isolated from young leaves, as reported by Doyle and Doyle (1990).

For bulked segregant analysis (BSA), equal quantities of DNA from 5 resistant (class 1) and 5 susceptible (class 4) F6 individuals were pooled to establish the resistant and susceptible bulks (Michelmore et al., 1991). For BSA, five resistant genotypes and five susceptible genotypes DNA bulks were generated from the RILs. Totally 134 SSR (Katzir et al., 1996; Danin-Poleg et al., 2000; Danin-Poleg et al., 2001; Monforte et al., 2003; Fukino et al., 2008; Watcharawongpaiboon and Chunwongse, 2008; Yuste-Lisbona et al., 2011a; Yuste-Lisbona et al., 2011b; Malik et al., 2011; Kacar et al., 2012; Solmaz et al., 2016), 108 ISSR (Levi et al., 2005; Yeboah et al., 2007; Parvathaneni et al., 2011), 26 TRAP (Hu and Vick, 2003; Fazza et al., 2013), 80 SRAP (Li and Quiros, 2001; Ferriol et al., 2003; Yeboah et al., 2007; Yuan et al., 2008; Solmaz et al., 2016), 2 CAPS and 5 SCAR (Yuste-Lisbona et al., 2011a; Daryono et al., 2011) markers were used to identify polymorphism between parents and resistant and susceptible bulks.

All PCR amplifications were carried out in a gradient thermal cycler (Biorad DNA-Engine Gradient Cycler, Hercules, CA, USA). PCR amplifications were performed in 18 µL volume containing 2xPCR Mastermix (Fermentas K0171), 2 µL (0.3 µM each primer) of forward and reverse primers, 2.5 µL double-distilled water, and 25 ng of DNA. PCR conditions for SSR markers were carried out according to Kacar et al. (2012) with some modifications. DNAs were amplified under the following protocol: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 50–55°C for 30 s, 72 °C for 1 min; and 72 °C for 10 min. To SRAP and TRAP markers, cycling parameters were included 5 min of denaturing at 94 °C, five cycles of three steps: 1 min of denaturing at 94 °C, 1 min of annealing at 35 °C and 1 min of elongation at 72 °C. In the following 35 cycles the annealing temperature was increased to 50 °C, and for extension, one cycle 5 min at 72 °C. To ISSR markers, cycling protocol: 94 °C for 3 min; 35 cycles of 94 °C for 45 s, 48 °C for 45 s, 72 °C for 90 s; and 72 °C for 10 min. Finally, the amplification for CAPS and SCAR markers were amplified under the following protocol: 94 °C for 3 min; 35 cycles of 94 °C for 45 s, 55 °C for 45 s, 72 °C for 90 s; and 72 °C for 10 min. PCR products were separated on 2% agarose gel in 1 × TAE buffer (Tris-acetate-EDTA) at 115 V for 3.5 h and photographed under UV light (ENDURO GDS Gel Documentation System) in dye (EZ-ONE N472-KIT, Ambresco) for further analysis. A 100 bp plus DNA ladder (VitaScientific, USA) was used as a molecular standard to confirm the appropriate markers. The PCR products of polymorphic markers acquired by BSA were used for all of the RIL population. Polymorphism rates (Pr) of primers and primer combinations and polymorphism information content (PIC) values were calculated as described by Smith et al. (1997).

2.4. Linkage map construction and QTL analysis

The linkage maps were constructed with JoinMap 4.0 software (Van Ooijen, 2006). Segregation distortion at each marker locus was tested against the expected ratios for an F6 RIL population using a chi-squared test. A logarithm of odds ratio (LOD) of 3.0 was used to establish significant linkage. Recombination values to map construction were converted into centiMorgans (cM) using the Kosambi mapping function with the following JoinMap parameters. QTL controlling melon powdery mildew was identified using software packages Qgene (Nelson, 1997). Additionally, the composite interval mapping (CIM) and single marker regression (SMR) approach were also applied by means of the QTL Qgene computer programme.

However, taken molecular markers closely linked to *Pm-R5* to gene were tested for molecular MAS analysis in twenty-nine hybrid genotypes and four commercial varieties. On the other hand, these genotypes were tested as classical method using isolate to race 5. In addition, Ediso 47, PMR45, TGR 1551, and PI 414723 were only molecular tested to obtain information on whether they are associated with other races.

3. Results

Powdery mildew resistance to race 5 was screened in 136 individuals of the RIL population. Also, four phenotypic classifications were recorded based on the level of sporulation on each individual RIL plant determined susceptible (scala 3 and 4) or resistant (scala 1 and 2).

