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Development of DNA markers for Slmlo1.1, a new mutant allele of the powdery mildew resistance gene SLMlo1 in tomato (Solanum lycopersicum)

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Abstract

Reductions in growth and quality due to powdery mildew (PM) disease cause significant economic losses in tomato production. *Oidium neolycopersici* was identified as the fungal species responsible for tomato PM disease in South Korea in the present study, based on morphological and internal transcribed spacer DNA sequence analyses of PM samples collected from two remote regions (Muju and Miryang). The genes involved in resistance to this pathogen in the tomato accession ‘KNU-12’ (*Solanum lycopersicum* var. *cerasiforme*) were evaluated, and the inheritance of PM resistance in ‘KNU-12’ was found to be conferred via simple Mendelian inheritance of a mutant allele of the PM susceptibility locus *Ol-2* (*SlMlo1*). Full-length cDNA analysis of this newly identified mutant allele (*Slmlo1.1*) showed that a 1-bp deletion in its coding region led to a frameshift mutation possibly resulting in SlMlo1 loss-of-function. An alternatively-spliced transcript of *Slmlo1.1* was observed in the cDNA sequences of ‘KNU-12’, but its direct influence on PM resistance is unclear. A derived cleaved amplified polymorphic sequence (dCAPS) and a high-resolution melting (HRM) marker were developed based on the 1-bp deletion in *Slmlo1.1*, and could be used for efficient marker-assisted selection (MAS) using ‘KNU-12’ as the source for durable and broad-spectrum resistance to PM.

Key words: disease resistance, molecular marker, *Oidium neolycopersici*, powdery mildew, *Solanum lycopersicum*. 
Introduction

Powdery mildew (PM) disease is caused by obligate biotrophic ascomycete fungi belonging to the order Erysiphales. The major pathogens causing PM in tomato (*Solanum lycopersicum*) are *Oidium* spp. (*O. neolycopersici* and *O. lycopersici*) and *Leveillula taurica* (Kiss et al. 2001; Kashimoto et al. 2003; Lebeda et al. 2015). *Oidium neolycopersici* forms a single spore on the conidiophore during its sporophyte development stage, therefore being morphologically distinct from *O. lycopersici*, which produces a chain of spores (Jones et al. 2001; Kiss et al. 2004). In terms of disease symptoms, *O. neolycopersici* and *O. lycopersici* are similar. Both produce signs resembling white powder on the upper surface of leaves and petioles and on stems, but not on leaf underside. *Leveillula taurica* produces the same signs in leaves, on both their upper and under sides, but not on stems (Jacob et al. 2008). The first incidence of PM caused by *L. taurica* in South Korea was reported from Jinju in 1995 (Kang et al. 1995), but few studies of *O. neolycopersici* and *O. lycopersici* in South Korea are publicly available.

Powdery mildew disease occurs on tomato cultivars at all growth stages from seedlings to mature plants, and in greenhouse and field cultures. Severe infection results in etiolation, early senescence, and reduction of fruit size and quality, leading to notable fruit losses, reduced yields, and economic damage (Jones et al. 2001; Jacob et al. 2008). By combining high temperature and low relative humidity (RH), PM disease severity can be reduced in tomato cultivated in greenhouse environments (Whipps and Budge 2000; Jacob et al. 2008). Foliar application of synthetic fungicides or potassium silicate are additional methods to manage PM disease (Yanar et al. 2011). Although cultivating PM-resistant (PMR) tomato cultivars is the most efficient way to prevent PM disease, there are not many commercial PMR cultivars available to farmers worldwide.

Tomato accessions resistant to PM caused by *Oidium* spp. have been reported, and the genetic inheritance of their resistances has also been studied (Seifi et al. 2014). In the wild tomato species *S. 
*habrochaites*, two dominant and possibly allelic PM-resistance genes, *Ol-1* and *Ol-3*, were detected in the long arm of chromosome (Chr.) 6, and gene *Ol-5* was closely linked to these loci (Bai et al. 2005). In *S. peruvianum*, another pair of possibly allelic and dominant genes, *Ol-4* and *Ol-6*, were found on the short arm of Chr. 6 (Bai et al. 2005). All the dominant genes referred above confer PM resistance through a hypersensitive reaction (HR) mechanism. One of the quantitative trait loci (QTLs) associated with PM resistance, *Ol-qtl1*, was detected in *S. neorickii* (Accession: G1.1601), in the chromosomal region proximal to *Ol-1*, *Ol-3*, and *Ol-5* (Bai et al. 2003). Additionally, the QTLs *Ol-qtl2* and *Ol-qtl3* were reported from Chr. 12, on the vicinity of *Lv*, which is a locus conferring resistance to PM caused by *L. taurica* in this accession (Chunwongse et al. 1994; Faino et al. 2012).

A single recessive PM resistance gene for *ol-2* (*Slmlo1*) was found on Chr. 4 of *S. lycopersicum* var. *cerasiforme* (Accession: LA1230) (Ciccarese et al. 1998), and, to date, it is the only PM resistance gene cloned in tomato.

As noted above, most sources of PM resistance in tomato have been identified among wild *Solanum* species. Due to linkage drag of unfavorable traits from wild strains, introgression of these genes into cultivated tomatoes can be a time-consuming process. Breeding efficiency can be greatly improved by using molecular markers, for example through marker-assisted selection (MAS), which are tightly linked with or developed from the gene controlling PM resistance. Although several genetic mapping studies of PM resistance genes using polymerase chain reaction (PCR) revealed a series of tightly linked markers, studies demonstrating their applicability to MAS have not been reported so far. Furthermore, an efficient method for the differential diagnosis of different PM pathogen races (isolates) in tomato is lacking (Kashimoto et al. 2003; Bai et al. 2005), and therefore the race-specificity for each PM resistance gene is not yet fully understood. This may hamper the use of published markers in MAS programs targeting cultivation areas for which the composition of PM pathogens in the population is not yet characterized. Thus, the current study aimed to discern which
pathogen species cause tomato PM disease in South Korea, and to characterize the PM resistance
genes in the tomato accession ‘KNU-12’. The results indicated PM resistance in ‘KNU-12’ might be
conferred by a novel mutant allele of ol-2 identified here. Based on this mutant allele of ol-2, gene-
based molecular markers that might be useful for MAS were developed.

Materials and methods

Plant materials

The tomato accession ‘KNU-12’ was selected for this study due to its natural resistance to PM
demonstrated in a greenhouse at Pusan National University (PNU, Miryang, South Korea) during
the summer of 2012. This inbred line (female) was crossed with ‘PMS’ (male), an advanced
breeding line susceptible to PM, to produce generation F\textsubscript{1}. A single F\textsubscript{1} plant was then self-pollinated
to produce generation F\textsubscript{2}, and F\textsubscript{2} individuals were subsequently self-pollinated to produce 111 F\textsubscript{3}
families (F\textsubscript{2:3}). All artificial pollination and progeny seed production was conducted in a greenhouse
at PNU, from 2013 to 2014. Commercial F\textsubscript{1} hybrid cultivars, either purchased or provided from
private seed companies in South Korea, were used for validating SCAR and derived cleaved
amplified polymorphic sequence (dCAPS) analyses.

