Identification of a New Race and Development of DNA Markers Associated with Powdery Mildew in Melon

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ABSTRACT Powdery mildew disease caused by an obligatory parasitic fungus *Podosphaera xanthii* is a serious problem of melon (*Cucumis melo* L.) production worldwide. Severity of problem is further associated with emergence of new races over the years. In this study a new race of powdery mildew fungus was discovered from Ibaraki, Japan. The race was different from all other existing races of *P. xanthii* occurring in Japan. Phenotypic and genetic analysis established the new fungus type as a new race, N5. Ten melon lines were infected with a total of eight fungal races including the new N5 race and it was found that all melon lines had different disease reactions against the new race compared to other seven races. Only four melon genotypes were found resistant out of 42 commercial cultivars and lines were tested. Disease reactions of two sets of F₂ populations and one set of backcross population revealed that two separate epistatic gene loci located in two different linkage groups (LG), LG II and LG XII, interact together for the resistant or susceptible reaction of melon lines. A total of six simple sequence repeat (SSR) markers were found polymorphic in melon lines out of 16 tested in response to N5 race. Two different sets of F₂ populations between resistant and susceptible melon lines were assessed with two polymorphic SSR markers located in two different groups, LG II and LG XII. SSR genotyping yielded 78% and 94% expected polymerase chain reaction fragments in favor of resistance or susceptibility of F₂ populations of CM17187×PMR5 and PMR45×PMR5 of melon lines, respectively.

Keywords Powdery mildew, Melon, *Podosphaera xanthii*, Simple sequence repeat markers, Genetic analysis

INTRODUCTION

Powdery mildew is one of the important diseases in melon (*Cucumis melo* L.) that commonly occurs in the cultivated fields. The causal agent of this disease, *Podosphaera xanthii*, is an absolute parasite. Occurrence of this disease was reported in many different countries over the last four decades including Israel (Cohen *et al.* 1984), United States (McCreight *et al.* 1987), France (Epinat *et al.* 1992), Sudan (Mohamed *et al.* 1995) and Japan (Hosoya *et al.* 1999). Powdery mildew resistant line PMR45 was reported for the first time in the United States in 1935, but that was found susceptible against a new powdery mildew fungus occurred later. Thus the fungus occurred later was named as race 2 and another fungus not endemic to PMR45 was named as race 1 (Jagger *et al.*

1938). In 1946, PMR5 and PMR6 with resistance lines against both race 1 and race 2 were bred, but again another new fungus (race 3) occurred against which the PMR5 became susceptible (Pryor *et al.* 1946). It is clearly evident that break-down of resistance by *P. xanthii* against the resistant melon lines is a recurring phenomenon. McCreight (2006) reported a total of 28 different races of *P. xanthii* that causing powdery mildew of melon worldwide.

In Japan, Hosoya *et al.* (1999) classified melon powdery mildew fungi into four different classes namely race 1, N1, N2, and race 5. The authors collected those fungi from Ibaraki region of Japan and inoculated those races into six selected melon lines (Fuyu3, PMR45, WMR29, Edisto47, PI414723, PMR5) (Hosoya *et al.* 1999). Later, Kuzuya *et al.* (2004) reported seven different races of powdery mildew fungi (race 1, 2, 3, 4, 5, 6, 7) tested against eight

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selected melon lines (Fuyu3, PMR45, WMR29, Edisto47, PI414723, PMR5, MR-1, and PI124112). A few new races identified by Kuzuya *et al.* (2004) were different from previously reported races by Hosoya *et al.* (1999). In addition, Izumikawa *et al.* (2008) reported three new races of powdery mildew fungi (race A, S, O) by using a modified testing method initially developed by Bardin *et al.* (1999). The authors collected fungi samples from the cultivated melon fields in 2006.

The existing inoculation test for screening out *P. xanthii* resistance is associated with some unstable disease index due to variability created by various environmental or cultural condition and differential growth status of leaf discs. In addition, phenotyping is generally difficult when the resistance is governed by recessive alleles. In such a condition it is difficult to identify the heterozygous state. Screening of a large number of samples is often required to make a decision.

