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ORIGINAL PAPER

Codominant PCR-based markers and candidate genes for powdery mildew resistance in melon (*Cucumis melo* L.)

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Abstract Powdery mildew caused by *Podosphaera xanthii* is a major disease in melon crops, and races 1, 2, and 5 of this fungus are those that occur most frequently in southern Europe. The genotype TGR-1551 bears a dominant gene that provides resistance to these three races of *P. xanthii*. By combining bulked segregant analysis and amplified fragment length polymorphisms (AFLP), we identified eight markers linked to this dominant gene. Cloning and sequencing of the selected AFLP fragments allowed the development of six codominant PCR-based markers which mapped on the linkage group (LG) V. Sequence analysis of these markers led to the identification of two resistance-like genes, MRGH5 and MRGH63, belonging to the nucleotide binding site (NBS)-leucine-rich repeat (LRR) gene family. Quantitative trait loci (QTL) analysis detected two QTLs, *Pm-R1-2* and *Pm-R5*, the former significantly associated with the resistance to races 1 and 2 (LOD score of 26.5 and 33.3; 53.6 and 61.9% of phenotypic variation, respectively), and the latter with resistance to race 5 (LOD score of 36.8; 65.5% of phenotypic variation), which have been found to be colocalized with the MRGH5 and MRGH63 genes, respectively. The results suggest that the cluster of NBS-LRR genes identified in LG V harbours candidate genes for resistance to races 1, 2, and 5 of *P. xanthii*. The evaluation of other resistant germplasm showed that the codominant markers here reported are also linked to the *Pm-w* resistance gene carried by the accession 'WMR-29' proving their usefulness as genotyping tools in melon breeding programmes.

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Introduction

Powdery mildew of melon (*Cucumis melo* L.) caused by *Podosphaera xanthii* (Castagne) U. Braun & N. Shishkoff (Shishkoff 2000) is a major disease in many areas of the world since it occurs during all seasons irrespective of the crop system (Sitterly 1978). Melon growers still rely on chemical treatments to control powdery mildew. However, the use of fungicides over many decades has resulted in the development of *P. xanthii* resistance to many chemical compounds, which have therefore lost their efficacy (Hollomon and Wheeler 2002). Moreover, the increasing concern for public health has motivated growers to seek alternative strategies to control plant diseases. The development of new resistant cultivars appears to be the most eco-compatible method of disease control (De Giovanni et al. 2004), hence the importance of characterizing new resistance sources. The melon genotype TGR-1551 was found to be resistant to races, 1, 2, and 5, of *P. xanthii* (Gómez-Guillamón et al. 1995, 1998) which occur most frequently in the melon crops of southern Europe (Bertrand 1991; Bardin et al. 1997; Del Pino et al. 2002). Interestingly, the resistance to powdery mildew described in TGR-1551 is conferred by a single genetic system which involves two independent genes, a dominant one and a recessive one (Yuste-Lisbona et al. 2010b). Therefore, TGR-1551 not only constitutes an

important resistance source for melon breeding but also a suitable genotype to study the genetic and molecular mechanisms underlying resistance to plant pathogens. Nevertheless, several factors limit studies on the genetic control of powdery mildew resistance, among them, the genetic variability in pathogen populations, the lack of uniformity in experimental assays, and the influence of environmental conditions on the expression of resistance (Cohen et al. 2002, 2004). Moreover, artificial inoculation methods used to assess powdery mildew resistance in melon are laborious and time-consuming, which hamper the development of new commercial varieties.

For breeding purposes, DNA markers tightly linked to a given resistance gene are useful tools to avoid the above-mentioned drawbacks as they provide significant advantages over traditional phenotypic screening. Marker-assisted selection (MAS) is a rapid, relatively inexpensive method, and it is not hampered by pathogen unavailability. In addition, there are no environmental limitations since MAS can be performed off-season, and it permits the simultaneous screening for several diseases. Bulk segregant analysis (BSA) (Michelmore et al. 1991) is an efficient method for identifying molecular markers linked to a specific gene using DNA bulks from segregating populations. The combination of BSA and highly polymorphic markers, such as amplified fragment length polymorphism (AFLP) markers, permits the identification of useful molecular markers for breeding purposes. However, AFLP markers are dominant, relatively costly, laborious, and unsuitable for routine screening. Hence, AFLP markers need to be converted into single locus PCR-based markers before they can be easily employed in breeding programmes. This strategy has been successfully used in many different plant species, particularly in melon, to develop codominant markers linked to important agronomic traits (Wang et al. 2000; Noguera et al. 2005).

At present, several genes which confer resistance to powdery mildew have been mapped in segregating populations generated from resistant other than TGR-1551, i.e. *Pm-x* (linkage group (LG) II; Périn et al. 2002), *Pm-y* (LG XII; Périn et al. 2002), *Pm-w* (LG V; Pitrat 1991; Périn et al. 2002), and *Pm-1* (LG IX; Teixeira et al. 2008). Similarly, two quantitative trait loci (QTL), named *PmV.1* and *PmXII.1*, have been detected on LGs V and XII, respectively (Perchepied et al. 2005), and more recently Fukino et al. (2008) have mapped two QTLs on LGs II and XII from the 'AR 5' breeding line. Genetic mapping of these genes and QTLs indicated that several different genomic regions seem to be involved in the resistance to powdery mildew in melon. Besides, only a few molecular markers have been identified as linked to this trait (Fukino et al. 2008; Teixeira et al. 2008), and they correspond to those genes and QTLs located in LGs II, IX and XII. None of these reported markers mapped in LG V, where the dominant powdery mildew resistance gene carried by TGR-1551 has previously been localized (Yuste-Lisbona et al. 2010a), and therefore, they cannot be used as selection markers in plant material developed from this resistant genotype. In the present paper, we report the identification of AFLP markers linked to the TGR-1551 dominant powdery mildew resistance gene, as well as their conversion into codominant PCR-based markers. A linkage analysis performed by including these codominant markers together with several sets of simple sequence repeats (SSR) and single nucleotide polymorphism (SNP) markers has led to the mapping of two new QTLs significantly associated with the resistance to powdery mildew in melon. Interestingly, a bioinformatic sequence analysis of such codominant markers has allowed us to identify two candidate genes involved in the resistance to this fungal pathogen, which open new opportunities for both their cloning and introgression in newly developed melon varieties.

