Fine mapping of *wmv*^{1551}, a resistance gene to *Watermelon mosaic virus* in melon

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Abstract

Recessive resistance to *Watermelon mosaic virus* (WMV) in melon has previously been reported in the African accession TGR-1551. Using a population of recombinant inbred lines (RIL), derived from a cross between TGR-1551 and the susceptible Spanish cultivar 'Bola de Oro' (BO), a major quantitative trait loci (QTL) controlling the resistance was previously mapped to a region of approximately 760 kb in chromosome 11. Minor QTLs were also reported with lower effects, dependent on the environmental conditions. A genotyping by sequencing (GBS) analysis of the RIL population has provided new information that allowed the better location of the major QTL in chromosome 11. Moreover, three minor QTLs in chromosomes 4, 5 and 6 were identified. Generations derived from the RIL population were subsequently phenotyped for resistance and genotyped with SNP markers to fine map the resistance derived from TGR-1551. The results obtained have allowed to narrow the position of the resistance gene on chromosome 11, designated as wmv^{1551}, to a 141 kb region, and the confirmation of a minor QTL in chromosome 5. The effect of the minor QTL in chromosome 5 was significant in heterozygote plants for the introgression in chromosome 11. The SNP markers linked to both QTLs will be useful in breeding programs aimed at the introgression of WMV resistance derived from TGR-1551. Future work will be directed to identifying the resistance gene, wmv^{1551}, in the candidate region on chromosome 11.

Keywords: *Cucumis melo*, WMV, potyvirus, SNP markers

Introduction

Watermelon mosaic virus (WMV) is a plus-strand RNA virus that belongs to the genus *Potyvirus* (family *Potyviridae*) and is transmitted by different aphid species. WMV infects melon (*Cucumis melo* L.) in the main production areas in countries with temperate climates worldwide (Lecoq and Desbiez 2008). Symptoms of infection include mosaic, leaf deformation, chlorosis and cessation of plant growth. Discoloration and slight deformation are observed in fruits, with early infections causing serious yield reduction. Different strategies, such as cultural practices (Fereres and Moreno 2011), have been used to control this disease in melons, although reduction in infection levels is not enough to ensure profitable yields. The introgression of the virus aphid transmission resistance gene (*Vat*) has been reported to have limited impact on WMV epidemics, probably due to the fact that *Aphis gossypii* Glover is not the main vector of the virus in fields (Schoeny et al. 2017). Thus, the identification of new plant resistance genes is necessary to fight this disease.

Tolerance to WMV has been reported in some melon genotypes (Webb 1967; Provvidenti et al. 1978; Sowell and Demski 1981; Moyer et al. 1985; Munger 1991). Up to date, only two accessions, PI 414723 and TGR-1551, have been identified as resistant to the disease. Resistance in PI 414723 is conferred by the dominant gene *Wmr* and it is characterized by a reduction in viral accumulation associated to mild symptoms after infection, with subsequent recovery (Gilbert et al. 1994). Usefulness of resistance derived from PI 414723 is limited, as it is not a full resistance.

Resistance to WMV in the African accession TGR-1551 has been reported as causing a reduction in virus titer, with infected plants remaining asymptomatic or exhibiting mild disease symptoms (Díaz-Pendón et al. 2003). Subsequent analysis showed that resistance is controlled by one recessive gene together with other additional genetic factors (Díaz-Pendón et al. 2005). Evaluation of the resistance in three different environments of a recombinant inbred lines (RIL) population derived from a cross between TGR-1551 and the susceptible Spanish cultivar 'Bola de Oro' allowed the identification of a major quantitative trait locus (QTL) responsible of the resistance on chromosome 11 (Palomares-Rius et al. 2011). These authors located the QTL between markers ECM215 and CMN04_35, in an interval of approximately 9 cM (ECM215 and CMN04_35 flank a physical region of 757.9 kb in the melon genome v3.6.1). This major QTL was associated with resistance in all three environments assayed. Other regions were also involved in the resistance, but they showed lower and non-stable effects, dependent on the environmental conditions (Palomares-Rius et al. 2011).

An expression analysis of 17,443 unigenes in the resistant and susceptible genotypes, TGR-1551 and 'Tendral' respectively, performed to study the regulation after inoculation with WMV, revealed extensive transcriptome remodeling in the resistant plants (Gonzalez-Ibeas et al. 2012). Genes differentially expressed in cotyledons and systemically infected leaves included those encoding proteins related to phytohormone biosynthesis and signaling, to endomembrane system functions and to defense and stress-response functions (Gonzalez-Ibeas et al. 2012). For most of them, deregulation was stronger in TGR-1551 than in the susceptible genotype 'Tendral'. These results suggested a complex resistance response of TGR-1551 plants to WMV infection. Although the recessive genetics of the resistance would suggest a passive resistance mechanism, the results obtained by Gonzalez-Ibeas et al. (2012) indicated that a defense response was activated in infected TGR-1551 plants. The micro RNA (miRNA) profiles were also analyzed in WMV infected plants of TGR-1551 and 'Tendral' genotypes, suggesting the potential involvement of the RNA silencing machinery in the TGR-1551 resistance to WMV (González-Ibeas et al. 2011).

Recently, a new source of resistance to WMV in melon has been reported (line ME8094), and argued to be different from those derived from PI 414723 and TGR-1551 (Bachlava et al. 2014, Patent No. US20140059712). Resistance to WMV from this source maps to the same region on chromosome 11 as the resistance from TGR-1551. However, in contrast to the recessive resistance derived from TGR-1551, inheritance of the resistance from this source has been reported as mainly dominant, although the level of resistance in heterozygotes depends on environmental conditions and inoculation pressure.

Genes conferring resistance to WMV have been studied in other plant species. A recessive gene responsible of resistance to WMV has been identified and cloned in *Arabidopsis thaliana* (L.) Heynh. The resistance gene, *rmv1*, encodes an evolutionary conserved nucleus-encoded chloroplast phosphoglycerate kinase (cPGK2; At1g56190), with a key role in cell metabolism (Ouibrahim et al. 2014). A single amino acid substitution that affected a putative phosphorylation site is involved in *rmv1*-mediated resistance. In the case of cucumber (*Cucumis sativus* L.) different results have been obtained when analyzing inheritance of WMV resistance derived from different sources. In a recent work, the resistance in the Northern Chine type inbred line '02245' was characterized (Tian et al. 2016). Resistance in this line is conferred by a recessive gene, wmv^{02245}, which maps to chromosome 6. The 134.7 kb candidate region contains 21 predicted genes from which two encode proteins with zinc finger structures, two encode proteins with nucleic acid and protein binding sites, and one corresponds to a pathogenesis-related transcriptional factor.