Since numbers of different races of powdery mildew fungi are available therefore to develop a race-specific resistance it is important to use race-specific markers (Kim *et al.* 2015). Thomas and Webb (1982) reported that *Pm-1* resistant gene of melon against race 1 fungus had dominant inheritance. In their study, powdery mildew resistant line PI78374 was used to develop F₂ populations. Similarly, Pitrat (1991), by using the powdery mildew resistant line WMR29 and PI414723 reported that *Pm-W* gene was resistant against the race 2 and *Pm-X* gene was resistant against race 1. The author also reported that the *Pm-X* gene resistant against the race 1 was inherited as a single dominant factor. Perin *et al.* (2002) obtained 163 recombinant inbred lines (RILs) from a crossing of Védrañtais×PI161375 of melon and the author developed a linkage map after phenotypic characterization and genotypic screening of those lines with 318 AFLP, IMAS, simple sequence repeat (SSR), RFLP markers. According to Perin *et al.* (2002) the resistant genes *Pm-x* (resistant against race 1) and *Pm-w* (resistant against race 2) were in the linkage groups (LG), LG II and LG V, respectively. Fukino *et al.* (2008) developed another linkage map and also analyzed the quantitative trait locus (QTL) with a population of recombinant inbred lines (RIL) between a resistant line AR5 and a susceptible line ‘Earl’s Favourite (Harukei 3)’

for melon powdery mildew resistance. The authors used 167 markers including SSR, SCAR, CAPS markers. The authors reported that melon powdery mildew resistant QTL was located to LG II and LG XII in the linkage maps and the resistant line to powdery mildew race 1 could be selected by using SSR markers (Fukino *et al.* 2008). By analysis of the RILs between AR5 (syn. PMAR5)×Harukei 3 it was found that powdery mildew resistance-related genes of AR5 were located at LG II and LG XII in the QTL map and the identified two genes (*Pm-II*, *Pm-XII*) were probably involved in powdery mildew resistance (Fukino *et al.* 2008, personal communication with Dr. Fukino). In addition, AR5 was bred by several backcrossing with PMR5 and thus, the PMR5 and AR5 considered inheriting the same powdery mildew resistance gene. In the present study we confirmed that, an incidentally found powdery mildew fungus in Ibaraki region of Japan in 2009 was a new race. We then assessed a large number of commercially cultivated melon cultivars and lines against that race to select out resistant lines. Thereafter, we screened two different F₂ populations and one backcross populations obtained by mating between one resistant and one susceptible line. Finally, we screened 16 SSR markers and two polymorphic markers associated with resistant genes present in two independent LGs were used for genetic analysis of F₂ and backcross populations.

MATERIALS AND METHODS

Plant materials

Ten melon lines namely: Fuyu3, PMR45, WMR29, Edisto47, PI414723, PMR5, PI124112, MR-1, ASL5, Ig1 were used for initial screening with newly identified *P. xanthii* race coupled with seven other reported races (Table 1). Later we screened a total of 42 melon germplasm including 19 commercial melon cultivars and some other melon inbred lines developed by the Plant Biotechnology Institute of Ibaraki Agricultural Center, Okayama University, United States Department of Agriculture and Institute National de la Recherche Agronomique, France (Table 2). We developed two different sets of F₂ populations and one backcross generation from the three different mating

Table 1. Response of 10 melon cultigens to 7 reported races and a new race N5 of *Podosphaera xanthii*.

Cultigen	Race							New
	1	N1	N2	5	A	S	O	N5
Fuyu3	S	S	S	S	S	S	S	S
PMR45	R	R	S	S	R	R	S	S
WMR29	R	R	R	S	R	R	S	S
Edisto47	R	R	R	S	R	R	S	S
PI1414723	R	S	S	R	R	R	S	I
PMR5	R	R	R	R	R	R	S	R
PI124112	R	R	R	R	R	R	S	R
MR-1	R	R	R	R	R	R	I	I
ASL5	R	R	R	S	S	S	S	S
Ig1	R	R	R	R	R	S	R	S

R: resistant, S: susceptible, I: Intermediate.