Materials and methods

Plant material and powdery mildew resistance test

Fifteen plants each of the resistant TGR-1551 genotype, the susceptible Spanish cultivar 'Bola de Oro', and their F_1 progeny, as well as 159 plants of their F_2 generation, were simultaneously inoculated on the second true leaf with race 1 (isolate 27), race 2 (isolate P-15.0), and race 5 (isolate C8) of powdery mildew (*P. xanthii*) following the methodology described by Yuste-Lisbona et al. (2010b). The race of the different fungal isolates was previously determined by analysing the reactions of a set of differential melon genotypes as described by Bardin et al. (1999). Artificial inoculations were carried out in a controlled growth chamber at 32°C day/22°C night with a 16:8 h (light:dark) photoperiod. Twelve days after inoculation, plants were scored according to the level of fungal sporulation using a scale of 1–4: class 1, no visible sporulation; class 2, low level of sporulation; class 3, moderate level of sporulation; and class 4, profuse sporulation (Fig. 1). Phenotypic classes 1 and 2 were considered to show a resistant response because the disease did not progress, whereas plants included in classes 3 and 4 showed evident symptoms of infection proving the progression of the disease; hence, the affected plants were considered as susceptible. A χ^2 test was performed to check the goodness-of-fit of resistance to powdery mildew.

In order to evaluate the utility of the markers developed, a new F_2 melon population was employed for powdery mildew inoculation and molecular genotyping. The melon genotype 'WMR 29' was selected as resistant parent since

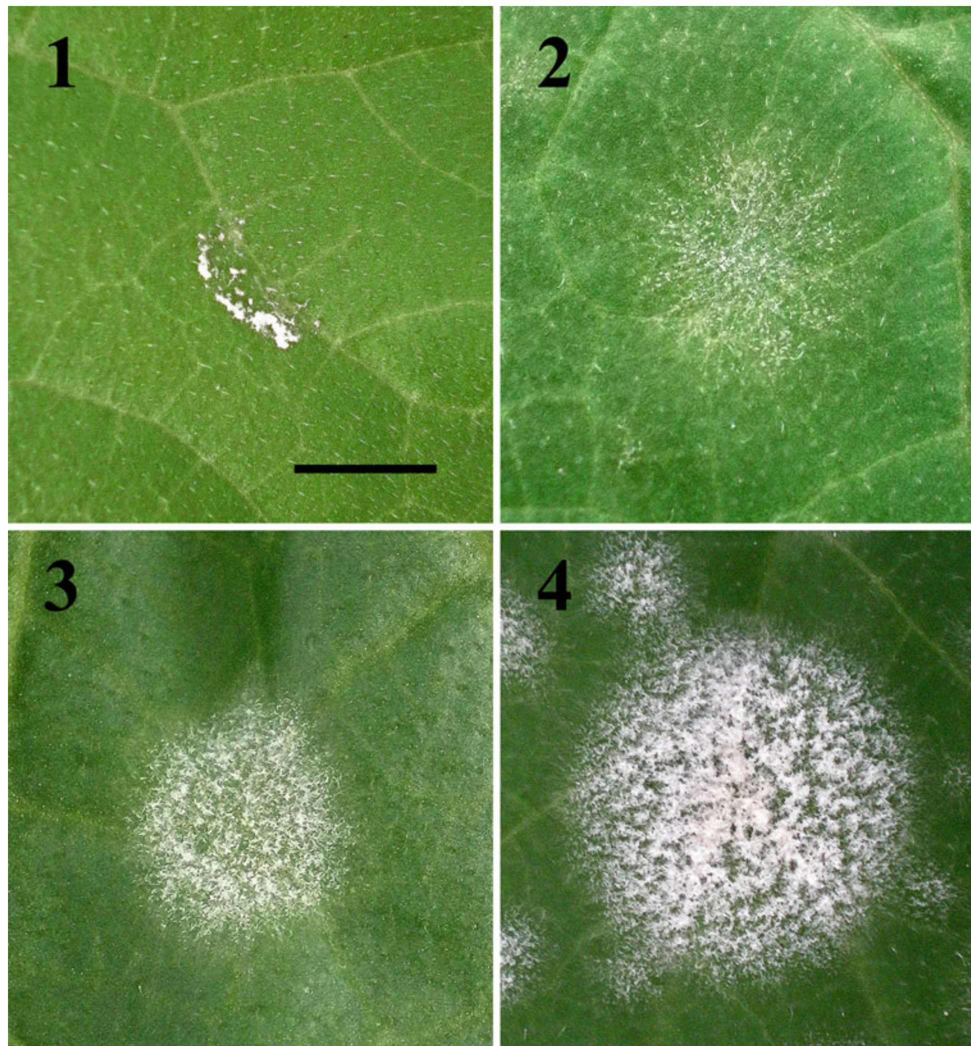

Fig. 1 Phenotypic classes established according to the sporulation level of *P. xanthii* detected on melon leaves 12 days after artificial inoculation. Class *1*, no visible sporulation; class *2*, low sporulation; class *3*, moderate sporulation; and class *4*, profuse sporulation. In classes *1* and *2*, the infection did not progress (resistant plants), whereas a significant fungal infection was detected in classes *3* and *4* (susceptible plants). *Scale bar* 1 cm

it carries the *Pm-w* gene involved in the resistance to powdery mildew race 2 (Pitrat 1991), which in turn had previously been located by Périn et al. (2002) on the LG V, as linked to *Virus aphid transmission* (*Vat*) gene. Hence, a population of 144 F_2 plants obtained by crossing 'WMR 29' (resistant parent) and 'Rochet' (susceptible parent) was inoculated with race 2 (isolate P-15.0) of powdery mildew. Phenotypic characterization of the parental lines, F_1, and F_2 progenies was performed following the methodology described above.