The objective of this work was to further map the *locus* derived from TGR-1551 conferring resistance to WMV by analyzing advanced segregating generations from the RILs evaluated by Palomares-Rius et al. (2011). Molecular-markers tightly linked to the resistance, useful in marker-assisted selection, and the identification of candidate genes for the resistance to WMV derived from this source are also presented.

Material and methods

Plant material

The plant material used in this work derives from the RIL population developed by Palomares-Rius et al. (2011). This population was initiated from a cross between TGR-1551 (TGR), an African genotype belonging to the *acidulus* group of *Cucumis melo*, which was WMV-resistant (Díaz-Pendón et al. 2003), and the Spanish cultivar 'Bola de Oro' (BO) (*C. melo* ibericus group), susceptible (Online resource 1) selfed up to the F7 generation. In previous works, 58, 77 and 66 RILs of this population were phenotyped for resistance to WMV in three different environments, to map the major QTL controlling resistance to WMV between two flanking simple sequence repeat (SSR) markers (ECM215 and CMN04.35) (Palomares-Rius et al. 2011). The whole RIL population has been now genotyped by sequencing (GBS) within this work to increase marker coverage and to conduct a new QTL analysis with SNP markers (see details below). Eight RILs with high resistance levels were selected from the whole RIL population and backcrossed to the susceptible parent BO. The selfing progenies (BC1S1) were then tested for resistance to WMV. The BC1S1 plants with the highest resistance levels derived from two of those RILs (RIL143 and RIL408) were backcrossed again to BO, selfed, and two BC1S1BC2S1 populations were generated and phenotyped for resistance. The selfing population derived from RIL143 was genotyped using a previously designed SNP-based Sequenom platform. Selected plants of the BC1S1BC2S1 generation obtained from RIL408 were used to construct advanced selfing generations (BC1S1BC2S2, BC1S1BC2S3 and BC1S1BC2S4) employed for fine mapping purposes using new Sequenom platforms designed for this study (see details below).

Markers and genotyping methods

Total DNA was extracted from young leaves following the method described by Doyle and Doyle (1990) with minor modifications (Esteras et al. 2013). DNA concentration was measured using spectrophotometry in a Nanodrop ND-1000 Spectrophotometer v.3.5. DNA was diluted to a concentration of 10 ng/µL and adjusted to the concentration suited for the different genotyping analysis.

Previously existing SNPs and new ones developed in this study were used for genotyping the different segregating populations. At the beginning of the study an existing panel of 124 SNPs evenly distributed throughout the genome was implemented in a Sequenom iPLEX® Gold MassARRAY platform by the Epigenetic and Genotyping unit of the University of Valencia (Unitat Central d´Investigació en Medicina (UCIM), Spain), and used to genotype the BC1S1BC2S1 population derived from RIL143. This SNP set had been previously validated in populations derived from ibericus x acidulus melon crosses (Esteras et al. 2013; Leida et al. 2015; Perpiñá et al. 2016; Sáez et al. 2017)

New SNPs were generated for this study. The whole RIL population (148 RILs), both parents (BO and TGR-1551) and their F1, were genotyped by GBS (GBS1 assay) and the generated SNP collection was used to construct a high density genetic map and to conduct a QTL analysis using the RILs population.

These new SNPs were also used for further QTL analyses and fine mapping purposes in advanced backcross/selfing generations derived from RIL408 (BC1S1BC2S2, BC1S1BC2S3, BC1S1BC2S4). For these analyses, SNPs derived from the GBS1 assay were combined with new SNPs identified in two additional GBS experiments (GBS2 and 3 assays), conducted to perform genetic diversity studies (including many genotypes, among others, BO and TGR-1551). Two SNP sets located in the candidate regions, selected from GBS1, 2 and 3 were implemented in two Sequenom iPLEX® Gold MassARRAY platforms, WMV1 and WMV2 (Online resources 2 and 3). The panel WMV1 included SNPs derived from GBS1 and GBS2 and was used to genotype generations BC1S1BC2S2 and BC1S1BC2S3 derived from RIL408. The panel WMV2 was designed with SNPs obtained in GBS3, prioritizing SNPs located in genes involved in resistance and defense responses, according to the information found in MELONOMICS (2018) (Online resource 3). BC1S1BC2S3 plants derived from RIL408 were genotyped with WMV2. Selected plants of generations BC1S1BC2S2, BC1S1BC2S3 and BC1S1BC2S4 included in the final offsprings assay were genotyped with both, WMV1 and WMV2.

The two SSR, ECM215 and CMN04_35, markers (Fukino et al. 2007; Fernández-Silva et al. 2008) that had previously been reported as the flanking markers for the candidate region in chromosome 11 in the preliminary study by Palomares-Rius et al. (2011) were used in some of the segregating populations.

High density linkage map and QTL analysis in the RILs population

A genetic map was constructed with the SNPs generated with the RILs population in GBS1. SNP calling was done in the Bioinformatics and Genomics Service of COMAV at the Universitat Politècnica de València. SNPs were filtered discarding those no biallelic, with more than 30% of missing data, with a minimum allele frequency < 20%, or with heterozygosity > 75%. The software used was MAPMAKER 3.0 (Lincoln et al. 1993). The map was generated using the Kosambi map function.

The genotyping results of GBS1 and previous phenotypic data of the evaluation for resistance to WMV of a total of 69 RILs were used to conduct a SNP-based QTL analysis with the RIL population. Phenotypic data were the same used in Palomares-Rius et al. (2011): averaging symptom scoring at 21 dpi of four plants per RIL, with a scale from 0 (no symptoms) to 5 (severe mosaic and leaf distortion in the five to six youngest leaves), after mechanical inoculation of the RIL population cultivated in three environments.

When QTLs for the different assays colocalized in the same map position, the overlapping region was considered as candidate region. Kruskal-Wallis non-parametric test was used for QTL detection, with MapQTL version 4.1 software (Van Ooijen 2009). In addition, a composite interval mapping approach was performed (CIM) (Zeng 1994), using a windows size of 15 cM and 5 cofactors, with Windows QTL Cartographer v.2.5-009 (Wang et al. 2012). QTLs retained were those with LOD scores higher than the threshold determined by a permutation test (1,000 cycles). *Loci* detected by both, Kruskal-Wallis and CIM methods, were considered sturdy QTLs. Map location of each QTL was determined using a drop interval of 2 from the peak LOD. The phenotypic effect, expressed as the percentage of phenotypic variance explained, R^2, and the additive (when possible) and dominance effects were estimated for each QTL.