A total of 310 primer combinations and primers were assayed to identify polymorphism between parents and resistant-susceptible bulked DNA samples. Of these primer combinations and primers, 49 were polymorphic between the parents. The primer and primer combinations used to amplify SRAP, TRAP, ISSR, SSR, and SCAR fragments resulted in the amplicon of 216, of which 12 (18%), 5 (22%), 12 (15%), 44 (100%), and 2 (100%), respectively, were polymorphic (Table 1) and mapped. A total of 216 markers for genetic linkage analysis were tested in the mapping population, and 77 markers were determined that showed initiative in the population. As a result of the grouping analysis, 44 markers were mapped, as shown in Figure 1.

Table 1. Marker names and the total number of amplicons, polymorphic amplicons, total alleles, polymorphic allele, percentages of polymorphism (%), and PIC as revealed by all markers among the mapping population.

Markers	Total primer/ combination	Polymorphic primer/ combination	Total alleles	Polymorphic allele	Mean allele	Mean polymorphic allele	Mean polymorphism rate (%)	Mean PIC
SRAP	**65**	**9**	**57**	**12**	**6.67**	**1.17**	**18**	**0.57**
TRAP	**26**	**5**	**33**	**5**	**6.22**	**1.22**	**22**	**0.64**
SSR	**117**	**22**	**44**	**44**	**2**	**2**	**100**	**0.71**
ISSR	**96**	**12**	**80**	**12**	**6.6**	**1**	**15**	**0.76**
SCAR	**5**	**1**	**2**	**2**	**2**	**2**	**100**	**0.66**
CAPS	**1**	**0**	**0**	**0**	**1**	**0**	**0**	-
TOTAL	**310**	**49**	**216**	**75**	-	-	-	-

When the results of composite interval mapping (CIM) and single marker regression (SMR) analysis were evaluated, it was recorded that three markers were tightly associated with the *Pm-R5* gene (Table 2). The reaction of RIL genotypes to *P. xanthii*, when scored as a phenotypic marker (scala 1–4), cosegregated with molecular markers on chromosome 1, indicating the likely position of resistance genes to races 5.

SSR-12202 (Figure 2), SSR-CMCT170b and SSR-CMMS30-3 (Figure 3) primers produced 250, 180, and 300 bp DNA fragments respectively that were found only in the resistant parent, resistant bulks, and resistant RIL plants (Table 3). The analysis of the segregation data from the markers on the 136 RIL individuals and the resistance trait allowed us to assign the position of these markers in the melon genomic region on chromosome 1 surrounding the *Pm-R5* locus with 2.1 cM distance. They were not converted to CAPS or SCAR markers because of SSR markers. Evaluation of the linkage markers for MAS of PM resistance were carried out in commercial 'Balözü', 'Cıtırex', 'Gediz', 'Ballıca' varieties and twenty-nine hybrid melon genotypes. These genotypes were also tested as classical method using isolate to race 5 (Table 4). However, Ediso 47, PMR45, TGR 1551, and PI 414723 were only molecular tested, and determined as susceptible. These results indicated that SSR-12202, SSR-CMCT170b, and SSR-CMMS30-3 primers could be used for screening and detection of *Pm-R5* in diverse melons for powdery mildew resistance breeding.