PM pathogen identification

Powdery mildew pathogens that occurred naturally on tomato plants in a greenhouse located in
Muju, Jeonnam Province (July 2014) and Miryang, Gyeongnam Province (July 2015), South
Korea were collected and subject to species identification. White mycelial mats (hyphae,
conidiophores, and conidia) were brushed off from the adaxial sides of fresh leaves of PM-infected
‘PMS’ plants, and then used for either microscopic observation or DNA analysis. A small quantity
of brushed samples was mounted on a microscope slide with a drop of double distilled water (ddH₂O) and examined using light microscopy.

Genomic DNA was extracted using the GenEx™ Plant KIT (GenEx™ Plant, GeneAll, Seoul, South Korea), and the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (rDNA) was amplified by PCR in 10-µL total volume reactions containing 1 µL genomic DNA (20 ng), 1 µL 10× buffer SolgTM (SolGent, Daejeon, Korea), 0.2 µL dNTPs (10 mM, SolGent), 0.5 µL of each forward (PMITS₁) and reverse (PMITS₂) primer (10 pmol) (Table 1) (Kiss et al. 2001), and 0.1 µL eTaqSolg™ Taq polymerase (5 U µL⁻¹, SolGent). Amplifications were conducted on the T100™ thermocycler (BIO-RAD, Hercules, CA, USA) as follows: denaturation for 10 min at 94 °C followed by 40 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 53 °C, and extension for 1 min at 72 °C. Amplicons were electrophoresed on a 2% agarose gel and purified from the gel using the Expin™ Gel SV gel extraction kit (GeneAll, Seoul, Korea). Purified PCR products were cloned using the pGEM T-Easy Vector System I (Promega, Madison, WI, USA).

Sequencing of recombinant plasmids was conducted using the dye terminator method of Genotech (Daejeon, Korea). For molecular identification of the fungi and respective taxonomic assignment, the resulting sequences (MJ_PM-1 and MR_PM-1) were compared with the ITS sequences of various PM pathogen species through the basic local alignment search tool using a nucleotide query (BLASTn) in the UNITE database (http://unite.ut.ee) (Abarenkov et al. 2010).

**PM disease evaluation**

Two independent bioassays for PM disease were conducted in 2014 and 2015. In 2014, 10 plants per F₂ family were evaluated with two replicates per family, while in 2015 five plants per F₂ family were evaluated with three replicates per family. The PM spore suspensions used in the bioassays were prepared by diluting the PM specimens obtained as described in the ‘PM pathogen...
identification’ section to $9.75 \times 10^4$ conidia mL$^{-1}$ with ddH$_2$O. These suspensions were spread three times on whole seedlings at the 4–5-true-leaf stage until run-off, at three-day intervals. After inoculation, seedlings were grown for two weeks in a greenhouse at PNU under 26–28 °C/10–15 °C day/night temperature conditions, and the PM disease symptoms developed were scored. The level of PM resistance was evaluated based on the Disease Symptom Index (DSI) as follows: DSI = 0, no symptoms; DSI = 1, symptoms covering less than 10% of the leaf area; DSI = 2, symptoms on 10–30% of the leaf area; and DSI = 3, symptoms covering more than 30% of the leaf area.

**Evaluation of molecular markers linked to PM resistance**

Cultivars ‘KNU-12’ and ‘PMS’ were genotyped using previously published molecular markers that were reported to be linked to PM resistance [dct136 for \(Ol\)-qtl1 (Bai et al. 2004); Y258 and tg111 for \(Ol\)-qtl3 (Bai et al. 2004); P2147, tg25-1, tg25-2, and H9A11 for \(Ol\)-1 and \(Ol\)-5 (Bai et al. 2005); Tom316, Tom332, U3-2, and M/SlMlo1 for \(ol\)-2 (Bail et al. 2008; Pavan et al. 2008); GP79L and 32.5Cla for \(Ol\)-4 and \(Ol\)-6 (Bai et al. 2005)]. Details of these markers and the primers used to amplify them are presented in Table 2. Genomic DNA of ‘KNU-12’ and ‘PMS’ was extracted from two or three true leaves using sodium dodecyl sulfate extraction buffer, and the PCR was conducted using the mixture described in the ‘PM pathogen identification’ section.

Amplifications were conducted as follows: denaturation for 2 min at 94 °C, 35 cycles of denaturation for 15 s at 94 °C, annealing for 30 s at 58 °C, and extension for 1 min at 72 °C. Amplicons were electrophoresed on a 1.0% agarose gel for 1 h at 130 V and visualized under a gel image analysis system (CoreBio-MAXTM, Davinch-K, Seoul, Korea) after ethidium bromide (EtBr) dying.

**Evaluation of \(ol\)-2 gene (\(Sl\)Mlo1) allele variation and development of gene-based SCAR**
The genomic DNA sequence of *ol-2* from ‘KNU-12’ and ‘PMS’ was determined using three primer pairs, 1-1, 1-2, and 1-3, which were designed based on the *SlMlo1* gene sequence (SGN accession number Solyc04g049090) using primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi/). To find a gene-based SCAR marker for *ol-2*, the primer pair SCAR_SlMlo1.1 (Table 1) was designed targeting an insertion/deletion (indel) positioned in an intron region of the gene. Primer amplifications and cloning and sequencing of the PCR products were conducted as described in the ‘PM pathogen identification’ section. SCAR_SlMlo1.1 marker genotyping of commercial F1 cultivars was evaluated by polyacrylamide gel using a Fragment Analyzer.

**Full-length cDNA cloning of *ol-2* and development of gene-based dCAPS**

True leaves (6-7th) of ‘KNU-12’ and ‘PMS’ seedlings were collected and immediately ground in liquid nitrogen. Total RNA was extracted using the RNeasy® Plant Mini Kit (QIAGEN, Germantown, MD, USA), and its quality and quantity were checked on the Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA, USA). Purified RNA was diluted to 10 ng μL⁻¹ and full-length cDNA of the *ol-2* gene was synthesized using a SMARTer® RACE 5'/3' kit (Takara, Kusatsu, Japan) following the manufacturer’s instructions. Gene-specific primers for rapid amplification of cDNA ends (RACE) PCR (5’ reverse primer: 5'-GATTACGCCAAGCTTTCACCCCATGGTTAGCCTTATGGCT-3, 3’ forward primer: 5'-GATTACGCCAAGCTTGATTTCTGGAGCAAGTCCCCCGTGTT-3) were designed based on the *SlMlo1* coding sequences (CDS). Cloning and sequencing of the RACE PCR products were carried out using the methods described by Kim et al. (2017). Sequence alignment and analysis of full-length cDNA were carried out using ClustalX 1.83.
Primers for ol-2 gene-based dCAPS (dCAPS_SlMlo1.1) (Table 1) were designed using dCAPS Finder (http://helix.wustl.edu/dcaps/dcaps.html), and the PCR for dCAPS genotyping was conducted as described in the ‘Evaluation of molecular markers linked to PM resistance’ section. Restriction enzyme digestion of PCR products was performed by adding 0.5 μL Time-Saver™ FokI (1,000 U mL⁻¹, NEB®, Ipswich, MA, USA), 1.5 μL 10× Cutsmart™ buffer (NEB®), and 3 μL of ddH₂O to 10 μL of the PCR products and letting the mixture sit for 1 h at 37 °C. Digested PCR products were electrophoresed on a 2.5% agarose gel for 1 h at 130 V, and visualized under a gel image analysis system (CoreBio-MAXTM) after being dyed with EtBr.