Fine mapping of WMV resistance genes

After screening the BC1S1 generations derived from eight selected RILs, we produced two BC1S1BC2S1 populations from the two BC1S1 plants with the highest level of resistance (derived from RILs 143 and 408). These two populations were phenotyped for resistance to WMV (227 and 168 plants, respectively). All these plants were genotyped with the two SSR markers reported to flank the major QTL in chromosome 11 controlling resistance to WMV in TGR (Palomares-Rius et al. 2011). Plants derived from RIL143 were genotyped using a Sequenom iPLEX® Gold MassARRAY with a set of 124 SNPs evenly distributed throughout the genome, available from previous genotyping assays (Esteras et al. 2013).

Nineteen selfing progenies (BC1S1BC2S2), 20 plants each, of selected plants of the BC1S1BC2S1 generation derived from RIL408 were phenotyped for resistance and genotyped using a Sequenom iPLEX® Gold MassARRAY with the new panel of SNPs WMV1, tagging the major and minor candidate regions in chromosomes 4, 5, 6 and 11.

Broad sense heritability was estimated as the ratio of genetic variance and total variance. Between-family variance component from the ANOVA was used as estimate of genetic variance.

Selfing progenies (BC1S1BC2S3) of six of them selected for their genotype in the candidate region in chromosome 11 were phenotyped to confirm the results and were further genotyped using the additional Sequenom iPLEX® Gold MassARRAY set WMV2, covering with a higher density the candidate regions in chromosomes 5 and 11. Also the progenies of three BC1S1BC2S2 plants heterozygous for the candidate region were selfed to construct a BC1S1BC2S3 population of 178 plants used to generate a new high density map of the candidate region of chromosome 11 and to perform an additional QTL analysis. Symptom scores at 30 dpi and virus detection by ELISA, as phenotypic data, and genotypes for the Sequenom WMV1 and WMV2 SNPs panels, were used.

A final phenotyping assay was conducted using 10 selfing progenies (three BC1S1BC2S2, five BC1S1BC2S3 and two BC1S1BC2S4) selected to represent homozygous TGR/TGR and BO/BO and heterozygous TGR/BO for the candidate final intervals. Ten plants were assayed in the offspring of homozygous plants and 20 in the case of those heterozygous. These plants were genotyped with the SNPs panel WMV2.

The combined effect of QTLs in different chromosomes was difficult to quantify because of the unbalance sizes of the samples obtained. The effect of the genotype for the minor QTL in chromosome 5 on plants with each of the genotypes for the major QTL in chromosome 11 was analyzed using one-way ANOVAs performed with the STATGRAPHICS Centurion XVI.I software. Symptom scores at 30 dpi and genotypic data for the linked SNPs in the BC1S1BC2S3 generation were used. The closest markers to the LOD peak, b11wmv09 in chromosome 11 and b5wmv11 in chromosome 5, were selected for the analysis.

Phenotyping for resistance to WMV

Virus inoculations were performed mechanically in plants at one-to-two true leaf stage. Firstly, inoculation was carried out at one cotyledon and the first true leaf; the other cotyledon and the second true leaf were inoculated one week later. The virus used in the experiments was originally isolated from naturally infected melon plants in Huerta de Vera (Valencia, Spain) in 2013. This isolate, WMV-Vera (accession number MH469650.1), has been recently characterized (Aragonés et al. 2018) and showed to be closely related to the WMV FMF00-LL1 isolate (EU660581.1), collected in France in year 2000 (Desbiez and Lecoq 2008). Inoculum was prepared by grinding symptomatic leaves of melon infected plants (Aragonés et al. 2018).

Symptoms were scored visually at 15 and 30 days post-inoculation (dpi), according to a scale from 0 (no symptoms) to 4 (severe mosaic and leaf distortion). Virus infection was assessed by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using the commercial polyclonal antiserum for WMV (Sediag, Longvic, France). Uncertain cases were confirmed by Western blot analysis (Cordero et al. 2017), using a polyclonal antibody against WMV coat protein conjugated to alkaline phosphatase (Bioreba).

Results

QTL analysis in the RILs population

The GBS analysis carried out with the RIL population, BO, TGR-1551 and their F1, allowed the identification of 5766 high quality SNPs, polymorphic between both parents. Markers with a significant segregation distortion were discarded to construct the genetic map. Bins were defined as groups of markers with the same genotyping pattern among all the RILs, i.e., completely linked markers. For map construction, only one SNP per bin was used. Samples with more than 20% missing values and those heterozygous for more than 50% of the markers were also discarded. A total of 126 RILs and 1713 SNPs met these criteria and were used for map construction (Online resource 4 and 5).

A revisited QTL analysis was performed using the genetic map constructed with the 1713 SNPs and the previous RIL phenotypic data, focusing on symptom score at 21 days after inoculation in three environments, spring greenhouse, fall greenhouse and climatic chamber (Palomares-Rius et al. 2011). A major QTL on chromosome 11 was identified in each of the three environments. The interval position of the putative QTL in the three assays overlapped. The overlapping region defined by the three QTLs spanned from 81.2 to 83 cM (positions 29,588,875-29,844,067 bp). LOD peaks (values 8.7, 11.8 and 10.8) were located at 82.6, 78.8 and 83.7 cM and the percentages of explained variance were 31, 44 and 46% in environment 1, 2 and 3, respectively (Table 1). This interval partially overlapped with the interval of 760 kb previously defined between markers ECM215 and CMN34_05 (physical positions between 28,895,450 and 29,653,352 bp), but was displaced to the region of marker CMN04_35 (Palomares-Rius et al. 2011).

Additional regions involved in WMV resistance were also detected on chromosomes 6, 4, and 5, in environments 1, 2 and 3, respectively, what suggests that they are dependent on the environmental conditions. The significance of these minor QTLs was lower and they explained a lower percentage of the variation (Table 1). Minor QTLs were previously reported also on chromosomes 4 and 5 by Palomares-Rius et al. (2011) using a low density SSR map, although their position did not overlap with the current report. These minor QTLs were targeted by SSR markers CMN06_25 (18,762,823 bp) and ECM203 (18,478,227 bp), respectively, located in the same chromosome but physically far from the intervals defined here with SNPs (chromosome 4: 23,744,558-28,597,859 and 5:

24,791,006-27,852,627). The minor QTL in chromosome 6 (4,552,376-6,043,604 bp) had not been previously described.