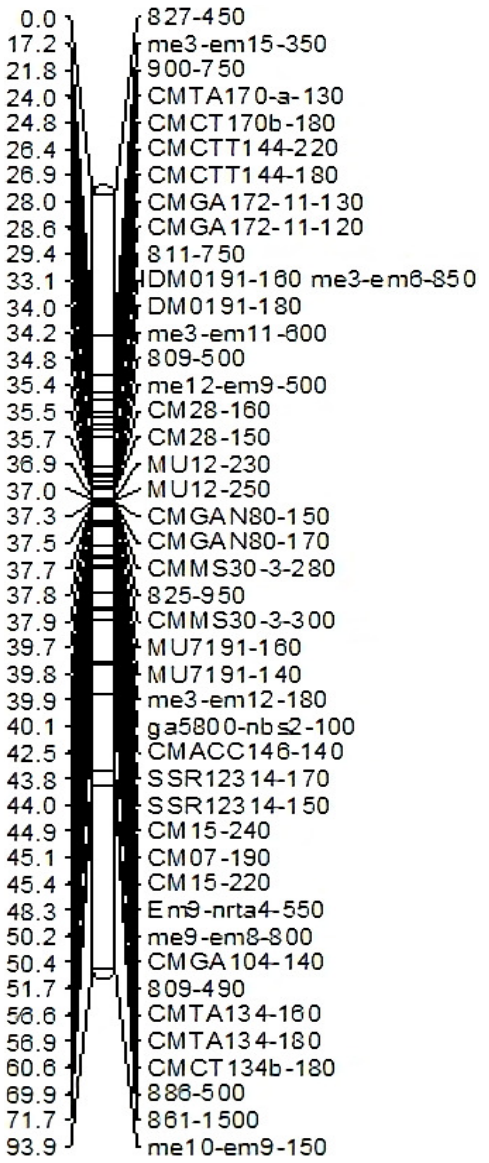

Figure 1. Map of genetic linkage.

4. Discussion

PMR6 is an important genetic source of resistance genes in melon (Kenigsbuch and Cohen, 1992; Hao et al., 2015), whose useful in breeding programs require further linkage genetic map information. This genetic linkage map defined herein is the first map derived using resistance PMR6 parent. A total of 310 molecular markers (65 SRAP,

Table 2. Quantitative trait locus tightly linked *Pm-R5* gene.

Quantitative trait locus	Primer sequences (5'-3')		LOD	R^2	Analysis method
SSR12202-220	F: AGA TTT GGA AGG ATG TTA GA	R: AAG TCG GGT GGT AGT TGT TT	5.971	0.183	SMR
CMCT170b-170	F: ATT GCC CAA CTA AAC TAA ACC	R: CAC AAC ACA ATA TCA TCC TTG	4.639	0.145	SMR
CMMS30-3-280	F: TTC CCA CCA GCC CAA CGG ACA CAC T	R: GAG ATA CAG AAA CGA CGT CTA ACC T	6.706	0.203	CIM

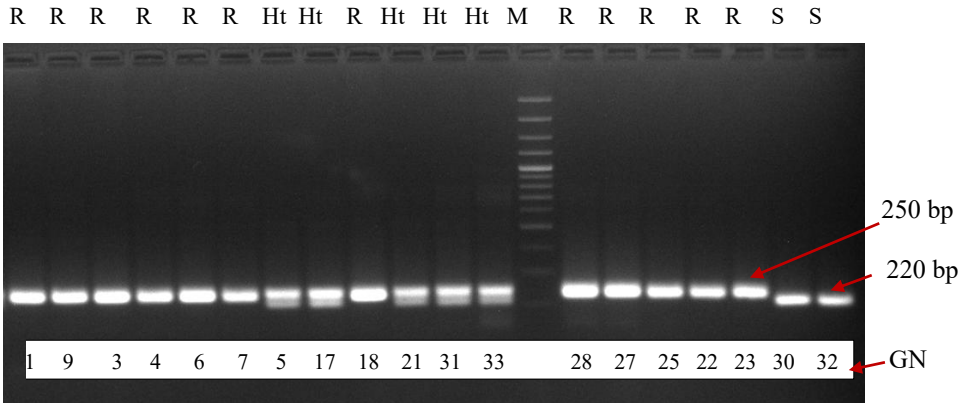

Figure 2. Analysis of 19 differential melon genotypes using the SSR-12202 marker. M: Ladder DNA, R: Homozygote resistance, HT: Heterozygote resistance, S: Susceptible, GN: Genotypes no.

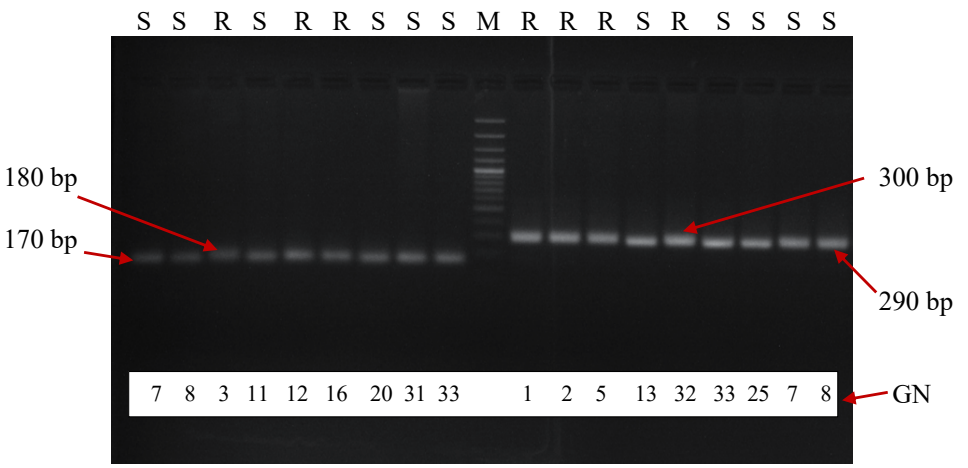

Figure 3. Analysis of 9 differential melon genotypes using the SSR-CMCT170b (left of M) and SSR-CMMS30-3 (right of M) marker. M: Ladder DNA, R: Homozygote resistance, S: Susceptible, GN: Genotypes no.