Table 2. Resistance and susceptibility of melon cultivars collected from different sources against the race N5 of *Podosphaera xanthii*.

Race N5	Cultigen	Seed source ^{z)}
Susceptible	Andes, Andes 3, Andes 5, Otome, Takami, Leina, Amus, Yuka, Quincy, Lenon, Rupia red, Mikoto, Festa, Earl's Arena, Sonata Syunjyu, Yellow king, Homerun star, Ginsen	Seed company, Japan
	P1, P2, P11, P14, P15, P18, P21, P22, P23, P25, P29, P32, L12	PBI
	PMR45, Edisto47, WMR29, Ig1, Im5, Im9, It2	USDA, INRA, Okayama University
	Resistant	Mirano natsu I
P3		PBI
PMR5, PI124112		USDA

²⁾PBI: Plant Biotechnology Institute, Ibaraki Agriculture Institute, USDA: United States Department of Agriculture, USA, INRA: Institut National de la Recherche Agronomique, France.

combinations. The first set of F₂ was obtained from a cross between CM17187 and PMR5, the second set of F₂ was obtained from a cross between PMR45 and PMR5 and the BC₁F₁ generation was obtained from a mating of PMR45×(PMR45×PMR5). The first and second set of F₂ populations had 50 and 49 genotypes respectively, while the BC₁F₁ generation had 94 genotypes.

Pathogen materials

A new race of *P. xanthii* causing powdery mildew disease against the resistant inbred line It2 was identified in Ibaraki region in Japan in 2009 (data unpublished). Ten melon lines were initially tested with seven reported races coupled with the newly reported race (Table 1). The new race was designated as N5. All later pathogenicity tests were conducted against the new N5 race.

Fig. 1. Interaction of ten melon cultigens to race N5 in the leaf disc test 2 weeks after inoculation. Conidia growth was observed in susceptible cultigens indicated red circles. Cultigens 1 to 10 are respectively Fuyu3, PMR45, WMR29, Edisto47, PI414723, PMR5, PI124112, MR-1, ASL5, and Ig1.

Inoculation methods

For the pathogenicity test, the leaf discs (2×2 cm) of melon lines were inoculated with the fungal spores on the upper surface of the leaves by a tree stick as a modified method of Bardin *et al.* (1999) (Fig. 1). The resistant, susceptible and intermediate genotypes were selected based on the severity of the spread of fungal spores on the leaf discs at two weeks after inoculation. Genotypes were scored resistant, intermediate and susceptible when infected area of leaf discs were 0%-10%, 11%-30%, and 31%-100%, respectively.

Selection of resistant lines and pathogenicity test of breeding populations

To find out powdery mildew resistant genetic resources

against the new fungal race, we inoculated leaf discs of a total of 42 melon germplasm including 19 commercial melon cultivars and some other melon inbred lines collected from three different sources (Table 2). The genotypes of F₂ and backcross populations obtained from the selected parental combinations were also screened against the N5 race.

Genetic analysis of resistance to melon powdery mildew new race

Kim *et al.* (2015) published the resistant genes present in different races of powdery mildew fungus along with their position in three different groups, LG II, LG V, and LG XII. Combining our screening results with the location of resistant genes in relevant LGs (Kim *et al.* 2015), we developed genotypic configuration of different melon lines

Table 3. Resistant and susceptible interaction of genes located in linkage groups (LG) II and XII for parents and their F₁ lines of melon against N5 fungal race.