Phenotyping for resistance and genotyping of BC1S1BC2S1 populations derived from RIL143 and RIL408

Before genotyping the RILs population with SSRs in Palomares-Rius et al. (2011), a backcross and selfing program was started with some selected RILs (the most vigorous RILs that showed the highest resistance levels in the three phenotyping assays were selected), conducting resistance selection in each generation to produce BC1S1BC2S1 populations. Once the SSR genotyping of RILs was available and used to conduct the preliminary QTL analysis (Palomares-Rius et al. 2011), two of these populations, the BC1S1BC2S1 derived from RILs 143 and 408, were selected for further phenotyping. As stated before in the study by Palomares-Rius et al. (2011), both RILs, 143 and 408, were homozygous for the TGR-1551 alleles in the major candidate region of chromosome 11 (flanked by SSRs ECM215 and CMN04_35), and in the candidate region of chromosome 4, targeted by the SSR CMN06_25. For the candidate region of chromosome 5, targeted by SSR ECM203, the line 143 was heterozygous and the line 408 homozygous for TGR-1551 alleles. These genotypes were later confirmed with the SNPs generated with the GBS (Online resource 4).

BC1S1BC2S1 populations derived from RILs 143 and 408 were phenotyped for resistance to WMV. In both assays, plants of the susceptible parent, used as susceptible controls, exhibited mosaic and leaf distortion, while plants of the resistant parent TGR-1551, used as resistant controls, remained asymptomatic or showed mild symptoms. Most of the F1 plants were susceptible, although around 20% of them showed only mild symptoms.

The progeny from RIL143, a total of 227 BC1S1BC2S1 plants, were grown and inoculated. However, many of them resulted to be weak plants that were strongly affected by the process of mechanical inoculation and, consequently, could not be clearly phenotyped. In any case, sets of the most resistant and susceptible plants were selected in this population, a total of 50 plants: 20 resistant plants that were asymptomatic and negative for the presence of virus as detected by Western blot and 30 susceptible plants with severe symptoms and the virus detected by Western blot. These plants were genotyped with a set of 124 SNPs evenly distributed throughout the genome. Cosegregation was detected between the marker PSI_41-B07 (chromosome 11, position 29,558,791 bp) and the resistant phenotype (χ^2=6.70, p=0.03), confirming the presence of the major QTL on chromosome 11.

To further study the effect of the major and the additional *loci*, we analyzed the population derived from the RIL408 (known to be carrier of homozygous resistant introgressions in chromosomes 11, 4 and 5). A total of 168 BC1S1BC2S1 descendants from RIL408 were phenotyped. Segregation was observed for symptom severity. Plants showing moderate to very severe symptoms were considered susceptible. The 48 plants that remained symptomless or showed mild symptoms were analyzed by Western blot for viral accumulation. All the plants analyzed, were virus-free, and thus were considered resistant. The observed ratio was 120 susceptible/48 resistant. The segregation observed fitted the expected (3 susceptible/1 resistant) (χ^2=1.14, p=0.29), which confirmed that the generation evaluated corresponds to the selfing progeny of a plant heterozygous for the major resistance gene.

All the plants were also genotyped with the two flanking SSR markers for the major resistance QTL in chromosome 11 (ECM215 and CMN04_35) described in Palomares-Rius et al. (2011). Segregation obtained for both markers fitted the expected ratio in the selfing progeny from a heterozygote (ECM215: χ^2=0.13, p=0.94: CMN04_35: χ^2=0.29, p=0.87). According to the segregation found in this population, these two SSR markers were located at 6.3 cM. As expected, genotype and phenotype segregation were not independent (ECM215: χ^2=11.20, p=0.004 4: CMN04_35: χ^2=10.81, p=0.004), but some plants showed unexpected phenotypes according to their genotype. A 5.4% of these plants were resistant to WMV but homozygous for the allele of the susceptible parent BO in both SSRs. These could be escapes from infection, or could derive from double recombination events. Additionally, an 8.9% of the plants were homozygous for the allele of the resistant parent, TGR-1551, but susceptible. This could also be a consequence of double recombination events, assuming that the resistance gene is flanked by these SSR markers. In any case, the occurrence of double recombinants would be expected in much lower proportion considering the size of the interval. The phenotype of plants recombinant between both markers, also pointed that the resistant gene is out of this interval and located below SSR CMN04_35 (position 29,653,352 bp), as suggested by RILs QTL analysis.

Phenotyping for resistance and genotyping of BC1S1BC2S2 offsprings derived from RIL408

A total of 19 BC1S1BC2S1 plants from the previously described population derived from RIL408 were selected according to their phenotype for resistance and their genotype for both SSRs, ECM215 and CMN04_35 (Table 2). The selfing progenies of these 19 selected plants were phenotyped for resistance to WMV. A perfect cosegregation was found between the CMN04_35 genotype of the parental plants and the progenies phenotype (all offsprings derived from plants homozygous for the BO allele, homozyogus for the TGR-1551 allele and heterozygous, were susceptible, resistant and segregant respectively) (Table 2). However, three recombinant plants were found according to the ECM215 genotype: two heterozygous (BC1S1BC2S1 plants 91 and 173) and one homozygous for the TGR allele (BC1S1BC2S1 plant 124), with susceptible and segregant progenies respectively (Table 2). Therefore, progeny test confirmed the hypothesis that the resistant gene is closer to CMN04_35. The selected plant set was genotyped with the SNPs panel WMV1 (panel selected to cover the region of the major QTL and the three minor QTLs detected in the QTL analysis performed with the RILs and the high density SNP-based map). Results obtained for the resistance phenotype and the genotype with WMV1 for the region on chromosome 11 (defining a candidate interval between markers ECM215 and SNP11, 28,895,450 bp-29,952,168 bp) were compatible with the candidate interval defined with the RILs population (Table 2).

The heritability value obtained with these offsprings was 0.46. The results showed that the generations obtained from RIL408 did not contain the TGR-1551 introgression for the regions corresponding to the minor QTLs associated with resistance in chromosome 4 and 6, while they kept the introgression for the region in chromosome 5. Most likely regions in chromosomes 4 and 6 were lost during the backcrossing and selection program, which suggest that they do not significantly increase the resistance levels in presence of the candidate regions of chromosomes 1 and 5.