26 TRAP, 117 SSR, 96 ISSR, 5 SCAR, and 1 CAPS) and 1 morphological marker (1–4 scala) were used to take QTL mapping to resistance race 5 of *P. xanthii*. It is important that the choice of molecular marker technique must be a compromise between reliability, labour, and ease to analyse, and confidence of revealing polymorphisms (Agarwal et al., 2008). In our study, SSR primers were successful in separating the resistance and susceptible genotypes. They represent some of the most useful tools for the analysis of genomes and enable the association of heritable traits with underlying genomic variation in mapping studies to PM (Yuste-Lisbona et al., 2011a; Yuste-Lisbona et al., 2011b; Hao et al., 2015).

Podosphaera xanthii causes serious destruction to melon production worldwide. However, frequent occurrences of the disease in a different country or different regions of

Table 3. SSR-12202, SSR-CMCT170b, and SSR-CMMS30-3 marker for MAS breeding and their base pairs (bp).

	SSR-12202	SSR-CMCT170b	SSR-CMMS30-3
Homozygote resistance (RR)	250	180	300
Heterozygote resistance (Rr)	250 and 220	180 and 170	300 and 280
Homozygote susceptible (SS)	220	170	280

Table 4. Evaluation of classical test and the SSR-12202, SSR-CMCT170b, and SSR-CMMS30-3 markers for MAS of powdery mildew resistance to race 5 as the molecular test in hybrid progenies and some commercial varieties.

Genotypes	Genotypes no	Classical test		Molecular test		
		Scala	Resistance (R) Susceptible (S)	SSR-12202	SSR-CMCT170b	SSR-CMMS30-3
T33 X T58 Hib 58	1	2	R	RR	RR	RR
T33 X T70 Hib 59	2	2	R	RR	RR	RR
T33 X T77 Hib 60	3	2	R	RR	RR	RR
T36 X T18 Hib 73	4	1	R	RR	RR	RR
T36 X T52 Hib 77	5	1	R	Rr	RR	RR
T56 X T11 Hib 111	6	1	R	RR	RR	RR
T56 X T52 Hib 115	7	1	R	RR	SS	SS
T56 X T58 Hib 116	8	2	R	RR	SS	SS
T56 X T77 Hib 118	9	1	R	RR	SS	RR
T57 X T77 Hib 124	10	2	R	RR	SS	RR
T63 X T18 Hib 127	11	1	R	RR	SS	RR
T65 X T32 Hib 137	12	2	R	RR	RR	RR
T65 X T70 Hib 142	13	2	R	RR	SS	SS
T65 X T77 Hib 143	14	2	R	RR	SS	RR
T75 X T11 Hib 145	15	2	R	RR	RR	RR
T86 X T70 Hib 169	16	2	R	RR	RR	RR
T98 X T5 Hib 177	17	2	R	Rr	RR	SS
T98 X T43 Hib 179	18	2	R	RR	SS	RR
T50 X T43 Hib 182	19	2	R	RR	SS	RR
T97 X T48 Hib 191	20	2	R	RR	SS	RR
T5 X T 50 Hib 196	21	2	R	Rr	RR	SS
T96 X T48 Hib 225	22	1	R	RR	SS	RR
T96 X T50 Hib 226	23	2	R	RR	SS	RR
T98 X T50 Hib 229	24	2	R	RR	RR	SS
T20 X T32 Hib 29	25	1	R	RR	RR	SS
T20 X T70 Hib 33	26	2	R	RR	RR	SS
T20 X T77 Hib 34	27	2	R	RR	RR	RR
T33 X T52 Hib 57	28	2	R	RR	RR	RR
T96 X T5 Hib 223	29	2	R	Rr	RR	RR
Balözü	30	4	S	SS	SS	RR
Çıtırex	31	3	S	Rr	SS	RR
Gediz	32	3	S	SS	SS	RR
Ballıca	33	4	S	Rr	SS	SS
		Rates of molecular test overlap with classical test (%)		93.9	63.6	66.6