Line	Resistant genes and locus of linkage map		Response of race N5 ²⁾
	LG II	LG XII	
Fuyu3	<i>pm-II/pm-II</i>	<i>pm-XII/pm-XII</i>	S
CM17187	<i>pm-II/pm-II</i>	<i>pm-XII/pm-XII</i>	S
PMR45	<i>Pm-II/Pm-II</i>	<i>pm-XII/pm-XII</i>	S
PMR5	<i>Pm-II/Pm-II</i>	<i>Pm-XII/Pm-XII</i>	R
RIL31	<i>pm-II/pm-II</i>	<i>Pm-XII/Pm-XII</i>	R
F ₁ line (Fuyu 3×RIL31)	<i>pm-II/pm-II</i>	<i>Pm-XII/pm-XII</i>	S
F ₁ line (CM17187×PMR5)	<i>Pm-II/pm-II</i>	<i>Pm-XII/pm-XII</i>	I
F ₁ line (PMR45×PMR5)	<i>Pm-II/Pm-II</i>	<i>Pm-XII/pm-XII</i>	R
F ₁ line (PMR5×RIL31)	<i>Pm-II/pm-II</i>	<i>Pm-XII/Pm-XII</i>	R

This interaction table has been developed comparing our screening results with mapping results of Fukino *et al.* (2008) and distribution information of resistant genes in different linkage groups (Kim *et al.* 2015).

²⁾R: resistant, S: susceptible, I: intermediate.

Table 4. Resistant, intermediate and susceptible (3 classes) response of 50 F₂ lines (CM17187×PMR5), 49 F₂ lines (PMR45×PMR5), and 94 BC₁F₁ lines (PMR45×[PMR45×PMR5]) against the race N5 of *Podosphaera xanthii* and the goodness of fit of 3 classes with expected ratios after χ^2 test.

F ₂ population	Response to race N5 resistant ²⁾			χ^2 test ³⁾		
	Resistant	Intermediate	Susceptible	χ^2	DF	P-value
F ₂ lines (CM17187×PMR5)	18	12	20	0.13	2	0.93
F ₂ lines (PMR45×PMR5)	35	0	14	0.33	1	0.56
BC ₁ F ₁ lines (PMR45×[PMR45×PMR5])	46	0	48	0.04	1	0.83

²⁾The number showed phenotype by inoculation of powdery mildew race N5.

³⁾The data shows the results of χ^2 test according to segregation ratio of resistance:intermediate:susceptibility=3:2:3 for F₂ lines of CM17187×PMR5, resistance:susceptibility=3:1 for F₂ lines of PMR45×PMR5, resistance:susceptibility=1:1 for BC₁F₁ lines of PMR45×(PMR45×PMR5).

corresponding with their disease reaction against the N5 race (Table 3).

For the genetic analysis of resistance against the new N5 race, F_1 of CM17187 (S)×PMR5 (R) and PMR45 (S)×PMR5 (R), their F_2 populations and BC_1F_1 lines of (PMR45×[PMR45×PMR5]) were inoculated and examined for the phenotypic resistance and the segregation ratio (Table 3). Chi-square test for goodness of fit with the expected ratio was conducted for each F_2 and BC_1F_1 generations (Table 4).

SSR marker analysis

Polymerase chain reaction (PCR) amplification of susceptible line CM17187, PMR45, and resistant line PMR5 against powdery mildew race N5 were initially performed by using 16 SSR markers that have been published by Fukino *et al.* (2008) and Izumikawa *et al.* (2009) but among them six SSR markers were found polymorphic (Table 5). The amplified products were analyzed with fluorescent labels following the method of Yamamoto *et al.* (2007) and then detected by an ABI3130xl sequencer. The fragments size of polymorphic SSR markers between susceptible and resistant lines were determined (Table 5). In addition, by using the two selected SSR markers (CMN01-38 associated with LG XII and CMBR120 associated with LG II; Fukino *et al.* 2008), the F_2 populations of CM17187×PMR5 and the F_2 populations of PMR45×PMR5 were investigated to detect the pattern of polymorphism present therein. We then selected the SSR markers linked to N5 race by comparing SSR marker genotypes with corresponding phenotypes for resistance/susceptibility in response to race N5.