BSA approach to identify AFLP markers linked to resistance

Genomic DNA was isolated from young leaves as described by Doyle and Doyle (1990). Total DNA was stored in sterile water, visualized after electrophoresis in 1% agarose gels in 1× TBE (Tris–borate–EDTA), and quantified by comparison with DNA standards (Lambda phage DNA digested with *Hin*dIII, Invitrogen Life Technologies). For BSA, eight DNA bulks were generated from the F_2 population composed of five plants each, four bulks comprising resistant plants and four including susceptible plants.

The AFLP analysis was carried out according to the procedure described by Vos et al. (1995), with some modifications (Noguera et al. 2005). A total of 500 ng of genomic DNA was digested with 5 U of *Mse*I (New England Biolabs) and *Eco*RI (Roche) enzymes for 2 h at 37°C in a final volume of 40 µl. The DNA fragments were ligated to appropriate adapters via addition of 1 U of T4-DNA Ligase (Roche) and incubated for 1 h at 37°C. The pre-amplification reactions were performed in a

volume of 20 μl using A as the selective nucleotide for the Eco primer (Eco + A) and three different Mse primers (Mse + C, Mse + G, and Mse + T). The PCR cycling parameters were 20 cycles at 94°C for 30 s, 56°C for 60 s, and 72°C for 60 s. Subsequent PCR amplifications were performed with primers that included three selective bases in their sequences, the Eco primer being radioactively labelled with γ[33P]-ATP (3,000 Ci/mmol). For the selective amplifications, the following PCR cycling parameters were used: an initial cycle at 94°C for 30 s, 65°C for 30 s, and 72°C for 60 s. During the next 12 cycles, the annealing temperature was lowered by 0.7°C per cycle. The temperature conditions for the next 23 cycles were 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s. AFLP products were separated in 6% denaturing polyacrylamide gels using Sequi-Gen Cell sequencing equipment (Bio-Rad). Electrophoresis was carried out for 2 h in 1× TBE. The gels were then lifted onto a Whatman paper sheet, dried for 2 h at 80°C, and exposed to radiographic Kodak film for 3 days at room temperature. All amplification reactions were performed in a GeneAmp PCR System 9600 (Perkin Elmer) thermal cycler.

DNA from the two parental lines, F_1 progeny, and the eight bulks mentioned above were simultaneously screened with 106 AFLP primer combinations. AFLP markers were named according to the standard list for AFLP primer nomenclature (Keygene, http://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html). The primer combinations that generated polymorphic bands between the bulks were tested on the bulked individuals to eliminate false-positive markers.

Conversion of AFLPs into PCR-based markers and sequence analysis

The F_2 population was genotyped with those AFLP markers which had revealed polymorphisms between all resistant and susceptible bulks. This allowed the estimation of the recombination frequencies between each marker and powdery mildew resistance (Allard 1956) and, therefore, the identification of the most linked markers for their conversion into codominant markers.

Isolation and cloning of the AFLP fragments identified were carried out as described by Noguera et al. (2005). Target AFLP bands on the autoradiograph were matched to the corresponding area in the gel, and the appropriate AFLP fragments were excised from the dried gel. Those fragments were then purified according to Qu et al. (1998), and the isolated DNA was re-amplified by PCR using non-labelled selective primers. The resulting PCR products were purified by size-exclusion chromatography using the GenElute PCR Clean-up kit (Sigma). The DNA fragments were then cloned into a pGEM-T vector (Promega, USA) and transformed into *Escherichia coli* strain DH5α. The DNA sequences of these fragments were obtained using an ABI PRISM® 3130 XL Genetic Analyser (Applied Biosystems) and used to develop SCAR primers (forward and reverse). Amplification of the SCAR markers was performed in a volume of 30 μl using 40 ng of total DNA, 50 ng of each SCAR primer, 0.25 mM dNTPs, 4 mM $MgCl_2$, and 2 U of *Taq* DNA polymerase (Bioline) in 1× *Taq* buffer. DNA was amplified under the following thermal cycling conditions: 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 52°C for 30 s, and 72°C for 2 min, and a final extension of 5 min at 72°C. PCR products were analysed in 3% agarose gels in SB buffer (10 mM sodium boric acid) and visualized with ethidium bromide. In the cases in which the SCAR products were not polymorphic between parental lines, the fragments were cloned and sequenced in order to detect possible polymorphisms in their sequences and to establish an adequate strategy to develop either cleaved amplified polymorphic sequence (CAPS) (Konieczny and Ausubel 1993) or derived cleaved amplified polymorphic sequence (dCAPS) (Michaels and Amasino 1998; Neff et al. 1998) markers.

Sequence homology searches were carried out by using the Basic Local Alignment Search Tool (BLAST) software through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/).

Linkage and QTL analysis

Based on the segregation data, the codominant PCR-based markers were located in a previously genetic linkage map constructed from the same F_2 population used for BSA approach (Yuste-Lisbona et al. 2010a). Different sets of SSR and SNP markers (Danin-Poleg et al. 2001; Fazio et al. 2002; Ritschel et al. 2004; Gonzalo et al. 2005; Fukino et al. 2007; Fernández-Silva et al. 2008; Deleu et al. 2009) previously described as located in LG V were assayed to saturate this genomic region. In addition, given that TGR-1551 bear the *Vat* gene and the aphid resistance is segregating in our mapping population, several markers described by Dogimont et al. (2004) as linked to this gene were analysed. PCR amplifications were carried out following the indications outlined in the above-mentioned references. JoinMap® 4.0 software (Van Ooijen 2006) was used to generate a new genetic linkage map. Map distances were based on Kosambi's (1944) mapping function, and the genetic map was drawn using the MapChart 2.2 software (Voorrips 2002).