Phenotyping for resistance and genotyping of BC1S1BC2S3 population derived from RIL408

One susceptible BC1S1BC2S2 plant from a uniformly susceptible BC1S1BC2S2 offspring (derived from BC1S1BC2S1 plant 21, Table 2) and two resistant BC1S1BC2S2 plants selected from two BC1S1BC2S2 offsprings, one uniformly resistant and the other segregant (derived from BC1S1BC2S1 plant 36 and plant 174, respectively, Table 2), were selected according to their genotype for the candidate regions. The susceptible plant, 21-8, was homozygous BO for all the markers analyzed on chromosome 11 and for SNP29 in chromosome 5, and homozygous TGR for SNPs 25 and 26 in chromosome 5, whereas the two resistant plants had a TGR introgression variable in length for chromosome 11 (from SNP2 to SNP17 in 36-13 and from SNP2 to SNP12 in 174-12) and heterozygous for the three SNPs in chromosome 5. Selfing progenies from these plants were phenotyped for resistance. Susceptibility was confirmed among descendants of 21-8 and uniform resistance among descendants of 36-13 and 174-12.

Moreover, three BC1S1BC2S2 plants heterozygous in the candidate region from SNP6 to SNP12 (selected from segregant BC1S1BC2S2 offsprings derived from BC1S1BC2S1 plants 75, 124 and 168, Table 2) were selfed to produce a recombinant segregating population of 178 BC1S1BC2S3 plants. A new SNPs panel was designed to saturate the chromosome 11 region (Online resource 3). The BC1S1BC2S3 population was then genotyped with this new SNP panel, generating a new map covering 19.1 cM, which corresponded to 2.1 Mb (Fig. 1). A QTL was detected explaining 23% of the variation in symptom scores and located at 5.5 cM, with LOD 10 (Table 1), being the closest marker b11wmv09 (29,724,835 bp).

This population also segregated for introgression in chromosome 5. The combined effect of QTLs in chromosome 11 and chromosome 5 was difficult to quantify because of the unbalanced size of the samples obtained. In any case, the marker with the most significant effect when analyzing separately each of the genotypes for the chromosome 11 (i.e., homozygotes for BO allele, heterozygotes and homozygotes for TGR-1551 allele) was marker b5wmv11 (27,806,146 bp). The putative effect of the region in chromosome 5 on the major QTL in chromosome 11 was analyzed (Fig. 2). Plants homozygous for the TGR introgression in chromosome 11 (marker b11wmv09) showed mild or no symptoms, independently of the genotype at marker b5wmv11. Similarly, there was not a significant effect on symptom severity in plants homozygous for the BO introgression in chromosome 11. However, the effect of the minor QTL in chromosome 5 was significant in plants heterozygote for the introgression in chromosome 11. Symptom severity was significantly lower in plants homozygous for TGR allele at b5wmv11 marker than in homozygotes for the BO allele.

Phenotyping for resistance of selected BC1S1BC2S3 and BC1S1BC2S4 populations derived from RIL408

Selfing offsprings from three selected BC1S1BC2S1, five BC1S1BC2S2 and two BC1S1BC2S3 plants, previously genotyped were phenotyped for resistance to confirm the candidate interval in chromosome 11 (Table 3). All the results obtained were compatible with the candidate interval obtained in the QTL analysis of BC1S1BC2S3 plants. Moreover, the fact that all descendants from plant 124-2 were resistant allowed the location of the QTL over marker b11wmv11 (29,794,533 bp), shortening the candidate interval. Thus, the final interval would be of approximately 141 kb, spanning from 29,653,352 bp (CMN04_35) to 29,794,533 bp (Fig. 1). This region has 11 annotated genes, some of which could be good resistance candidates (Online resource 6).

Discussion

In this work, two *loci* derived from TGR-1551 conferring resistance to WMV have been mapped. Resistance to WMV derived from TGR-1551 was previously described as monogenic recessive, with modifier genes affecting symptom severity (Díaz-Pendón et al. 2005; Palomares-Rius et al. 2011). The segregation observed in this work among BC1S1BC2S1 descendants from RIL408 fitted the expected for a monogenic recessive model. The existence of a major QTL on chromosome 11, designated as wmv^{1551}, was confirmed here, initially with the subset of the RILs that have been both, phenotyped for resistance and genotyped by sequencing. Palomares-Rius et al. (2011) located the major QTL between markers ECM215 and CMN04_35, which corresponds to a region of 760 kb, being CMN04_35 the closest marker. The highest density of markers used here to genotype this population allowed the narrowing of the physical region and the better location of the interval, which is displaced to the region of marker CMN04_35. Moreover, the QTL analyses developed with descendants from RIL408 and the selfing progenies analyses have allowed a more accurate location of the interval, reducing the candidate region to approximately 130 kb (from 29,667,149 to 29,794,533 bp) not comprising CMN04_35. The chromosome interval containing WMV resistance derived from the other melon line ME8094 (Bachlava et al. 2014, Patent No. US20140059712) includes the candidate region defined here for resistance derived from TGR-1551.

Three minor QTLs were also identified in this work using the RILs population, on chromosomes 4, 5 and 6, each of them in one of the assays, thus, dependent on environmental conditions. Several minor QTLs were described by Palomares-Rius et al. (2011), two of them on chromosomes 4 and 5, respectively. However, their physical positions differ from those obtained here. The highest density of markers has allowed a better delimitation of the position of the minor QTLs. The descendants from RIL408 segregated for the QTL in chromosome 5. The effect of this minor QTL has been confirmed in plants heterozygous for the QTL in chromosome 11. Saez et al. (2017) obtained a similar interaction between the major resistance QTL and one of the minor QTLs affecting resistance to *Tomato leaf curl New Delhi virus* in melon. The effect of this minor QTL could explain the discrepancies between the phenotype for resistance and the genotype for the candidate region on chromosome 11 when analyzing heterozygous plants in segregant generations. QTL on chromosome 11 showed consistent important effects across experiments and generations, what would explain the high heritability. Other QTLs with minor effects may not be detected across experiments due to QTL x environment interactions or by sampling. Those minor QTLs probably would not contribute to the high heritability estimate.

The recessive nature of *wmv*^{1551}contrasts with the defense response activated in infected TGR-1551 plants (González-Ibeas et al. 2012). Recessive resistance is frequent against potyviruses, if compared with viruses belonging to other families (Díaz-Pendón et al. 2004). However, dominant resistant genes have also been reported against some potyviruses, as is the case of *RTM1* and *RTM2* genes effective in *Arabidopsis thaliana* against *Tobacco etch virus* (TEV) (Maule et al. 2007) or the *Pvr7* gene conferring resistance to *Pepper mottle virus* (PepMoV) in pepper (*Capsicum annuum* L.) (Venkatesh et al. 2018). Different alternatives of recessive resistance genes compatible with the transcriptome remodeling observed in infected TGR-1551 plants were proposed by González-Ibeas et al. (2012), such as mlo-like genes, stearoyl-ACP desaturases or traslational initiation factors. More specifically, eukaryotic translation initiation factors (eIF4Es)-mediated resistance against potyviruses has been found in several resistant crops, such as pepper (*Capsicum annuum*), lettuce *(Lactuca sativa* L.), and wild tomato (*Solanum habrochaites* S. Knapp & D.M Spooner) (Hashimoto et al. 2016). None of these genes has been found among the annotated sequences in the candidate regions in chromosome 11 or chromosome 5 (Online resource 6). Melon lines silenced for *eIF4E* did not result resistant to WMV, suggesting either that the virus is able to use the isoform6 *eIF(iso)4E* or that WMV does not need these factors (Rodríguez-Hernández et al. 2012).