RESULTS

Screening melon lines against *P. xanthii*

We performed an inoculation test with a total of eight different races of *P. xanthii* including the collected new fungus race N5. Most of the melon lines were resistant against the previously reported six different races: race 1, N1, N2, 5, A, and S (Table 1). However, against the race O only one line, Ig1, was found resistant (Table 1). Against the new race N5 the following six lines Fuyu3, PMR45, WMR29, Edisto47, ASL5, Ig1 were susceptible (Fig. 1, Table 1). Only two lines PMR5 and PI124112 had the resistant reactions against the new race (Fig. 1, Table 1). Two other lines MR-1 and PI414723 showed intermediate type of disease reactions in response to the N5 race (Fig. 1, Table 1).

Screening melon commercial cultivars and lines against new N5 race

In response to new N5 race, out of 42 melon genotypes (commercial cultivars and lines) only four genotypes (a commercial cultivar 'Mirano natsu I' and three bred line P3, PMR5, and PI124112) were found resistant (Table 2). All other 38 lines had susceptible disease reaction against this fungal race (Table 2).

Genetic analysis of resistance to melon powdery mildew (*P. xanthii*) race N5

The melon line CM17187 that was used in breeding program had susceptibility reaction against four reported races (race 5, A, S, and O) (data not shown) and also against the new N5 race (Fig. 2). We, therefore, used CM17187 in the genetic analysis as it was presumed that this line did not

Table 5. Polymorphism of PCR fragments obtained by using 6 SSR marker located in LG XII in susceptible lines CM17187, PMR45, and resistant line PMR5 of melon.

Line	Marker (bp)					
	CMN22-45	CMN01-38	CMBR150	CMBR14	CMGAN80	CMAGN32
PMR45	269	225	283	175	171	191
CM17187	269	215	279	175	171	191
PMR5	264	219	281	158	183	193

PCR: polymerase chain reaction, SSR: simple sequence repeat, LG: linkage group.

inherit any of the following resistant genes: *Pm-II* and *Pm-XII* similar to PMR5. A further inoculation test by race N5 fungus resulted in susceptible reaction for following three lines: Fuyu3, CM17187 (*pm-II* homo, *pm-XII* homo) and PMR45 (*Pm-II* homo, *pm-XII* homo) but RIL31 (*pm-II* homo, *Pm-XII* homo) and PMR5 (*Pm-II* homo, *Pm-XII* homo) resulted in resistant reaction (Fig. 2, Table 3). F₁ line (*Pm-II* hetero, *Pm-XII* hetero) of CM17187×PMR5 had intermediate type of disease reaction between resistant and susceptible types (Fig. 2). The first set of F₂ populations obtained by selfing of the F₁ line of CM17187×PMR5 were inoculated with race N5 fungus. The disease response of 50

Fig. 2. Resistant (red letters), susceptible (white letters), and intermediate (yellow letters) disease reactions of melon lines and their F₁s against the race N5 of *Podosphaera xanthii*.

F₂ populations against the race N5 fungus separated them into resistant:intermediate:susceptible=18:12:20≐3:2:3 ratios for a χ^2 test (Table 4). The frequency of the three different phenotypes fitted well with the presumed segregation ratio 3:2:3 but not with 1:2:1 (Table 4).

F₁ line (*pm-II* homo, *Pm-XII* hetero) of Fuyu 3×RIL31 showed susceptibility, F₁ line (*Pm-II* homo, *Pm-XII* hetero) of PMR45×PMR5 showed resistance and F₁ line (*Pm-II* hetero, *Pm-XII* homo) of PMR5×RIL31 showed strong resistance against the race N5 (Fig. 2, Table 3). The second set of F₂ populations having 49 lines obtained from the selfing of F₁ (*Pm-II* homo, *Pm-XII* hetero) of PMR45×PMR5 were separated into resistant:susceptible=34:14≐3:1 ratio against race N5 fungus for a χ^2 test (Table 4). The frequency of the two different phenotypes fitted well with the expected segregation ratio 3:1 (Table 4).