Conversion of dCAPS to a high-resolution melting marker

To develop a high-resolution melting (HRM) marker, a 3′-blocked and unlabeled oligonucleotide probe (Luna Probe; BioFire Defense, Salt Lake, UT, USA) was designed based on the targeted indel mutation (Table 1). Using the saturating dye EvaGreen® Plus (Biotium, Hayward, CA, USA), a PCR was performed to generate melting curves characteristic of the probed genotype using the LightScanner® Instrument System (Roche, Basel, Switzerland). This PCR involved pre-denaturation at 95 °C for 5 min followed by cycles of denaturation at 95 °C for 20 s, annealing at 60 °C for 20 s, and extension at 72 °C for 30 s, and a final extension for 40 s at 72 °C. Primer and probe sets for indel detection are described in Table 1.

Detection of alternative transcripts

Ten ‘KNU-12’ seedlings at the 4–5-true-leaf stage were inoculated with the PM suspension as described in the ‘PM disease evaluation’ section. Control plants were treated with ddH₂O for mock inoculation. Inoculated leaves were collected from three seedlings at 0, 12, 24, and 48 h post-inoculation (hpi), and immediately frozen in liquid nitrogen. Total RNA extraction and cDNA
synthesis were performed as described in the ‘Full-length cDNA cloning of ol-2 and development of gene-based dCAPS’ section.

Complementary DNAs were PCR-amplified using the primer set ‘ASC-1’ (Table 1) that was designed to flank the 9th exon of ol-2. Amplifications were carried out in a 10-μL mixture containing 1 μL cDNA (20 ng), 1 μL 10× SolgTM buffer (SolGent), 0.2 μL dNTPs (10 mM, SolGent), 0.5 μL of each forward and reverse primer (10 pmol), and 0.1 μL eTaqSolg™ Taq polymerase (5U μL⁻¹, SolGent), as follows: denaturation for 10 min at 94 °C followed by 35 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 61 °C, and extension for 1 min at 72 °C. Amplicons were electrophoresed on a 2.5% agarose gel for 1 h at 130 V and visualized under a gel image analysis system (CoreBio-MAXTM) after being dyed with EtBr.

Results

PM pathogen identification

The morphological characteristics and the rDNA ITS sequences of PM pathogen samples collected in Muju (MJ_PM1) and Miryang (MR_PM1), South Korea were analyzed. Microscopic observation clearly showed that the type of conidiogenesis displayed by MJ_PM1 and MR_PM1 specimens (Fig. 1) corresponded to that of *O. neolycopersici*; formation of non-catenate, single conidia with ellipsoid-ovoid shape and mean length greater than 30 μm (Kiss et al. 2001).

A DNA fragment of 579 bp was sequenced from the ITS of MJ_PM1 and MR_PM1 (Supplementary File 1). No ITS sequence variation was observed between these samples. Comparison of these ITS sequences with that of other Erysiphales species showed that MJ_PM1 and MR_PM1 were most closely related to *O. neolycopersici*. The BLASTn search conducted on the UNITE database retrieved 29 sequences that were 100% identical (E-value = 0.0) to the query
sequences and annotated as *O. neolycopersici*. Among them, 22 sequences originated from fungi isolated from tomato hosts in Japan, China, South Korea, and many unspecified countries (Kiss et al. 2001) (Supplementary File 1). Thus, microscopical examination and ITS sequence analysis indicated that *O. neolycopersici* might be the predominant *Oidium* anamorph causing tomato PM in South Korea.

**Bioassay of PM resistance**

The inheritance of PM resistance in ‘KNU-12’ was investigated using generation F$_1$ and 111 F$_3$ family populations. In the first evaluation, generation F$_1$ and 87 F$_3$ families were scored as susceptible (S, DSI = 1–3), while 24 F$_3$ families were scored as resistant (R, DSI = 0–0.9). In the second evaluation, generation F$_1$ and 89 F$_3$ families were scored as S (DSI = 1–3), while 22 F$_3$ families were scored as R (DSI = 0–0.9). The DSIs of F$_3$ families were consistent between the two evaluations except for two F$_3$ families (F$_3$-121 and F$_3$-144, Table 2). The DSIs values of the two replicates per F$_3$ family were averaged, and the final disease reaction was scored based on the average DSI per-family. The ratio of R (21 F$_3$ families) and S (90 F$_3$ families) in the final evaluation was 1:3 ($X^2 = 0.68$ and $P = 0.41$), indicating that PM resistance in ‘KNU-12’ is conferred via a simple Mendelian model of inheritance of a single recessive locus (Table 3).

**Development of DNA markers for PM resistance**

Cultivars ‘KNU-12’ and ‘PMS’ were genotyped for DNA markers that were previously reported to be linked to PM resistance (Table 2, Fig. 2). However, all markers except GP79L were monomorphic between ‘KNU-12’ and ‘PMS’. This marker, which has been reported as linked to the dominant loci *Ol-4* and *Ol-6* (Fig. 2) (Bai et al. 2005), was genotyped for the 111 F$_3$ plants examined here, but no significant linkage to PM resistance was observed (data not shown).
indicating that the resistance in ‘KNU-12’ is not conferred by those loci. Phenotype evaluation of
generation F₁, which showed the single recessive inheritance of PM resistance in ‘KNU12’, also
supported this assumption. Although considering the inheritance pattern ol-2 could be associated
with PM resistance in ‘KNU-12’, none of the ol-2 linked markers, including the gene-based marker
M/SIMlo1 showed polymorphisms between ‘KNU-12’ and ‘PMS’ cultivars. Thus, PM resistance in
‘KNU-12’ seems to be mediated by an allele of ol-2 that is different from the original mutant allele
of Ol-2 in ‘LA1230’ (S. lycopersicum var. cerasiforme).