The responsible gene of WMV resistance in *A. thaliana*, *rwm1*, a recessive gene, encodes a nucleus-encoded chloroplast phosphoglycerate kinase (Ouibrahim et al. 2014). No similar gene is annotated in the candidate region in chromosome 11. In fact, the melon orthologue of *rwm1* maps to chromosome 11 (MELO3C019634.2, position 25,348,721 to 25,351,874 bp) outside the candidate interval. Moreover, this gene, although represented in the expression array studied in Gonzalez-Ibeas et al. (2012), was not differentially expressed after inoculation with WMV in TGR-1551 compared to the susceptible genotype 'Tendral'.

In the case of cucumber, the candidate region, on chromosome 6, contains 21 predicted genes, 18 of them annotated (Tian et al. 2016). Some of the predicted functions match with those identified in our candidate region on melon chromosome 11, such as dual specificity phosphatase. In any case, the orthologues in melon of the genes identified in cucumber are mainly located on melon chromosome 5 (between 2,353,694 and 2,504,064 bp), in the syntenic region of the candidate cucumber region of chromosome 6, which does not include the minor QTL identified here in melon. Three of the genes in the candidate region in cucumber (Csa6G421630, Csa6G421640 and Csa6G421660) have also significant blast hits with melon genes on chromosome 11 (MELO3C019735, MELO3C019734, and MELO3C019725 located between 23,244,951 and 23,527,925 bp), again, outside the candidate region for WMV resistance identified here.

Several of the annotated genes in the melon candidate region on chromosome 11 play roles related to plant defense responses and also some of them were found to be differentially expressed after WMV infection by Gonzalez-Ibeas et al. (2012). One example was a heavy metal-associated isoprenylated plant protein (HIPP; MELO3C021404). HIPP are metallochaperone proteins exclusive to plants, which have been reported to be involved in plant defense responses (Abreu-Neto et al. 2013; Zschiesche et al. 2015). This gene was found to be differentially expressed in cotyledons non-infected and infected with WMV (González-Ibeas et al. 2012). Other examples were the dual specificity protein phosphatase 1 (MELO3C021405) or the mitogen-activated protein kinase (MAPK) (MELO3C021394), both involved in the MAPK cascade in plant defense (Colcombet and Hirt 2008), but that were not differentially expressed in Gonzalez-Ibeas et al. (2012). Another interesting gene is the Serine incorporator (MELO3C021398), a vesicle-mediated transport gene that was reported to be up-regulated under the potyvirus PVY infection in tobacco. The interest of these genes relays in the fact that it has been suggested that mutations in genes encoding a component of plant defense responses could confer resistance to viruses (Hashimoto et al. 2016).

The melon interval in chromosome 5 obtained from the QTL analysis of the RILs included the 700 kb region of chromosome 5 with the highest concentration of resistance genes in the melon genome (González et al. 2013). Thus, several resistance genes were annotated in this region. Among them, the *virus aphid transmission resistance gene* (*Vat*) is located in this region. This gene that is carried by TGR-1551 prevents melon colonization by *Aphys gossypii*, and subsequent aphid virus transmission, through a microscopic hypersensitive response (Sarria-Villada et al. 2009). However, no interference of this gene in the results of our work is expected because WMV was mechanically inoculated in all our experiments. Moreover, a QTL associated with resistance to *Cucurbit yellow stunting disorder virus* (CYSDV) (Palomares-Rius et al. 2016) and the major QTL for resistance to powdery mildew caused by *Podosphaera xanthii* (Castagne) U. Braun & N. Shishkoff races 1, 2, and 5 (Yuste-Lisbona et al. 2009) derived from TGR-1551 also map to this region on chromosome 5.

A total of 28 NBS-LRR genes were previously reported in the 700 kb region of chromosome 5 (González et al. 2013). In any case, it is not probable that the minor QTL corresponds to this type of dominant resistant genes. Several genes associated with plant defense responses were also annotated in the candidate region, some of them similar to those aforementioned for chromosome 11, such as phosphatase 2C family proteins

(MELO3C004209.2 and MELO3C004439.2, differentially expressed in susceptible and resistant genotypes after infection in Gonzalez Ibeas et al., 2012), heavy metal-associated isoprenylated plant protein 3-like (MELO3C004225.2, differentially expressed only in the susceptible genotype), a mitogen-activated protein kinase (kinase NPK1 isoform X2 (MELO3C004269.2), or a receptor-like cytosolic serine/threonine-protein kinase (MELO3C004315.2), among others. Several pentatricopeptide repeat-containing proteins were annotated in this region. Recent findings have identified them as RGA (Sekwahl et al. 2015). However, the analysis of the segregating populations derived from RIL408 suggested the location of the minor QTL near the end of the candidate interval, which has a lower density of resistance-related genes. Further work will be done to confirm the location of this minor QTL.

The virus isolate used in the analysis was WMV-Vera (accession number MH469650.1), a wild-type virus collected in Spain in 2013 on infected melon plants (Aragonés et al. 2018). Additionally, a uniformly resistant response was reported previously in TGR 1551 against a selection of Spanish isolates (Díaz-Pendón et al. 2005). All isolates included in this previous study belonged to the group described as 'classical' isolates, whereas the WMV-Vera isolate showed the highest similarity with FMF00-LL1 (EU660581.1), which belongs to the group of 'emerging' isolates (Desbiez and Lecoq 2008). The 'emerging' isolates are characterized by being more aggressive and by rapidly replacing the 'classical' isolates when both groups occurred, as was the case in France (Desbiez et al. 2009) and in Spain (Juárez et al. 2013). Therefore, the resistance derived from TGR1551 could be of interest to breed new varieties with wide resistance to different isolates of WMV.