In addition, 94 BC₁F₁ populations (*Pm-II* homo, *Pm-XII* hetero or without) of PMR45×(PMR45×PMR5) were separated into resistant:susceptible=46:48≐1:1 for a χ^2 test (Table 4). The frequency of the two different phenotypes fitted well with the segregation ratio 1:1 (Table 4).

Selection of DNA markers for the powdery mildew resistant gene of race N5 in melon

We tested 16 SSR markers for their polymorphism for *Pm-XII* gene located in LG XII in response to race N5. PCR products obtained by six markers were polymorphic between the susceptible lines CM17187, PMR45 and resistant line PMR5 of melon (Table 5). SSR genotyping revealed that melon line was resistant because of the presence of *Pm-XII* homo gene in LG XII irrespective of the status of another locus located in LG II (Table 3, Table

Table 6. Distribution of 50 F₂ population (CM17187×PMR5) to three different categories (resistant homo, hetero, and susceptible homo against N5 fungal race) according to SSR genotyping by two markers.

SSR marker	Marker type	CMBR120 (LG II)		
		Resistant homo (196 bp)	Hetero (184/196 bp)	Susceptible homo (184 bp)
CMN01-38 (LG XII)	Resistant homo (219 bp)	Resistant (2/2) ²⁾	Resistant (4/4)	Resistant (3/3)
	Hetero (215/219 bp)	Resistant (7/7)	Intermediate (12/18)	Susceptible (4/5)
	Susceptible homo (215 bp)	Susceptible (1/1)	Susceptible (3/6)	Susceptible (3/4)

²⁾The number of the matched line of genotype with phenotype for race N5/the number of genotyping line.
SSR: simple sequence repeat, LG: linkage group.

Table 7. Distribution of 49 F₂ population (PMR45×PMR5) to three different categories (resistant homo, hetero, and susceptible homo against N5 fungal race) according to SSR genotyping by two markers.

Marker	Marker type	CMBR120 (LG II)		
		Resistant homo (196 bp)	Hetero	Susceptible homo
CMN01-38 (LG XII)	Resistant homo (219 bp)	Resistant (11/11) ^{z)}	- ^{y)}	-
	Hetero (219/225 bp)	Resistant (24/27)	-	-
	Susceptible homo (225 bp)	Susceptible (11/11)	-	-

^{z)}The number of the matched line of genotype with phenotype for race N5/the number of genotyping line.

^{y)}- sign indicates absence of heterozygous or homozygous susceptible allele. The two parents are homozygous for resistant allele (located in LG II) and therefore F₂ populations of PMR45×PMR5 were neither heterozygous nor homozygous susceptible.

LG: linkage group.

6). SSR genotyping further revealed that *Pm-II* homo gene present in LG II was required for the resistance reaction against the race N5, when in another LG (LG XII) was hetero *Pm-XII/pm-XII* (Table 3, Table 6). When *Pm-II/pm-II* hetero gene was present in LG II then another gene present in LG XII was in homozygous dominant for a resistant reaction (Table 3, Table 6). Thus the two SSR markers associated with resistance against the race N5 were selected from LG II (CMBR120) and LG XII (CMN01-38) (Table 6). The F₂ populations of CM17187×PMR5 (consists of 50 offspring having 18 resistant lines, 12 intermediate lines, and 20 susceptible lines) and PMR45×PMR5 (consists of 35 resistance lines and 14 susceptible lines) were assessed with two selected SSR markers CMN01-38 (linked with LG XII) and CMBR120 (linked with LG II) (Table 6, 7). The SSR genotypes of two markers, CMBR120 and CMN01-38, matched highly with resistant or susceptible phenotypes against the race N5 fungus. Out 50 F₂ populations of CM17187×PMR5, 78% SSR genotypes matched with expected phenotypes (either resistant or susceptible types) and out of 49 F₂ populations of PMR45×PMR5 94% SSR genotypes matched with expected phenotypes (Table 6, 7).