To develop a gene-based marker for Ol-2 that is polymorphic between ‘KNU-12’ and ‘PMS’,
three primer pairs (1-1, 1-2, and 1-3) encompassing the genomic DNA sequence of the Ol-2 gene
(SGN accession number: Solyce04g049090; 5344 bp) (Fig. 3) were designed, and each region was
amplified by PCR. A polymorphism in amplicon size was identified by the primer set ‘1-3’ that was
designed to amplify the region between the 13th and 15th exon. Cloning this amplicon using primer
‘1-3’ revealed a 14-bp indel in the intron between the 14th and 15th exons, and a 1-bp indel in the
15th exon (Fig. 4). The 14-bp indel occurred within a simple sequence repeat (SSR) region that
comprised 21 TA dinucleotide motifs [(TA)₂₁] in ‘PMS’ and 14 TA dinucleotide motifs [(TA)₁₄] in
‘KNU-12’. Based on these indels, SCAR (SCAR_SIMlo1.1) and dCAPS (dCAPS_SIMlo1.1)
markers were developed to genotype the ol-2 allele in ‘KNU-12’, ‘PMS’, and F₂ hybrid plants. The
SCAR_SIMlo1.1 marker used a forward primer and a reverse primer flanking the 14-bp indel and
amplified 194-bp DNA fragments from ‘KNU-12’ and 208-bp DNA fragments from ‘PMS’. The
dCAPS_SIMlo1.1 marker used a forward primer and a reverse primer flanking the 1-bp indel and
produced 200-bp DNA fragments from ‘KNU-12’ and 170-bp and 30-bp DNA fragments from
‘PMS’ after restriction enzyme digestion of the PCR amplicon using BclI (Fig. 5).

Genetic association between the markers and PM resistance
The SCAR_SlMlo1.1 marker was used to genotype 111 ‘KNU-12’ × ‘PMS’ F₂ plants (Fig. 5).

The F₂ plants that were scored as having a PM-resistant phenotype among the F₃ families (F₂:₃) showed the marker genotype for ‘KNU-12’, while plants that were scored as having the susceptible phenotype among their F₃ families showed the marker genotype for either ‘PMS’ or heterozygosity (57 plants) (Table 3). Furthermore, all F₂ plants for which resistant and susceptible individuals were segregated in their F₃ families (Table 3) showed the marker genotype for heterozygosity. To further verify this result, we selected 10 seedlings from each F₃ family of heterozygous F₂, extracted their genomic DNA, and pooled DNA samples for PCR. All PCR analyses of pooled DNA samples from each F₃ plant revealed the PCR bands specific to ‘KNU-12’ and ‘PMS’. All F₂ plants were also genotyped using dCAPS_SlMlo1.1 markers, and the results matched those for SCAR_SlMlo1.1 (Fig. 5). Therefore, we confirmed that PM resistance in ‘KNU-12’ is conferred by a single recessive gene that is cosegregated with SlMlo1.

Applicability of the gene-based markers in MAS and marker conversion to HRM

The applicability of the SCAR_SlMlo1.1 and dCAPS_SlMlo1.1 markers in MAS was validated by testing 96 commercial F₁ hybrid cultivars. None of these cultivars were labeled as PM-resistant in their commercial bulletins, and therefore were considered susceptible to PM. Tests with dCAPS_SlMlo1.1 markers, revealed that all F₁ cultivars had the marker genotype for ‘PMS’, except for two that showed the marker genotype for ‘KNU-12’. These results indicated that the 1-bp indel in the 15th exon of the SlMlo1.1 is unique to PM-resistant ‘KNU-12’ plants. Therefore, the dCAPS_SlMlo1.1 marker can be effectively used as a universal gene-based marker for MAS of the PM resistance allele of ‘KNU-12’.

Because dCAPS marker genotyping based on agarose gel electrophoresis is a time-consuming and costly procedure, a marker suitable for automated high-throughput genotyping platforms, such
as real time PCR-based HRM is required. To convert the dCAPS marker into a HRM markers, we used 3’-blocked and unlabeled oligonucleotide probes for melting curve analysis of the 1-bp indel. This HRM marker (HRM_SlMlo1.1) was evaluated using ‘KNU-12’, ‘PMS’, and F₁ and F₂ plants derived from ‘KNU-12’ × ‘PMS’. Three different melting curves were distinguishable with two homozygous types (A/A for ‘PMS’ and one F₂ plant, -/- for ‘KNU-12’) and a heterozygous type (A/- for the F₁ plant) (Fig. 6).

307 **Full-length cDNA sequencing of SlMlo1.1**

308 The full-length cDNA sequence of the SlMlo1 gene was analyzed by RACE PCR to identify any additional sequence variations in its coding region in ‘KNU-12’ and ‘PMS’ plants (Supplementary File. 2). For each line, three cDNA molecules (three colonies) were sequenced, and the 1-bp indel in the 5ᵗʰ exon was confirmed for all sequences. No additional sequence variation was detected between ‘KNU-12’ and ‘PMS’. However, a frameshift mutation caused by the 1-bp deletion possibly resulted in the loss of function of the Ol-2 gene product (designated as SlMlo1.1 in this study) in ‘KNU-12’ plants (Bai et al. 2008).

315 Interestingly, a deletion of the entire 9ᵗʰ exon was also detected in one of the three cDNA molecules cloned from ‘KNU-12’. Therefore, we attempted to verify whether this cDNA molecule originated from a SlMlo homolog that is present in a different genomic location (Zheng et al. 2016), or if it originated from alternative splicing during the RNA processing of Slmlo1.1. The PCR amplification of genomic DNA of ‘KNU-12’ using the primer set for the two introns flanking the 9ᵗʰ exon resulted in a wild-type PCR band of 842 bp without the deletion, indicating that the deletion of the 9ᵗʰ exon might be due to alternative splicing. We further investigated the level of alternative transcripts by PCR amplification of the cDNA samples synthesized as described in the ‘Detection of alternative transcripts’ section. The cDNA samples were PCR amplified using the primer set ‘ASC-
1’ designed for binding to the 8th exon (forward primer) and 10th exon (reverse primer) flanking the 
9th exon. In addition to the strong PCR band with the wild-type allele size (172 bp), a very weak 
PCR band showing around 122 bp indicative of the deletion of the 9th exon was observed in all 
amplified cDNA samples, including non-inoculated leaves (Fig. 7). Thus, alternative splicing 
possibly occurred in both ‘KNU12’ and ‘PMS’ samples, but it does not seem to be event-specific or 
related to the inoculation of the PM pathogen.

Discussion

Gene SlMlo1 is a member of the SlMlo family, and it is homologous to the Mlo (Mildew locus 
O) gene (Zheng et al. 2016) of barley that has been shown to be involved in broad-spectrum PM 
resistance (Bai et al. 2008). Although PM resistance involving ol-2 is not associated with the race-
specific HR observed in dominant resistance loci, it is known to have a non-race-specific 
mechanism involving the formation of papillae (plant cell wall appositions) that arrest fungal 
penetration (Bai et al. 2005).