The SNPs tightly linked to the WMV-resistance QTLs on chromosome 11 and chromosome 5 identified in this work will be useful in marker-assisted selection in the context of melon breeding programs. Future work will include the expression analysis and co-segregation assays of the most interesting genes in the candidate regions, in order to clarify the mechanisms underlying resistance.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Table 1. Quantitative trait loci (QTLs) identified in two different populations. RILs1, RILs2 and RILs3 correspond to analysis in the recombinant inbred lines RILs population derived from the cross between TGR-1551 and the cultivar 'Bola de Oro', phenotyped for resistance to *Watermelon mosaic virus* by Palomares-Rius et al. (2011) and genotyped by sequencing. The trait used was symptom score at 21 days post-inoculation. Generation RIL408 corresponds to the BC1S1BC2S3 population derived from RIL408, phenotyped for resistance to *Watermelon mosaic virus* and genotyped with SNP panel WMV2. The trait used was symptom score at 30 days post-inoculation. See Materials and methods section for details.

Gen[a]	Chr [b]	Interval [c]	Nearest marker[d]	Kruskal-Wallis			Composite interval mapping				
				K[e]	Mean TGR[f]	Mean BO[g]	LOD[h]	Add[i]	Dom[j]	d/a[k]	R^2 [m]
RILs1	6	42.2-54.5 cM 4,552,376-6,043,604 bp	S6_5175540	0.001	1.10	2.90	4.6	0.70	-	-	0.13
	11	81.2-89.9 cM 29,588,875-30,547,485 bp	S11_29844067	0.0001	0.29	2.93	8.7	1.12	-	-	0.31
RILs2	4	79-94.6 cM 23,744,558-28,597,859 bp	S4_25496808	0.05	1.45	2.75	6.4	0.67	-	-	0.15
	11	79-83 cM 29,314,773-29,844,067 bp	S11_29588837	0.0001	0.40	2.87	11.8	1.26	-	-	0.44
RILs3	5	65.4-86.3 cM 24,791,006-27,852,627 bp	S5_26193386	0.05	1.32	2.67	3.3	0.51	-	-	0.07
	11	77.9-88.3 cM 29,213,661-30,188,068 bp	S11_29455618	0.0001	0.51	3.23	10.8	1.41	-	-	0.46
RIL408	11	3.0-7.5 29,276,266-29,952,168	b11wmv09	0.0001	0.29	2.05	10	0.88	0.88	1.00	0.23

[a] Gen: Generation and experiment. RILs1, RILs2 and RILs3 correspond to the three experiments with the RILs population; RIL408 corresponds to the BC1S1BC2S3 population derived from RIL408

[b] Chromosome

[c] Interval position of the putative QTL on the genetic and the physical map according to a LOD drop of 2

[d] Closest marker to the LOD peak

[e] Significance level in the Kruskal-Wallis test

[f] Mean of the genetic class TGR-1551 for the corresponding marker

[g] Mean of the genetic class 'Bola de Oro' for the corresponding marker

[h] Higher logarithm of the odds score

[i] Additive effect of the BO allele

[j] Dominant effect of the BO allele

[k] Degree of dominance

[m] Percentage of phenotypic variance explained by the QTL

Table 2. Genotype for the SSRs ECM215 and CMN04_35 and for SNPs in the panel WMV1, for the BC1S1BC2S1 plants selected to evaluate their descendants (A: homozygous for 'Bola de Oro' allele; H: heterozygous; B: homozygous for TGR-1551 allele). The phenotype of the descendants is indicated (SU: susceptible; R: resistant; SE: segregating). Markers in the candidate interval for chromosome 11 are highlighted in grey.

			Number of BC1S1BC2S1 plant																		
Marker	Chr[a]	Position (bp)	36	54	88	107	137	160	170	21	91	100	173	8	10	62	75	104	124	168	174
SNP1	11	27320918	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
SNP2	11	28286948	B	B	B	H	B	B	B	A	H	A	H	H	H	H	H	H	B	H	H
ECM215	11	28895450	B	B	B	B	B	B	B	A	H	A	H	H	H	H	H	H	B	H	H
SNP6	11	29455618	B	B	B	B	B	B	B	A	A	A	A	H	H	H	H	H	H	H	H
SNP7	11	29630096	B	B	B	B	B	B	B	A	A	A	A	H	H	H	H	H	H	H	H
CMN04_35	11	29653352	B	B	B	B	B	B	B	A	A	A	A	H	H	H	H	H	H	H	H
SNP9	11	29694206	B	B	B	B	B	B	B	A	A	A	A	H	H	H	H	H	H	H	H
SNP11	11	29952168	B	B	B	H	B	B	B	A	A	A	A	H	H	H	H	H	H	H	H
SNP12	11	30188018	B	B	H	H	B	B	B	A	A	A	A	H	H	H	H	H	H	H	H
SNP 15	11	31264349	B	B	H	H	B	B	H	A	A	A	H	H	H	B	H	H	H	H	A
SNP16	11	32304043	B	B	H	H	B	B	H	A	A	A	H	H	A	B	H	H	H	A	H
SNP17	11	32731899	H	B	H	H	B	B	H	A	A	A	H	H	A	B	H	H	A	A	H
SNP18	11	33796187	A	B	H	H	H	H	H	A	A	A	H	H	H	B	H	H	A	A	H
SNP19	11	34367855	A	B	H	H	H	H	H	A	A	A	H	B	H	B	H	H	A	A	H
SNP24	4	21507155	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
SNP23	4	23744558	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
SNP21	4	25496808	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
SNP20	4	28057027	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
SNP25	5	25081882	H	B	H	H	A	A	H	B	H	H	H	H	B	H	H	A	H	B	H
SNP26	5	25229866	H	B	H	H	A	A	H	B	H	H	H	H	B	H	H	A	H	B	H
SNP29	5	27772725	H	H	H	B	H	A	A	H	H	A	A	B	B	H	A	H	H	H	H

SNP32	6	4785824	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**
SNP31B	6	5541959	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**
Phenotype			R	R	R	R	R	R	R	SU	SU	SU	SU	SE	SE	SE	SE	SE	SE	SE	SE

[a] Chromosome

Table 3. Genotype for the SSRs ECM215 and CMN04_35 and for SNPs in the panels WMV1 and WMV2, for the BC1S1BC2S1, BC1S1BC2S2 and BC1S1BC2S3 plants selected to evaluate their descendants (A: homozygous for 'Bola de Oro' allele; H: heterozygous; B: homozygous for TGR-1551 allele). The phenotype of the descendants is indicated (SU: susceptible; R: resistant; SE: segregating). Markers in the candidate interval for chromosome 11 are highlighted in grey.