Powdery mildew resistance was mapped as a quantitative trait using classes 1–4 as numerical values. Thus, QTL analyses of *P. xanthii* resistance were performed using interval mapping and multiple QTL model (MQM) mapping with the MapQTL® 5.0 software (Van Ooijen 2004). Once potential QTLs were detected by interval mapping

analysis, markers closely linked to the QTLs were selected as cofactors and tested using the automatic cofactor selection procedure in MapQTL (default *P* value cut off for elimination of a cofactor set of 0.02). Using the set of selected cofactors, MQM mapping analyses were carried out. A permutation test (10,000 cycles) was used to determine the LOD threshold score at which the QTL was deemed to be present in a particular genomic region with a confidence interval of 99%.

Results

Evaluation of powdery mildew resistance

Twelve days after inoculations with races 1, 2, and 5 of *P. xanthii*, melon plants of either the resistance donor parent TGR-1551 (class 1) or the F_1 progeny (class 2) were resistant to powdery mildew, while plants of the parent 'Bola de Oro' showed severe infection symptoms (class 4). Different levels of fungal sporulation were recorded on F_2 plants although each individual plant showed the same susceptible (classes 3 and 4) or resistant (classes 1 and 2) response to the three races of powdery mildew. Thus, the segregation ratio of F_2 plants observed was 130 resistant:29 susceptible, which is consistent with a 13:3 ratio corresponding to the independent segregation of two genes, one dominant gene and one recessive gene ($\chi^2 = 0.008$; $P = 0.929$), which confer resistance to races 1, 2, and 5 of *P. xanthii* in TGR-1551 (Yuste-Lisbona et al. 2010b).

Identification of AFLP markers linked to powdery mildew resistance

Twenty F_2 plants of each extreme phenotype, i.e. class 1 (resistant) and class 4 (susceptible), were selected for bulk construction. A total of 106 AFLP primer combinations were assayed to identify DNA polymorphisms useful for BSA. The results of the amplification allowed over 6,850 AFLP fragments to be scored. Of these, 1,390 (20%) were polymorphic between the parents although only 16 of these fragments were polymorphic between the resistant and susceptible bulks. PCR assays performed in the F_2 population led to the selection of eight AFLP fragments linked to powdery mildew resistance at a genetic distance of less than 10 cM. These were named as E42M51-B6, E42M51-T8, E38M43-B1, E38M43-B2, E38M82-B5, E38M43-T9, E31M66-B1, and E31M66-T3.

Conversion of AFLPs into PCR-based markers

The E42M51 primer combination amplified an AFLP fragment in the susceptible parental line, named E42M51-B6, which was also present in the F_1 progeny and the F_2 susceptible bulk. The same primer combination amplified another AFLP fragment in the resistant parental line, E42M51-T8, which was not detected in the susceptible parent. The E42M51-T8 marker showed a size of 81 bp, and its nucleotide sequence contained a 2 bp deletion when compared to the sequence of E42M51-B6, indicating that both fragments were alleles of the same locus. Two specific PCR primers were designed to recognize the ends of both allelic markers, which allowed us to develop the SCAR marker named PM1-SCAR (Fig. 2).

The PCR amplification products obtained with the primers designed from the sequence of the E38M43-B1 marker revealed a 3-bp indel polymorphism (Fig. 2) between the resistant and susceptible alleles (297 bp in TGR-1551 and 294 bp in 'Bola de Oro', respectively), which avoided distinguishing them by routine electrophoresis. For this reason, the corresponding sequences were analysed in order to detect restriction enzyme recognition sites. A restriction site for the *Psy*I enzyme was identified at position 116 in the PCR fragment amplified from TGR-1551. After digestion, two fragments of 181 and 116 bp were obtained, while the 294-bp fragment amplified in 'Bola de Oro' (susceptible parent) was not digested (Fig. 3). This new CAPS marker was named PM2-CAPS.

From the DNA sequence of the AFLP fragment E38M43-B2, two specific primers were designed for PCR assays, which resulted in the amplification of a monomorphic marker of 220 bp in the parental lines. Both sequences differed in a SNP at position 113 (Fig. 2), which meant a recognition site for the *Tai*I restriction enzyme in the 'Bola de Oro' allele. Once digested, two fragments of 113 and 107 bp were generated, while the fragment amplified for TGR-1551 was not digested (Fig. 3). The CAPS marker thus generated was named PM3-CAPS.

The primers designed according to the sequence of E38M82-B5 marker amplified one fragment of 130 bp in both parents. The two sequences differed in three SNPs at positions 56, 69, and 86, respectively. However, no restriction sites could be identified in these polymorphic sites. For this reason, a new primer was designed by including a T-to-G transversion (position 90) in order to generate a dCAPS marker. The PCR product obtained with the new primer combination was 109 bp long in both parental lines. Due to the SNP present at position 86, a recognition site for the restriction enzyme *Nco*I was generated in the 'Bola de Oro' fragment. After digestion of the PCR products, two fragments of 86 and 23 bp were generated in the susceptible parental line, but not in TGR-1551. This dCAPS marker was termed PM4-dCAPS (Fig. 2).

The PCR fragments amplified with specific primers deduced from the E38M43-T9 AFLP marker were