DISCUSSION

Background of this study lay on an incidental observation in the cultivated melon fields of Ibaraki region of

Japan during summer season of 2009. In that season, powdery mildew fungi were collected in Ibaraki prefecture of Japan from the cultivated fields of melon. The inbred line of melon It2 was screened against seven races (race 1, N1, N2, 5, A, S, O) of *P. xanthii* and was found to be resistant against all seven races (data unpublished). But that inbred line we found susceptible against another race (that was the focus of this study) which was virulent to that resistant line It2. Afterwards, the new powdery mildew fungus virulent to It2 inbred line was inoculated to leaf disc of the selected melon lines. We then screened a collection of commercial melon cultivars and lines to select out resistant (R) and susceptible (S) genotypes. Later we produced several F₁, F₂ and back-cross populations for genetic analysis and used molecular markers to analyze their resistance response against the new fungal race.

Confirmation of new fungal race

The response of 10 melon lines against the new fungus race N5 was different from previously reported seven races by Izumikawa *et al.* (2008). The results thus confirmed that the N5 race was a new fungal race of *P. xanthii*. Disease response of 10 lines against the N5 race had partial similarity with race 5 although the line Ig1 had a resistance to the race 5, but it showed susceptibility to the new race. Thus, it could be speculated that the new 'race N5' is a mutant of the race 5 (Table 1). Two lines resistant against the N5 race, PMR5, and PI124112, were used in genetic analysis. We recommend that these two lines could be used

for transferring resistance to other commercially important melon lines using them as a parent in any future breeding program. Also these two lines could be used for marker development and linkage map development.

Epistatic gene interaction is present

SSR genotyping revealed that interaction between genes located in two different LGs was required for resistant reactions of the melon lines against the N5 race (Table 6, 7). For the F₂ populations of CM17187×PMR5, a resistant phenotype was observed when gene present in LG XII was in homozygous dominant state (Table 4). When gene present in LG XII was heterozygous then another gene present in LG II was required to be in homozygous dominant state for their resistant reactions (Table 4). When the genes in two different LGs were in heterozygous state an 'intermediate type' disease response was observed (Table 6). Absence of dominant gene allele in LG XII always resulted in susceptibility irrespective of the status of the gene present in LG II (Table 6). Absence of any dominant gene allele in LG II group in presence of one dominate gene allele in LG XII also resulted in susceptible disease reaction (Table 6). Since expression of the gene located in locus LG II and LG XII were dependent on each other therefore it could be commented that an epistatic gene interaction was present in favor of the powdery mildew resistance in melon. In the second set of F₂ populations of PMR45×PMR5 susceptible disease reactions was observed when gene present in LG XII was in homozygous recessive state in presence of homozygous dominant status of another locus present in LG II (Table 7). The results indicated that at least one dominant allele is required in LG XII in presence of homozygous dominant status of LG II locus (Table 7). In a recent study, differential expression of 14 *MLO* genes have been reported in the same melon line in response to seven different fungal races of *P. xanthii* (Howlader *et al.* 2016) which suggested possible existence of inter-genic gene interaction.

SSR genotyping confirmed phenotypic ratios

The two selected SSR markers (CMBR120, CMN01-38) which were able to separate the resistant and susceptible genotypes against N5 race were located in LG II and LG

XII. For different other races, resistant QTL were reported to be located in LG II, LG V, and LG XII (Fukino *et al.* 2008; Izumikawa *et al.* 2009; Kim *et al.* 2015). Comparing our results with that of previous studies it can be commented that the resistant genes for powdery mildew race N5 were located in the same or near the other resistant genes of powdery mildew against other different races, for example: *Pm-XII* of AR5 and *Pm-y* of VA435 were located in LG XII (Pitrat 1991; Fukino *et al.* 2008). By using the SSR markers, the resistant lines against the powdery mildew N5 race were selected in high accuracy between 78% and 94% from the F₂ populations (Table 6, 7). Furthermore, these markers could be used for selecting resistant line and cultivars with unknown disease response against the race N5.

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