A previous genome-wide study of the tomato SlMlo gene family characterized 15 SlMlo 
homologs (Zheng et al. 2016). Using transgenic plants, Zheng et al. (2016) also demonstrated that 
simultaneous silencing of SlMlo1 and two of its closely related homologs, SlMlo5 and SlMlo8, 
conferred a higher level of resistance than the ol-2 mutation. However, the possibility that PM 
resistance in ‘KNU-12’ arose from a naturally occurring mutant allele of SlMlo5 or SlMlo8 has been 
rulled out, as the Slmlo1.1 sequence in ‘KNU-12’ was a perfect match, except for a single deletion. 
Furthermore, the previously reported ol-2 gene-based marker [M/SlMol, a sequence characterized 
amplified region (SCAR) marker targeting the 19-bp deletion in the 7th exon of SlMlo1] (Bai et al. 
2008) was not polymorphic between ‘KNU-12’ and ‘PMS’, suggesting that PM resistance in ‘KNU-
12’ is conferred by another resistance allele of Slmlo1 (ol-2). This allele was newly identified in the present study based on gene sequence analysis and an association study using an F2:3 population. The ol-2 allele was originally identified from ‘LA1230’, an accession of S. lycopersicum var. cerasiforme (a cherry tomato-type cultivar) (Jones et al. 2001; Bai et al. 2005). Horticultural traits of ‘KNU12’ are similar to that of S. lycopersicum var. cerasiforme (data not shown) and a phylogenetic study based on the SoICAP single nucleotide polymorphism array showed that ‘KNU-12’ was clustered in the group of S. lycopersicum var. cerasiforme accessions (Sim et al. 2015). This implies that ‘KNU12’ belongs to the subspecies S. lycopersicum var. cerasiforme. The ol-2 gene (Slmlo1) in S. lycopersicum var. cerasiforme, which is an ortholog of Mlo in barley, has advantages with respect to tomato breeding. Although other PM resistance genes are found in wild species of tomato, the ol-2 gene originated from cherry tomato-type accessions that represent an admixture of wild (S. pimpinellifolium) and cultivated species (S. lycopersicum). By using this subspecies as a source of PM resistance genes, breeders can easily introduce the gene into cultivated cherry or round tomato cultivars while avoiding the adverse effects of linkage drag that may result from using wild sources of resistance genes. In addition, the loss-of-function mutation in Ol-2 in tomato (SlMlo) has been reported to result in a broad-spectrum, non-race-specific resistance to PM, like that in barley (Mlo) and Arabidopsis (AtMlo) homologs (Pavan et al. 2010). It has also been suggested that wild-type Mlo homologs are plant factors required and targeted by both adapted and non-adapted PM to induce PM susceptibility (Appiano et al. 2015). Tomato Slmlo mutants overexpressing barley wild-type Mlo (HvMlo) were susceptible to O. neolycopersici and to a non-adapted PM species, Blumeria graminis f. sp. hordei. Similarly, tomato Slmlo1 mutants proved to be less susceptible to L. taurica compared to a wild-type control cultivar, but they restored susceptibility when transformed with pepper wild-type CaMlo2 (Zheng et al. 2013).
During the validation of *SlMlo1.1* gene-based markers using commercial *F*₁ cultivars susceptible to PM, PCR bands of various sizes were detected by SCAR_SlMlo1.1 among different cultivars, indicating that the 14-bp deletion in an intron is not unique to ‘KNU-12’ and is not associated with the PM resistance phenotype. Diverse polymorphisms at this intron may be attributed to the different number of (TA)ₙ SSR motifs carried by different cultivars. However, for the 1-bp indel marker, most cultivars showed the marker genotype for ‘PMS’. This implies that the 1-bp deletion at 3rd amino acid in 15th exon might induce a frameshift in the coding region, altering the function of SlMlo1. The *Mlo* gene family codes for proteins harboring seven transmembrane domains (TMs) and a calmodulin-binding site (CaMBD) that are functionally conserved among monocot and dicot species (Appiano et al. 2015). The CaMBD is positioned in the 15th exon of *Mlo*, which codes for the extreme carboxy-terminal end of the protein. Thus, the frameshift mutation induced by the 1-bp deletion in this region might significantly impair the molecular function of SlMlo.

In addition, in terms of gene evolution, finding the intron deletion allele in many *F*₁ cultivars, while the exon-15 deletion was only observed in two cultivars, indicated that exon deletion might have occurred after the mutation in the intron. The two *F*₁ cultivars that showed the dCAPS marker genotype for ‘KNU-12’ need to be further evaluated for their resistance phenotype. Nevertheless, the uniqueness of the 1-bp indel found in elite commercial cultivars indicated that the dCAPS marker can be used for efficient and effective MAS of the *Slmlo1.1* allele of ‘KNU-12’ as a source of PM resistance. Incorporating recessive gene(s) such as *Slmlo1.1* can be more difficult and time consuming than incorporating dominant allele(s) in a conventional breeding process. Thus, dCAPS is also advantageous for selecting recessive homozygous plants at early growth stage using molecular markers.

The putative alternative splicing of *SlMlo1* observed in the present study might be related to the diversification of disease resistance genes (R genes) and their mechanism of conferring resistances.
The crucial role of alternative splicing in regulating plant defense responses has been reported for many R genes, including \textit{RPS4} in \textit{Arabidopsis} (Zhang and Gassmann 2007), \textit{Mla6} and \textit{Mla13} in barley (Dennis et al. 2003), and \textit{Pi-ta} in rice (Yang et al. 2014). Isoforms produced by alternatively spliced transcripts may suppress the negative regulation of immunity by pathogens or directly engage in effector-triggered signaling (Yang et al. 2014). In tobacco, the tobacco mosaic virus (TMV)-resistance gene \textit{N} specifically recognizes the 50 kDa helicase (p50) of TMV. Alternative splicing of \textit{N} resulted in translation of an isoform (\textit{N}_{AT}) in addition to the usual isoform (\textit{N}_{RT}), and the ratio of these isoforms was related to TMV tolerance (Dinesh-Kumar and Baker 2000). However, it is unclear whether truncated transcripts of \textit{Slmlo1.1} in ‘KNU-12’ have any influence on its level of resistance to PM.

In conclusion, PM resistance in a cherry tomato-type accession ‘KNU-12’ is conferred by a natural null allele (\textit{Slmlo1.1}) of \textit{SlMlo} that forms multiple alleles of \textit{ol-2}. A 1-bp deletion in a coding region of \textit{Slmlo1.1} causes a frameshift mutation that possibly results in the loss-of-function of \textit{SlMlo1}. The dCAPS and HRM markers developed in the present study for detecting this deletion could be efficiently used for MAS where ‘KNU-12’ is utilized as a source for durable and broad-spectrum resistance to PM.