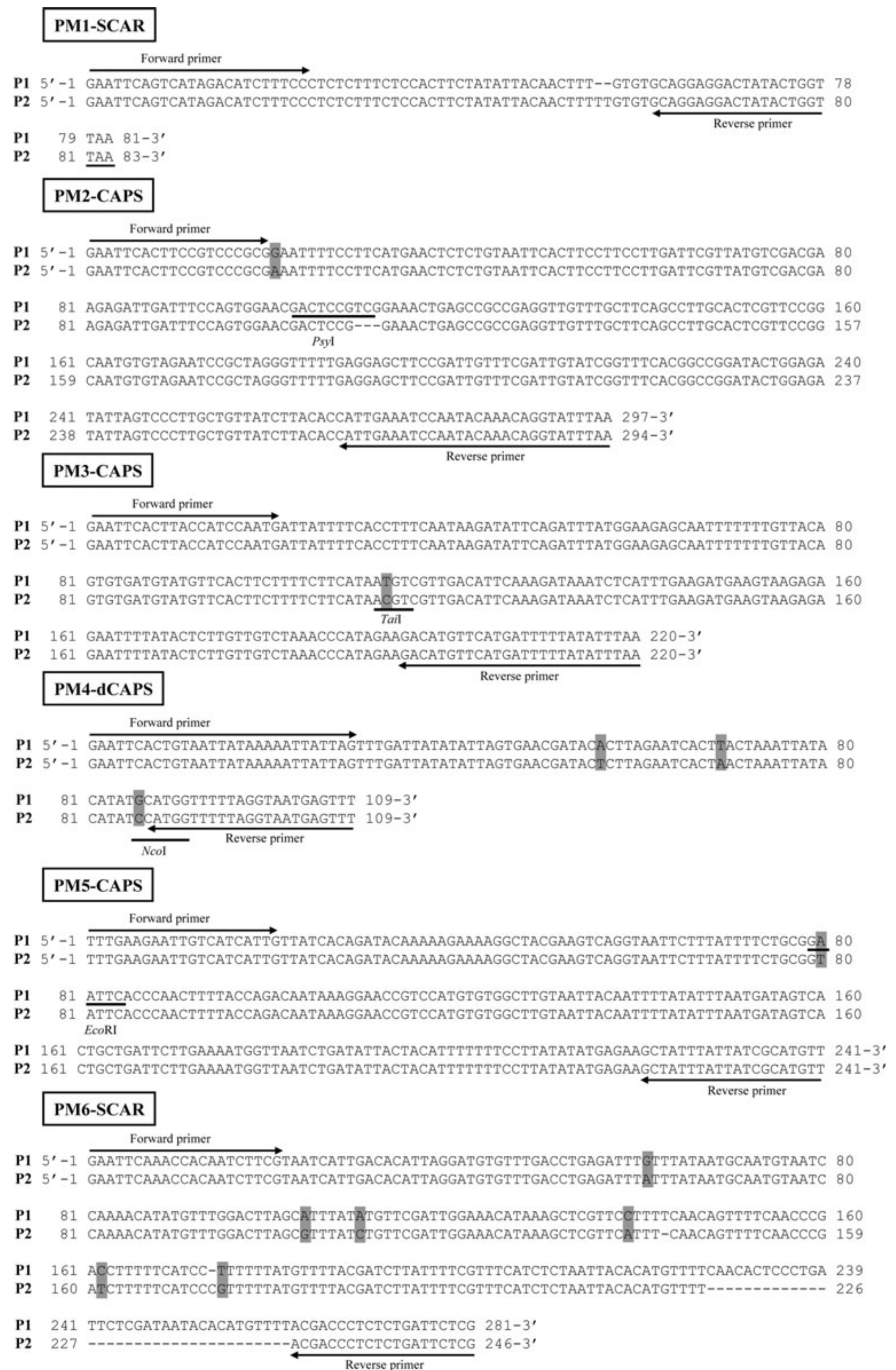

Fig. 2 Alignment of allelic sequences for each codominant marker developed in this work, PM1-SCAR, PM2-CAPS, PM3-CAPS, PM4-dCAPS, PM5-CAPS, and PM6-SCAR. Nucleotide sequences of the forward and reverse primers and the recognition sites for the restriction enzymes are *underlined*. *P1* TGR-1551 (resistant), *P2* 'Bola de Oro' (susceptible)

monomorphic, i.e. they showed 73 bp size in both parents. They were sequenced, but no differences were detected. Therefore, the flanking sequences of the AFLP fragments were rescued using ligation-mediated suppression PCR methods as previously described (Schupp et al. 1999). Based on the sequence information of the cloned flanking region of the AFLP markers, primers were designed for PCR amplification, which in turn generated one fragment of 241 bp in the parental lines. The sequences differed in a single nucleotide polymorphism at position 79. This SNP