			Generation assayed and parent genotype										
			BC1S1BC2S2	BC1S1BC2S3	BC1S1BC2S3	BC1S1BC2S3	BC1S1BC2S3	BC1S1BC2S2	BC1S1BC2S2	BC1S1BC2S2	BC1S1BC2S3	BC1S1BC2S4	BC1S1BC2S4
Marker	Chr[a]	Position (bp)	107	10-3	124-2	163-3	163-11	91	100	173	75-9	75-7-49	168-7-65
SNP1	11	27320918	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**
b11wmv01	11	28181279	**H**	**B**	**B**	**B**	**B**	**H**	**A**	**H**	**B**	**A**	**H**
b11wmv01B	11	28182749	**H**	**B**	**B**	**B**	**B**	**H**	**A**	**H**	**B**	**A**	**H**
SNP2	11	28286948	**H**	**B**	**B**	**B**	**B**	**H**	**A**	**H**	**B**	**A**	**H**
ECM215	11	28895450	**B**	**B**	**B**	**B**	**B**	**H**	**A**	**H**	**-**	**A**	**-**
b11wmv02	11	29113537	**B**	**B**	**B**	**B**	**B**	**H**	**A**	**H**	**A**	**A**	**A**
b11wmv03	11	29216444	**B**	**B**	**B**	**B**	**B**	**H**	**A**	**H**	**A**	**A**	**A**
b11wmv04	11	29276266	**B**	**B**	**B**	**B**	**B**	**H**	**A**	**H**	**A**	**A**	**A**
SNP6	11	29455618	**B**	**B**	**B**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	**A**
b11wmv05	11	29570883	**B**	**B**	**B**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	**A**
b11wmv06	11	29596257	**B**	**B**	**B**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	**A**
b11wmv7B	11	29630012	**B**	**B**	**B**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	**A**
SNP7	11	29630096	**B**	**B**	**B**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	**A**
CMN04_35	11	29653352	**B**	**B**	**B**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	**A**

b11wmv8	11	29667149	**B**	**B**	**B**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	**A**
SNP9	11	29694206	**B**	**B**	**B**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	**A**
b11wmv9	11	29724835	**B**	**B**	**B**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	**A**
b11wmv10	11	29756985	**B**	**B**	**B**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	**A**
b11wmv11	11	29794533	**B**	**B**	**H**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	**A**
b11wmv12	11	29813505	**B**	**B**	**H**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	**A**
b11wmv13	11	29843972	**H**	**B**	**H**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	**A**
b11wmv13C	11	29846583	**H**	**B**	**H**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	**A**
b11wmv15B	11	29887364	**H**	**B**	**H**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	**A**
SNP11	11	29952168	**H**	**B**	**H**	**B**	**B**	**A**	**A**	**A**	**A**	**A**	**H**
b11wmv16	11	30046950	**H**	**H**	**H**	**B**	**B**	**A**	**A**	**H**	**A**	**H**	**H**
b11wmv17	11	30063592	**H**	**H**	**H**	**B**	**B**	**A**	**A**	**A**	**A**	**H**	**H**
b11wmv18	11	30136174	**H**	**H**	**H**	**B**	**B**	**A**	**A**	**A**	**A**	**H**	**H**
b11wmv19	11	30162440	**H**	**H**	**H**	**B**	**B**	**A**	**A**	**H**	**A**	**H**	**H**
SNP12	11	30188018	**H**	**H**	**H**	**B**	**B**	**A**	**A**	**A**	**A**	**H**	**H**
b11wmv20	11	30284318	**H**	**H**	**H**	**B**	**B**	**A**	**A**	**H**	**A**	**H**	**H**
b11wmv21	11	30547485	**H**	**H**	**H**	**B**	**B**	**-**	**A**	**H**	**A**	**H**	**H**
SNP15	11	31264349	**H**	**H**	**H**	**A**	**A**	**A**	**A**	**H**	**A**	**H**	**H**
SNP16	11	32304043	**H**	**A**	**H**	**A**	**A**	**A**	**A**	**H**	**A**	**H**	**A**
SNP17	11	32731899	**H**	**A**	**A**	**A**	**A**	**A**	**A**	**H**	**H**	**H**	**A**
SNP18	11	33796187	**H**	**A**	**A**	**A**	**A**	**A**	**A**	**H**	**H**	**B**	**A**
SNP19	11	34367855	**H**	**A**	**A**	**A**	**A**	**A**	**A**	**H**	**H**	**B**	**A**
SNP24	4	21507155	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**
SNP23	4	23744558	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**
SNP21	4	25496808	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**
SNP20	4	28057027	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**
b5wmv1	5	4723072	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**

b5wmv2	5	14945725	**A**	**B**	**H**	**A**	**A**	**H**	**H**	**H**	**H**	**H**	**B**
b5wmv3	5	20677985	**A**	**B**	**H**	**A**	**A**	**H**	**H**	**H**	**A**	**H**	**B**
SNP25	5	25081882	**H**	**B**	**H**	**A**	**A**	**H**	**H**	**H**	**A**	**H**	**B**
SNP26	5	25229866	**H**	**B**	**H**	**A**	**A**	**H**	**H**	**H**	**A**	**H**	**B**
b5wmv4	5	25314558	**H**	**B**	**H**	**A**	**A**	**H**	**-**	**B**	**A**	**A**	**B**
b5wmv4C	5	25326396	**H**	**-**	**H**	**A**	**A**	**H**	**H**	**H**	**A**	**A**	**B**
b5wmv6	5	26629651	**H**	**B**	**H**	**A**	**A**	**H**	**H**	**H**	**A**	**A**	**A**
b5wmv7C	5	26940315	**H**	**B**	**H**	**A**	**A**	**H**	**H**	**H**	**A**	**A**	**A**
b5wmv8	5	27194925	**H**	**B**	**H**	**A**	**A**	**H**	**H**	**H**	**A**	**A**	**A**
b5wmv9	5	27509294	**H**	**B**	**H**	**A**	**A**	**H**	**A**	**B**	**A**	**A**	**A**
b5wmv10	5	27698241	**H**	**B**	**H**	**A**	**A**	**H**	**A**	**H**	**A**	**A**	**A**
SNP29	5	27772725	**B**	**B**	**H**	**A**	**A**	**H**	**A**	**A**	**A**	**A**	**A**
b5wmv11	5	27806146	**H**	**B**	**H**	**A**	**A**	**H**	**A**	**A**	**A**	**A**	**A**
SNP32	6	4785824	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**
SNP31B	6	5541959	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**
Phenotype			**R**	**R**	**R**	**R**	**R**	**SU**	**SU**	**SU**	**SU**	**SU**	**SU**

[a] Chromosome