\textbf{Acknowledgments}

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References


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Figure Captions

**Fig. 1.** Powdery mildew disease in tomato. (A) Tomato leaf showing symptoms of powdery mildew (*Oidium neolycopersici*) infection in Miryang, South Korea. Light microscopy photographs of tomato powdery mildew pathogen collected from Miryang, Gyeongnam Province (B) and Muju, Jeonnam Province (C) in South Korea. Conidiophores producing single conidia (anamorph of *Pseudoidium* sp.) indicate that these two isolates correspond to *O. neolycopersici*. Bar = 30 μm.

**Fig. 2.** Evaluation of public markers linked to powdery mildew (PM) resistance in the tomato inbred accessions ‘KNU-12’ (PM-resistant) and ‘PMS’ (PM-susceptible). DNA samples of ‘KNU-12’ and ‘PMS’ were tested using two replicates per marker. Polymorphic bands (red arrows) were only detected in marker GP79L. The SlMlo1 gene-based marker M/SlMlo1 for the *ol-2* locus is monomorphic between ‘KNU-12’ and ‘PMS’.

**Fig. 3.** Location of the mutation sequences and primer sets used for genomic DNA amplification of *SlMlo1* (SGN gene annotation number: Solyc04g049090). The first exon and exons 7\(^{th}\) to 15\(^{th}\) are displayed in gray boxes. Exons 2\(^{nd}\) to 6\(^{th}\) are not represented. Introns are displayed as the gray diagonal line. Numbers below exons indicate the location of the first nucleotide of each exon based
on the genomic sequence of \textit{SlMlo1}. The 19-bp deletion in powdery mildew (PM) resistance line LA1230 is shown as a yellow vertical line in the 7\textsuperscript{th} exon. This 19-bp deletion was used to develop the SCAR marker M/\textit{SlMlo1} (Bai et al. 2008). The 14-bp deletion in an intron between the 14\textsuperscript{th} and 15\textsuperscript{th} exons and the 1-bp deletion in the 15\textsuperscript{th} exon that were observed in the PM-resistance line ‘KNU-12’ are indicated by the red horizontal and vertical lines, respectively. Genomic regions amplified by the primer sets ‘1-1’, ‘1-2’, and ‘1-3’ are indicated by horizontal lines above the gene sequence. The 9\textsuperscript{th} exon, which is predicted to be alternatively spliced in ‘KNU-12’, is presented in black. Blue arrows above the 8\textsuperscript{th} and 10\textsuperscript{th} exons represent the forward (ASC-1F) and reverse (ASC-1R) primers that were used for assessing alternative splicing of the 9\textsuperscript{th} exon.

\textbf{Fig. 4.} Comparison of partial \textit{SlMlo1} gene sequences that were PCR-amplified by primer set ‘1-3’ and cloned from tomato inbred line ‘KNU-12’ (powdery mildew resistance) and ‘PMS’ (powdery mildew susceptibility). A 14 bp insertion/deletion (indel) in the 14\textsuperscript{th} intron (in red) and a 1 bp indel (bold letter) in the 15\textsuperscript{th} exon were detected. The 14\textsuperscript{th} exon (>14) and 15\textsuperscript{th} exon (>15) are indicated by gray shading. Solyc04g049090 is the genomic DNA sequence of \textit{SlMlo1} in the reference genome of tomato.

\textbf{Fig. 5.} Genotyping of the F\textsubscript{2} population derived from ‘KNU-12’ [powdery mildew (PM)-resistant] × ‘PMS’ (PM-susceptible) using \textit{ol-2} gene-based SCAR_\textit{SlMlo1.1} (upper lanes) and dCAPS_\textit{SlMlo1.1} (lower lanes) markers. M, 100-bp marker; K, ‘KNU-12’; P, ‘PMS’. From the 3\textsuperscript{rd} lane to the right, 46 F\textsubscript{2} individuals were tested as indicated in Table 2 (F\textsubscript{2:3} \textendash 1–F\textsubscript{2:3} \textendash 66). Cosegregation between the marker genotype and disease reaction was observed for both markers. The relatively weak band corresponding to the ‘PMS’ allele in heterozygous dCAPS_\textit{SlMlo1.1} was
possibly due to the forward primer (enzyme site-derived primer) having lower PCR efficiency on the wild-type allele compared to the mutant allele.

**Fig. 6.** Development of the high-resolution melting (HRM) maker (HRM SlMlo1.1) based on the 1-bp indel in the 15\textsuperscript{th} exon of the powdery mildew (PM) resistance gene Slmlo1.1. The HRM marker genotype of PM-resistant tomato ‘KNU-12’ and PM-susceptible tomato ‘PMS’ and their F\textsubscript{2} progeny are shown in blue and yellow melting curves, respectively. The red melting curve with two peaks indicates the maker genotype of heterozygous F\textsubscript{1} progeny.

**Fig. 7.** Assessment of alternative splicing in the 9\textsuperscript{th} exon of Slmlo1.1. M, 100-bp marker; gDNA-Control, amplified using gDNA of powdery mildew-resistant tomato inbred line ‘KNU-12’ by a primer set designed for the two introns flanking the 9\textsuperscript{th} exon (PCR amplicon size: 842 bp); SP-Clone, amplified using the plasmid of the 9\textsuperscript{th} exon deletion mutation by a primer set (ASC-1) binding to the 8\textsuperscript{th} and 10\textsuperscript{th} exons (122 bp); WT-Clone, amplified using the plasmid of the wild-type sequence (172 bp), cDNAs-0, 12, 24, 48; amplified from cDNAs extracted from ‘KNU-12’ at 0, 12, 24, and 48 h post inoculation using the primer ‘ASC-1’. Weak PCR bands of cDNAs-0, 12, and 24 specific to SP-Clone indicate a possible alternative splicing of SlMlo1.
Supplementary File Captions

Supplementary File 1. DNA sequence of rRNA ITS of PM pathogen isolate from Muju (MJ_PM1) and Miryang (MR_PM1) (A) and 29 ITS sequence accessions (GeneBank ID) that showed 100% identity to MJ_PM1 and MR_PM1 (B).

Supplementary File 2. Multiple sequence alignment of full-length cDNA sequences revealed by the RACE PCR of ‘PMS’ and ‘KNU12’. KNU12-1 and KNU12-2 are two different colonies with sequence variation at the 9\textsuperscript{th} exon (the 9\textsuperscript{th} exon sequence is missing in KNU12-2 possibly due to alternative splicing). The first base of each exon is marked by > and by the number of the corresponding exon. The deleted 9\textsuperscript{th} exon in KNU-12 and the 1-bp indel for dCAPS_SlMlo1.1 are marked by red dashes. Primer sequences for ASC-1 and dCAPS_SlMlo1.1 are shown in gray blocks. Start (ATG) and stop (TGA) codons are shown in bold.
Table 1. Primers used in the present study.