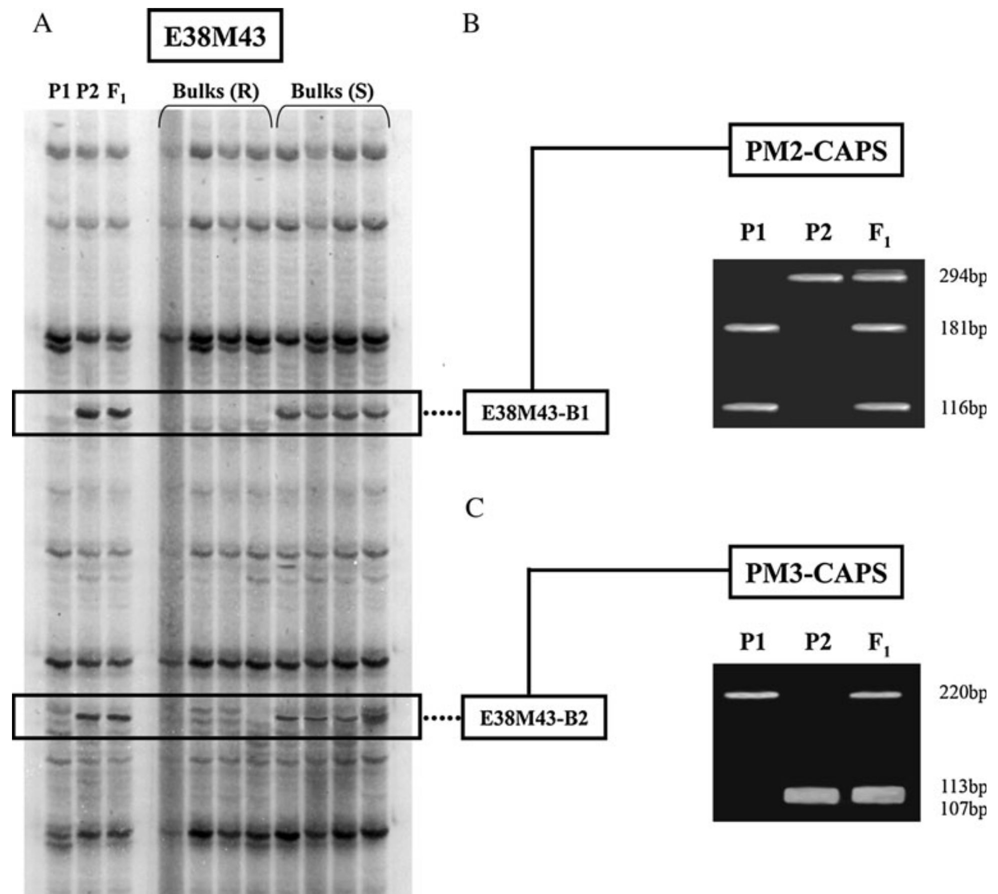

Fig. 3 Identification of molecular markers linked to powdery mildew resistance. **a** BSA approach and PCR fingerprinting obtained by using the E38M43 AFLP combination. Two AFLP fragments (*boxed*) were selected for further conversion into codominant markers. **b** PM2-CAPS generated from the AFLP fragment E38M43-B1. **c** PM3-CAPS developed from the AFLP fragment E38M43-B2. The size of the fragments (bp) is indicated on the *right*. *P1* TGR-1551 (resistant), *P2* 'Bola de Oro' (susceptible), F_1 F_1 progeny, *R* resistant, *S* susceptible

marker included a recognition site for the restriction enzyme *Eco*RI in the TGR-1551 allele which explained the original AFLP marker detected. After digestion, two fragments of 162 and 79 bp were obtained, while the fragment amplified for 'Bola de Oro' was not digested. The CAPS marker developed was called PM5-CAPS (Fig. 2).

The E31M66 primer combination amplified an AFLP fragment in the susceptible parental line, named E31M66-B1, which was also present in the F_1 progeny and the F_2 susceptible bulk. The same primer combination amplified another AFLP fragment in the resistant parental line, called E31M66-T3, also detected in the F_1 progeny and the F_2 resistant bulk. The E31M66-B1 nucleotide sequence contained a 35-bp deletion when compared to the sequence of E31M66-T3. Sequencing of the two AFLP allelic markers allowed us to design two specific PCR primers. The new SCAR marker developed was named PM6-SCAR (Fig. 2).

Sequence analysis of developed markers

DNA sequences of the developed markers (abbreviated as PM markers) were analysed by a BLAST search for the presence of homology in the GenBank database. The sequence of the PM3-CAPS marker showed 99% identity to the *C. melo* Bacterial Artificial Chromosome (BAC) clone PIT92-60K17 (van Leeuwen et al. 2003), between positions 16,198 and 16,417 bp. The sequence of PM4-dCAPS marker had 99% identity to *C. melo* BAC clone 13J4 (Deleu et al. 2007), which is located from 48,954 to 49,062 bp in this BAC. DNA sequences of the remaining PM markers did not reveal significant homologies when compared with the GenBank data base.

Interestingly, the BAC clone PIT92-60K17 (van Leeuwen et al. 2003) contained a resistance gene cluster of the nucleotide binding site (NBS)-leucine-rich repeat (LRR) family, which included the complete genomic sequence of two genes, MRGH5 (position 4,078–8,874 bp) and MRGH63 (24,292–30,897 bp), and a partial genomic sequence of the gene MRGH6 (1–342 bp). The PM3-CAPS marker was located in a non-coding region between MRGH5 and MRGH63 genes. PM3-CAPS was the most tightly linked marker to the dominant powdery mildew resistance gene carried by TGR-1551, which would indicate that both MRGH5 and MRGH63 are candidate genes for resistance to *P. xanthii* in this melon genotype. In addition, we have sequenced intragenic regions of these NBS-LRR resistance genes, and several polymorphisms between both parents were found, which allowed us to

map them in the segregating population derived from TGR-1551.

Linkage and QTL analysis

The dominant powdery mildew resistance gene carried by TGR-1551 has previously been localized in the LG V of the melon genome (Yuste-Lisbona et al. 2010a). Therefore, in order to saturate this genomic region, 21 new SSR markers and 8 SNP markers previously reported in the literature as located in LG V were evaluated in the parental lines and their F_1 progeny. A total of five SSR and two SNP markers were polymorphic in our mapping population. These markers together with the six codominant PCR-based markers developed as well as the markers for the resistance genes MRGH5 and MRGH63 were screened in the F_2 population. The segregation data of all these markers and SSR markers previously reported by Yuste-Lisbona et al. (2010a) were used for the construction of a new LG V (Fig. 4), which mainly included codominant markers.

By using interval mapping, putative QTLs for powdery mildew resistance were detected. The closely linked markers to the QTLs were chosen as cofactors and tested using the automatic cofactor selection procedure in MapQTL. The marker MRGH5 for resistance to races 1 and 2 of powdery mildew and the marker MRGH63 for resistance to race 5 of this fungus were selected and used as cofactors for MQM mapping. With this approach, one QTL was detected for resistance to races 1 and 2 of *P. xanthii*, and it was designated as *Pm-R1-2* and colocalized with the MRGH5 gene. This QTL was supported by a LOD score of 26.5 and 33.3 and explained 53.6 and 61.9% of the phenotypic variance in the mapping population for resistance to powdery mildew races 1 and 2, respectively (Fig. 4; Table 1). The MRGH63 gene colocalized with the QTL *Pm-R5*. This QTL was supported by a LOD score of 36.8 and explained 65.5% of the phenotypic variance observed for resistance to race 5 of *P. xanthii* (Fig. 4; Table 1).