the country hint at the potential existence of several races which need to be identified. At a minimum, twenty-two putative races of *P. xanthii* have been identified in different countries (Cohen et al., 1996; Bardin et al., 1997; Hosoya et al., 1999; Del Pino et al., 2002; McCreight, 2006; Ma et al., 2011; McCreight and Coffey, 2011). In Turkey, only one race, race 5, has been reported (Ünlü et al., 2010). The predominant race can change depending on cultivars, cultivation season, geographic area, and country (Hosoya et al., 1999), so determination of *P. xanthii* race is necessary when working with PM resistance (Zhang et al., 2013).

The traditional breeding approach of morphological selection is time-consuming, laborious, and expensive. Molecular markers for MAS can be more efficient and rapid selection method of desired phenotypes, which could save much effort, time, and expenses of field work, and eliminate the need for pathogen inoculums. Identification of the target genes and the development of molecular markers tightly linked with them are a prerequisite for efficient MAS (Zhu et al., 2016). Unfortunately, markers linked to race 5 of PM in melon are scarce. Although the determination of genes and QTL associated with PM resistance in melon (Fukino et al., 2008; Wang et al., 2011; Beraldo-Hoischen et al., 2012; Fazza et al., 2013), few markers linked to this trait have been identified.

The difficulty of working with this pathogen and the low number of markers linked to this resistance makes that the marker developed could be useful in breeding programs for the selection of PM resistance to race 5. Yuste-Lisbona et al. (2011a) constructed an integrated genetic linkage map using an F2 population derived from a cross between the TGR-1551 (resistant to races 1, 2, and 5 of *P. xanthii*) and the Spanish cultivar 'Bola de Oro' (susceptible). *Pm-R5* is a PM resistance gene which provides resistance to race 5 of *P. xanthii* in TGR-1551. This QTL was confirmed by a LOD score of 36.8% and 65.5% of the phenotypic variance determined for resistance to race 5. In the other work, genome-wide association studies with PM resistance were detected at the previously reported *Pm-x1.5* gene to race 1 and 5 closely linked of *P. xanthii* located 5.1 cM apart using F2 population taken from crossing PI 414723 and Védrantais (Fazza et al., 2013).

As stated before, mapping with molecular markers could provide valuable information for estimating genetic position and location of genes or QTL associated with disease for melon breeding (Yuste-Lisbona et al., 2011a; Yuste-Lisbona et al., 2011b). The codominant PCR-based markers developed by this study, especially the SSR-12202 located within the support interval of the *Pm-R5* QTL, could be applied in MAS, easily improvement of cultivars resistant to race 5 of *P. xanthii*. This QTL marker was supported by a LOD score of 5.971 and explained 93.9% of the phenotypic variance observed for resistance to race 5.

PMR 6 has similar parentage as PMR 5 (Cohen and Eyal, 1988; Harwood and Markarian, 1968). PMR5 has also resistance to race 0, 1, 2F, 2US, 4, and 5 of *P. xanthii* (Kuzuya et al., 2006; Rabelo et al., 2017). PMR6 genotype contains resistance genes that other powdery mildew agent *Golovinomyces cichoracearum* and the other *P. xanthii* races in addition to race 5 of PM resistance. Therefore, information regarding the linkage map presented here, together with the use of the RIL population for multiple evaluations, could be significant tools for examining the location of disease resistance genes in the melon genome and for identifying markers tightly linked to these resistances.

5. Conclusion

The findings of the current study obtained from a well-structured population indicate that some SSR markers are a valuable application for detecting marker–resistance associations in melon breeding for powdery mildew resistance to race 5. In this study, the *Pm-R5* gene locus was detected on chromosome 1. Furthermore, codominant markers linked to powdery mildew resistance have been developed and the linkage distance between them is 2.12 cM, which is the closest molecular marker that could be used in MAS breeding for powdery mildew resistance to race 5 described until now.

Funding

This work was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) with grant number 116O200.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgement

The authors gratefully acknowledge the financial support of the Scientific and Technological Research Council of Turkey (TÜBİTAK). The authors would like to thank Pınar AKKELLE, a scholarship student, for her help with the laboratory work. Thanks also to Dr. İbrahim ÇELİK, lecturer at the University of Pamukkale Çal Vocational School, for his greatly appreciated statistical analyses to QTL mapping.

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