<table>
<thead>
<tr>
<th>Use</th>
<th>Primer name</th>
<th>Primer sequence (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITS sequencing</td>
<td>PMITS₁</td>
<td>F: TCGGACTGGCCTCAGGGAGA</td>
</tr>
<tr>
<td></td>
<td>PMITS₂</td>
<td>R: TCACTCGCCGTTACTGAGGT</td>
</tr>
<tr>
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<td>1-1</td>
<td>F: AACATGTGTGCCTATTGTTCG</td>
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<td></td>
<td>R: TCATTGAAGTTTTGTGCAAC</td>
</tr>
<tr>
<td></td>
<td>1-2</td>
<td>F: CGTATCTTTGGTGCCATT</td>
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<td></td>
<td></td>
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<td></td>
<td>1-3</td>
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<td></td>
<td></td>
<td>R: TTGGTTCCAAAAAGTAAAATCTGACA</td>
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<td>R: ATGGCACCCCAAAGATACGAG</td>
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Table 2. Published DNA markers that are linked to the loci associated with powdery mildew (PM) resistance in tomato.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Primer sequence (5’-3’)</th>
<th>Marker type (enzyme)</th>
<th>Chr.</th>
<th>Target locus</th>
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</table>
| dct136  | F: CGAAGTGTCGGATCCGAAGGTTTT  
R: AACACAATCGGAaaaaa  
(\textit{XmnI}) | dCAPS  
(\textit{XmnI}) | 6  
\textit{Ol-qtl1} |
| Y258    | F: GTAATTCAAAAAGTGAGGT  
R: TTTGCGTCTAGAGTTATTTT  
(\textit{MboI}) | CAPS  
(\textit{MboI}) | 12  
\textit{Ol-qtl3} |
| tg111   | F: TGGCAACCGGACAAAGA  
R: TGGGAAAGTGATTAGACAGGACA | SCAR  
(\textit{DdeI}) | 12  
\textit{Ol-qtl3} |
| P2147   | F: TAAACATCTGACCATAGTTCC  
R: CACATCCGAATTTCCCTCC  
(\textit{Ddel}) | CAPS  
(\textit{Ddel}) | 6  
\textit{Ol-1, Ol-5} |
| tg25-1  | F: TAAATTTGCGACTCGCGT  
R: TTGTYATRTTGYTATTCG | SCAR  
(\textit{DdeI}) | 6  
\textit{Ol-1, Ol-5} |
| tg25-2  | F: TAAATTTGCGACTCGCGT  
R: CATGTGGYGTATATCGAGT | SCAR  
(\textit{DdeI}) | 6  
\textit{Ol-1, Ol-5} |
| H9A11   | F: TGCTCTAAACAAACTACCAAAATC  
R: AAATGTTCAACAACACAGACAGTATGAG | SCAR  
(\textit{DdeI}) | 6  
\textit{Ol-1, Ol-5} |
| Tom316  | F: GAGTTGTACCTTGTAGATG  
R: TAGATATTCCTGATGATGT | SSR  
(\textit{ApoI}) | 4  
\textit{Ol-2} |
| Tom332  | F: GATACCATTTAAAGCTCATTTCC  
R: GGTTCGCTCATATATGCTAG  | SSR  
(\textit{ApoI}) | 4  
\textit{Ol-2} |
| U3-2    | F: AGTTGTTGCGGATAGGTG  
R: TTGGCAACGTTGGGAAAACT | SCAR  
(\textit{ApoI}) | 4  
\textit{Ol-2} |
| M/SIMlo1| F: ACCCTTAAGAAACTAGGCAAA  
R: ACCATCATGAACCATGACGTCT  | SCAR  
(\textit{ApoI}) | 4  
\textit{Ol-2} |
| GP79L   | F: CACTCAATGGGGGAAAGCAAC  
R: AATGGAAACGCCGGGACT  
(\textit{ApoI}) | CAPS  
(\textit{ApoI}) | 6  
\textit{Ol-4, Ol-6} |
| 32.5Cl  | F: ACACGAACAAAAGTGCCAAG  
R: CCACCACAAACACAGGAGTGTG  
(\textit{HinfI}) | CAPS  
(\textit{HinfI}) | 6  
\textit{Ol-4, Ol-6} |
**Table 3.** Disease assay of powdery mildew (PM) and genotyping of *ol-2* gene-based SCAR_SlMlo1.1 marker for 111 F$_{2:3}$ families derived from the tomato (*Solanum lycopersicum*) inbred line ‘KNU-12’ (PM-resistant) x ‘PMS’ (PM-susceptible).

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<th>F$_{2:3}$</th>
<th>Phenotype$^a$</th>
<th>Genotype$^b$</th>
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<th>Phenotype$^a$</th>
<th>Genotype$^b$</th>
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|  17 | 0.0 | 0.0 | R | R | R | 72 | 2.5 | 2.8 | S | S | S | 123 | 2.0 | 2.6 | S | H | Seg |
|  19 | 1.6 | 1.8 | S | H | Seg | 74 | 2.1 | 1.9 | S | H | Seg | 124 | 1.5 | 2.2 | S | H | Seg |
|  20 | 1.6 | 2.9 | S | S | S | 75 | 2.6 | 2.0 | S | H | Seg | 125 | 1.1 | 1.8 | S | H | Seg |
|  21 | 0.0 | 0.0 | R | R | R | 76 | 1.4 | 1.9 | S | H | Seg | 126 | 1.5 | 1.8 | S | H | Seg |
|  25 | 1.7 | 2.7 | S | S | S | 77 | 1.1 | 1.7 | S | H | Seg | 127 | 1.7 | 3.0 | S | S | S |
|  26 | 0.0 | 0.0 | R | R | R | 80 | 1.0 | 2.0 | S | H | Seg | 128 | 1.6 | 2.1 | S | H | Seg |
|  28 | 1.5 | 1.8 | S | S | S | 81 | 1.2 | 2.3 | S | H | Seg | 131 | 1.5 | 1.6 | S | H | Seg |
|  30 | 2.6 | 2.8 | S | S | S | 82 | 0.1 | 0.2 | R | R | R | 132 | 1.8 | 2.0 | S | H | Seg |
|  32 | 2.3 | 2.9 | S | S | S | 83 | 1.7 | 2.4 | S | H | Seg | 133 | 1.4 | 2.1 | S | H | Seg |
|  35 | - | 0.0 | R | R | R | 84 | 2.1 | 1.7 | S | H | Seg | 134 | 0.0 | 0.1 | R | R | R |
|  36 | 2.0 | 2.0 | S | H | Seg | 85 | 0.0 | 0.0 | R | R | R | 135 | 1.5 | 1.9 | S | H | Seg |
|  37 | 1.5 | 1.4 | S | H | Seg | 86 | 1.0 | 2.1 | S | H | Seg | 136 | 1.7 | 2.0 | S | H | Seg |
|  38 | 1.9 | 2.8 | S | S | S | 87 | 1.1 | 2.6 | S | H | Seg | 137 | 1.4 | 2.3 | S | H | Seg |
|  41 | 1.1 | 2.0 | S | H | Seg | 88 | 2.2 | 2.6 | S | S | S | 138 | 0.0 | 0.2 | R | R | R |
|  42 | 0.0 | 0.0 | R | R | R | 91 | 1.6 | 2.5 | S | H | Seg | 139 | 1.2 | 2.5 | S | H | Seg |
|  43 | 2.1 | 2.6 | S | S | S | 94 | 1.8 | 2.1 | S | H | Seg | 140 | 2.1 | 1.3 | S | H | Seg |
|  44 | 2.3 | 2.9 | S | S | S | 95 | 1.9 | 1.8 | S | H | Seg | 141 | 2.4 | 2.7 | S | S | S |
|  45 | 2.1 | 2.9 | S | S | S | 96 | 1.7 | 1.7 | S | H | Seg | 142 | 1.4 | 2.6 | S | S | S |
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|  46 | 1.9 | 2.8 | S | S | S | 97 | 1.7 | 2.0 | S | H | Seg | 143 | 2.5 | 2.4 | S | S | S | S |
|  48 | 1.8 | 2.6 | S | S | S | 99 | 0.0 | 0.0 | R | R | R | 144 | 0.9 | 2.3 | S | H | Seg |
|  49 | 0.0 | 0.1 | R | R | R | 101 | 1.6 | 2.6 | S | H | Seg | 145 | 1.0 | 1.8 | S | H | Seg |
|  50 | 2.0 | 1.5 | S | H | Seg | 102 | 1.6 | 2.5 | S | S | S | 147 | 1.4 | 2.0 | S | H | seg |
|  52 | 0.0 | 0.0 | R | R | R | 103 | 1.6 | 2.5 | S | H | Seg | 150 | 2.5 | 2.6 | S | S | S |
|  53 | 1.5 | 2.1 | S | H | Seg | 104 | 1.8 | 2.3 | S | H | Seg |
|  54 | 0.0 | 0.0 | R | R | R | 105 | 1.5 | 2.7 | S | H | Seg |
|  55 | 0.0 | 0.1 | R | R | R | 106 | 1.8 | 2.5 | S | S | S |