Usefulness of the codominant markers for powdery mildew resistance

The most closely linked markers (PM2-CAPS, PM3-CAPS, and PM4-dCAPS) to the QTLs detected were screened in a new F_2 population generated from the cross between 'WMR 29' (resistant) and 'Rochet' (susceptible) with the aim to check the utility of these codominant markers for breeding purposes. The phenotypic analysis of the F_2 population revealed 100 plants resistant and 44 susceptible to powdery mildew. The segregation ratio observed fits with the expected 3:1 ratio for monogenic dominant inheritance ($\chi^2 = 2.37$; $P = 0.124$). The marker analysis

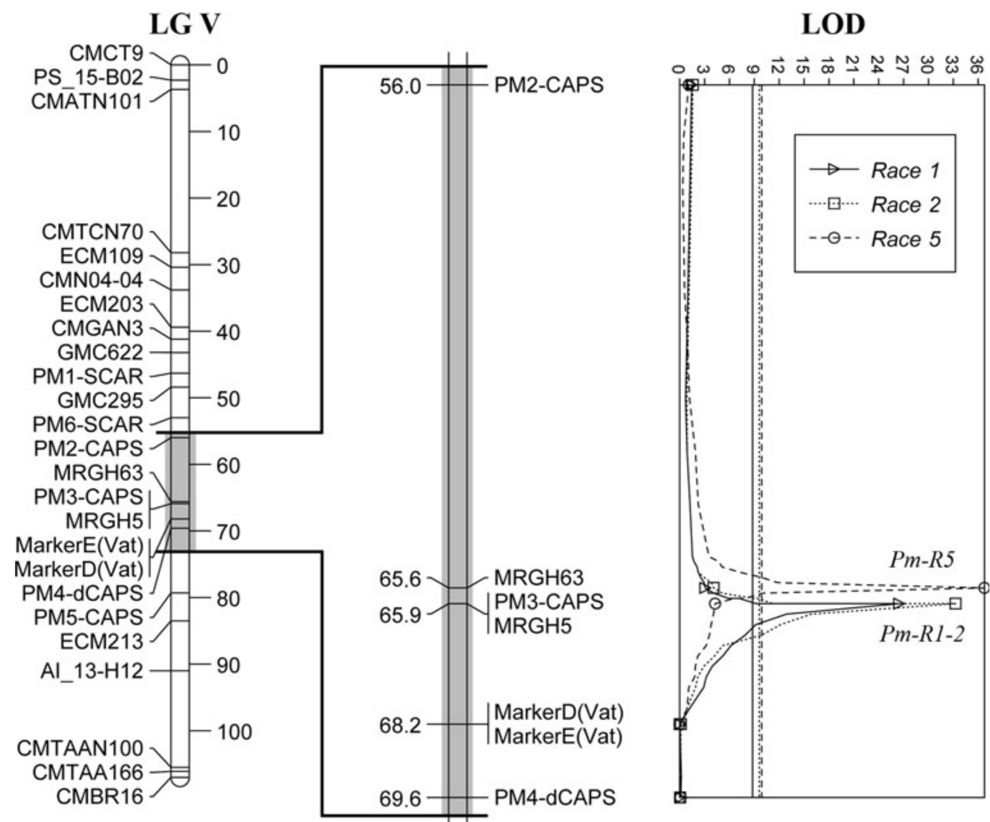

Fig. 4 Linkage group V and QTL-likelihood curves of LOD scores showing the locations of the QTL *Pm-R1-2* for resistance to races 1 and 2 of powdery mildew and the QTL *Pm-R5* for resistance to race 5. The LOD values for permutation test are also presented. Map distances (cM) were estimated using Kosambi's function

Table 1 QTL mapping results for resistance to race 1, 2, and 5 of *P. xanthii* in the F_2 population derived from the cross TGR-1551 × 'Bola de Oro'

Map location[a]	Marker	Powdery mildew QTLs					
		Race 1		Race 2		Race 5	
		LOD	R^2 (%)	LOD	R^2 (%)	LOD	R^2 (%)
56.0	PM2-CAPS	1.44	1.9	1.59	1.7	1.11	2.1
65.6	MRGH63	0.01	0	0.01	0	36.76	65.5
65.9	PM3-CAPS/MRGH5	26.48	53.6	33.28	61.9	0.29	0.3
68.2	MarkerD(Vat)/MarkerE(Vat)	0.02	0	0.14	0.2	0.01	0
69.6	PM4-dCAPS	0.13	0.2	0.06	0.1	0.12	0.1

R^2 proportion of the phenotypic variance explained by the QTL

[a] Position on linkage group V. Expressed in Kosambi cM

results showed that the resistant genotypes TGR-1551 and 'WMR 29' bear the same alleles for the three markers evaluated although they are different from those carried by the susceptible 'Bola de Oro' and 'Rochet' genotypes. The recombination frequency analyses indicated that the estimated genetic distance between the *Pm-w* gene and PM2-CAPS, PM4-dCAPS, and PM3-CAPS markers were 4.2, 3.8, and 2.4 cM, respectively.

Discussion

When working with powdery mildew resistance, the major problem is usually the clear characterization of *P. xanthii* races. The identification of resistance genes and clarification of the plant–pathogenic race interaction are necessary. The development of differential cultivars each carrying a race-specific resistance gene could help to clarify the pathogenesis of powdery mildew races and the gene–race interactions (Fukino et al. 2008). In this context, the objective of this study was to identify and develop PCR-based molecular markers for genotypic selection in melon breeding programmes.

Although the resistance to powdery mildew showed by TGR-1551 is inherited following a double dominant-recessive epistasis (13 resistant: 3 susceptible segregation pattern; Yuste-Lisbona et al. 2010b), the combination of the BSA methodology and AFLP techniques allowed us to identify several molecular markers linked to the dominant powdery mildew resistance gene. Within the group of F_2 resistant plants, only 8% (1/13) of the plants would be resistant exclusively due to the action of the recessive gene (*aabb* genotype, considering *b* as the recessive gene), while 92% (12/13) of the F_2 resistant plants would carry the dominant gene (*A-B-* and *A-bb* considering *A* as the dominant gene). Consequently, the resistant bulk used in the BSA was likely composed of plants whose resistance was conferred by the dominant gene. Even if some F_2 resistant individuals carrying the recessive gene (*aabb* genotype) were included in the resistant bulk (along with individuals belonging to the *A-B-* and *A-bb* genotypes), the comparative analysis with the susceptible bulk (composed by *aaB-* plants) should only reveal molecular differences due to the dominant gene.

Mapping of the SSR, SNP, and PM markers enhances the marker density of the genomic region of LG V where the dominant powdery mildew resistance gene carried by TGR-1551 is located. The newly constructed LG V mainly includes codominant markers and displays a higher saturation level than the previous map (Yuste-Lisbona et al. 2010a), allowing for more accurate and precise QTL mapping. In fact, two new QTLs have been detected in TGR-1551, namely *Pm-R1-2* and *Pm-R5*, which makes them important loci for both genetic and breeding purposes. The QTLs detected in TGR-1551 together with the other ones previously mapped in different melon genotypes indicate that at least four genomic regions appear to be involved in powdery mildew resistance in this species. The existence of different genomic regions involved in resistance to a pathogen is common in plant–fungus interactions. Indeed, over 35 powdery mildew resistance loci distributed on most chromosomes have been reported in wheat (http://wheat.pw.usda.gov/GG2/pubs.shtml).

Among the powdery mildew resistance genes described to date, *Pm-w* seems to be a true allele of the aphid resistance *Vat* locus in the melon accession 'WMR29' (Dogimont et al. 2008). However, we have found a F_2 plant carrying the *Vat* gene that is nonetheless susceptible to powdery mildew, and this result was confirmed by phenotypic characterization of the corresponding F_3 family. This result indicates that the dominant powdery mildew resistant gene carried by TGR-1551 is not allelic to the *Vat*/*Pm-w* locus. However, given that the *Pm-w*/*Vat* locus and the dominant powdery mildew resistance locus are closely linked in the LG V, we tried to ascertain whether the PM markers were also linked to the powdery mildew resistance displayed by 'WMR 29', and hence their utility for genotyping selection in different genetic backgrounds. The evaluation of these codominant markers in a F_2 population derived from the crossing of 'WMR 29' and 'Rochet' demonstrated that they are linked to the *Pm-w* gene, which proved the usefulness of these PM

markers. Despite the identification of genes and QTL associated with powdery mildew resistance in melon, few markers linked to this trait have been identified previously as useful tools for breeding selection. Prior to this work, the SSR markers described by Fukino et al. (2008) and the AFLP marker identified by Teixeira et al. (2008) were distributed among the LGs II, IX, and XII. These markers do neither seem to be related to the *Pm-w* locus nor to the dominant gene conferring resistance to powdery mildew in TGR-1551. Thus, the codominant markers reported in this work are the first ones reported as being linked to the powdery mildew resistance locus which maps in the LG V.

In addition, the PM markers developed have been analysed on a wide range of resistant and susceptible melon genotypes (data not shown) in an attempt to test the universality of these markers. However, no significant results have been obtained other than the utility of the markers in TGR-1551 and 'WMR 29' resistant genotypes. Resistance to powdery mildew in most species is race-specific, and therefore, the available resistance markers may only be useful for those genotypes characterized for the specific resistance for which the marker is developed. For example, markers linked to more than 32 powdery mildew resistance genes have been reported in wheat, but universal markers have not been identified (Xu et al. 2008). The coevolution of pathogen virulence and host resistance and the extensive use of race-specific resistance genes can lead not only to the rapid emergence of new virulent pathogen strains, but also the appearance of new powdery mildew resistance alleles or loci (McDonald and Linde 2002). Consequently, the development of universal markers for powdery mildew resistance remains a challenge for breeders. This situation is quite similar to that observed in melon where more than four LGs containing powdery mildew resistance genes have been reported (Pitrat 1991; Périn et al. 2002; Perchepied et al. 2005; Fukino et al. 2008; Teixeira et al. 2008; this work).

Among the several approaches proposed to generate more durable and broad-spectrum resistance, the combination of different resistance genes to the same pathogen in one breeding line or cultivar is perhaps the most successful strategy (Liu et al. 2000) to control powdery mildew. In general, deployment of the different resistance genes can lower the probability of the pathogen overcoming the resistance provided by these genes and lengthen their effectiveness. The use of codominant markers (SCAR, CAPS, and dCAPS) described in this work, together with other markers linked to powdery mildew resistance genes, such as the SSR described by Fukino et al. (2008), could facilitate pyramiding of different resistance genes in a single genotype, providing more comprehensive and durable protection.

Sequence analysis of these codominant markers has allowed us to identify two resistance-like genes, MRGH5 and MRGH63, belonging to the NBS-LRR family as candidate genes for powdery mildew resistance in TGR-1551. Linkage analysis and QTL mapping showed that MRGH5 and MRGH63 were closely linked at 0.3 cM and cosegregated with the QTLs *Pm-R1-2* and *Pm-R5*, respectively, suggesting that there is a powdery mildew resistance gene cluster in TGR-1551, which provides resistance to races 1, 2, and 5 of *P. xanthii*. The aphid resistance gene *Vat*, which is also a NBS-LRR gene, is localized in the same genomic region where the powdery mildew candidate genes have been mapped. Cluster organization of NBS-LRR resistance genes is quite common in plant genomes (Michelmore and Meyers 1998) as has been demonstrated for other species such as *Arabidopsis thaliana* (Meyers et al. 2003) and *Oryza sativa* (Zhou et al. 2004). This also seems to be the most probable case for the melon genome.

This is the first time that the NBS-LRR genes MRGH5 and MRGH63 have been described as candidate genes for powdery mildew resistance although further studies will be necessary to clarify the interaction between *P. xanthii* races and these resistance genes. The results of this work may contribute not only to better understanding of the plant–pathogen interaction but also to initiating a map-based cloning approach of the powdery mildew resistance genes present in the TGR-1551 genome. Initiatives on sequencing and functional genomics of the melon genome currently in progress (http://www.gen-es.org) will provide an excellent means to achieve this objective.

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