*aPhenotyping followed the disease assay method described in the text. ‘aver’ indicates the average value of disease index (DSI) in 2014 and 2015. R, resistant (average DSI < 1.0); S, susceptible (average DSI ≥ 1.0).*

*bF₂ individuals and 10 seedlings per F₃ family were genotyped using SCAR_SlMlo1.1 and dCAPS_SlMlo1.1 markers. R, homozygous marker genotype for ‘KNU-12’ (PM-resistant); S, homozygous marker genotype for ‘PMS’ (PM-susceptible); H, heterozygous marker genotype; seg, segregation.*
Fig. 1. Powdery mildew disease in tomato. (A) Tomato leaf showing symptoms of powdery mildew (Oidium neolycopersici) infection in Miryang, South Korea. Light microscopy photographs of tomato powdery mildew pathogen collected from Miryang, Gyeongnam Province (B) and Muju, Jeonnam Province (C) in South Korea. Conidiophores producing single conidia (anamorph of Pseudoidium sp.) indicate that these two isolates correspond to O. neolycopersici. Bar = 30 μm.
Fig. 2. Evaluation of public markers linked to powdery mildew (PM) resistance in the tomato inbred accessions 'KNU-12' (PM-resistant) and 'PMS' (PM-susceptible). DNA samples of 'KNU-12' and 'PMS' were tested using two replicates per marker. Polymorphic bands (red arrows) were only detected in marker GP79L. The SlMlo1 gene-based marker M/SlMlo1 for the ol-2 locus is monomorphic between 'KNU-12' and 'PMS'.
Fig. 3. Location of the mutation sequences and primer sets used for genomic DNA amplification of SlMlo1 (SGN gene annotation number: Solyc04g049090). The first exon and exons 7th to 15th are displayed in gray boxes. Exons 2nd to 6th are not represented. Introns are displayed as the gray diagonal line. Numbers below exons indicate the location of the first nucleotide of each exon based on the genomic sequence of SlMlo1. The 19-bp deletion in powdery mildew (PM) resistance line LA1230 is shown as a yellow vertical line in the 7th exon. This 19-bp deletion was used to develop the SCAR marker M/SlMlo1 (Bai et al. 2008). The 14-bp deletion in an intron between the 14th and 15th exons and the 1-bp deletion in the 15th exon that were observed in the PM-resistance line 'KNU-12' are indicated by the red horizontal and vertical lines, respectively. Genomic regions amplified by the primer sets '1-1', '1-2', and '1-3' are indicated by horizontal lines above the gene sequence. The 9th exon, which is predicted to be alternatively spliced in 'KNU-12', is presented in black. Blue arrows above the 8th and 10th exons represent the forward (ASC-1F) and reverse (ASC-1R) primers that were used for assessing alternative splicing of the 9th exon.
Fig. 5. Genotyping of the F2 population derived from ‘KNU-12’ [powdery mildew (PM)-resistant] × ‘PMS’ (PM-susceptible) using ol-2 gene-based SCAR_SlMlo1.1 (upper lanes) and dCAPS_SlMlo1.1 (lower lanes) markers. M, 100-bp marker; K, ‘KNU-12’; P, ‘PMS’. From the 3rd lane to the right, 46 F2 individuals were tested as indicated in Table 2 (F2:3-1–F2:3-66). Cosegregation between the marker genotype and disease reaction was observed for both markers. The relatively weak band corresponding to the ‘PMS’ allele in heterozygous dCAPS_SlMlo1.1 was possibly due to the forward primer (enzyme site-derived primer) having lower PCR efficiency on the wild-type allele compared to the mutant allele.
Fig. 6. Development of the high-resolution melting (HRM) maker (HRM_Slmlo1.1) based on the 1-bp indel in the 15th exon of the powdery mildew (PM) resistance gene Slmlo1.1. The HRM marker genotype of PM-resistant tomato ‘KNU-12’ and PM-susceptible tomato ‘PMS’ and their F2 progeny are shown in blue and yellow melting curves, respectively. The red melting curve with two peaks indicates the maker genotype of heterozygous F1 progeny.
Fig. 7. Assessment of alternative splicing in the 9th exon of SlMlo1.1. M, 100-bp marker; gDNA- Control, amplified using gDNA of powdery mildew-resistant tomato inbred line 'KNU-12' by a primer set designed for the two introns flanking the 9th exon (PCR amplicon size: 842 bp); SP-Clone, amplified using the plasmid of the 9th exon deletion mutation by a primer set (ASC-1) binding to the 8th and 10th exons (122 bp); WT-Clone, amplified using the plasmid of the wild-type sequence (172 bp), cDNAs-0, 12, 24, 48; amplified from cDNAs extracted from 'KNU-12' at 0, 12, 24, and 48 h post inoculation using the primer 'ASC-1'. Weak PCR bands of cDNAs-0, 12, and 24 specific to SP-Clone indicate a possible alternative splicing of SlMlo1.