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Marker-Assisted Evaluation of Two Powdery Mildew Resistance Candidate Genes in Korean Cucumber Inbred Lines

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Abstract: Two genes, *CsLRR-RPK2* (*CsGy5G015660*) and *CsaMLO8* (*Csa5G623470*), have been considered as powdery mildew (PM) resistance genes in cucumbers. In this study, we evaluated the involvement of the alleles of these two genes in PM resistance in 100 commercial Korean cucumber inbred lines. To achieve this, we developed cleaved amplified polymorphic sequences (CAPS) and InDel markers from *CsLRR-RPK2* and *CsaMLO8*. Genotyping analysis indicated that the *CsLRR-RPK2*-CAPS marker showed a stronger correlation with the PM-resistant phenotype, with an 84% consistency compared to the *CsaMLO8*-InDel marker. The use of the *CsaMLO8*-InDel marker showed a 70% consistency between phenotype and genotype results. It was proposed that the *CsLRR-RPK2*-CAPS marker successfully eliminated PM-susceptible inbred lines, since both genotype and phenotype results were 100% identical. Furthermore, the present study revealed that the introduction of one of these alleles is probably enough to confer PM resistance in cucumbers. However, seven PM-resistant inbred lines harbored either *CsaMLO8* or *CsLRR-RPK2* alleles, indicating that there is another PM-resistant resource(s) besides *CsaMLO8*- and *CsLRR-RPK2*-originated resistance in the commercial Korean inbred lines. Our results provide reliable evidence confirming two PM-resistant candidate genes for the detection of PM resistance resources in cucumber inbred lines.

Keywords: *Cucumis sativus* L.; *Podosphaera xanthii*; PM-resistance resource; *MLO*; *LRR-RPK2*

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1. Introduction

The cucumber [*Cucumis sativus* L. (2n = 2x = 14)] is a member of the Cucurbitaceae family, which is the same botanical family as melons and squashes [1]. It is an economically important vegetable cultivated worldwide [2]. Powdery mildew (PM) is one of the most serious productivity and quality-loss-inducing diseases in cucumber-growing fields and greenhouses worldwide. Two pathogens, *Podosphaera xanthii* and *Golovinomyces cichoracearum*, have been recognized as the hard-to-control fungi that cause PM disease [3–10]. Among them, *P. xanthii* is predominant in most countries, including Korea [3–7,9–12]. To date, the application of protective fungicides has been considered a vital way to control *P. xanthii*. However, *P. xanthii* has become resistant to fungicides for many years [13–18]. In addition, many consumers have become focused on social issues, such as environmental protection and public health. Accordingly, the most efficient and environmentally friendly way to control the spread of *P. xanthii* is the breeding of PM-resistant cultivars [7].

It is necessary to understand the genetic and molecular mechanisms of PM resistance in the breeding of PM-resistant cucumber cultivars. Several efforts have been made to identify the PM resistance loci in cucumber. As a result of these efforts, six temperature-dependent QTLs (chromosomes 1, 5, 6, and 7) from PI 197088-1, five PM-resistance QTLs (*PM1.1*, *PM1.2*, *PM2.1*, *PM2.2*, and *PM5.1*) from S06, two QTLs (chromosome 5) from PI 250147, four QTLs (*pm5.1*, *pm5.2*, *pm5.3*, and *pm6.1*) from K8, six QTLs (*pm1.1*, *pm1.2*, *pm3.1*, *pm4.1*, *pm5.1*, and *pm5.2*) from WI 2757, nine QTLs (*pm1.1*, *pm1.2*, *pm2.1*, *pm3.1*, *pm4.1*, *pm5.1*,

pm5.2, *pm5.3*, and *pm6.1*) from CS-PMR1, and four QTLs (*pm1.1*, *pm2.1*, *pm5.1*, and *pm 6.1*) from PI 197088 [1,6,19–23] have been identified. Although we have deep insight into the genetic inheritance of PM resistance, it is very difficult to directly use the information on PM-resistant QTLs to select the PM-resistant germplasm.

From a practical point of view, molecular markers for the selection of the PM-resistant germplasm from the above QTL regions should be developed. Several studies have reported candidate gene(s) that govern PM resistance in different cucumber germplasms. For example, Xu et al. [24] identified two genes (*Csa1G064780* and *Csa1G064790*) encoding a putative cysteine-rich receptor-like kinase from the 41.1-kb region of the Jin5-508-derived SSSL0.7 line. Three possible candidate genes, *Csa5G622800*, *Csa5G622830*, and *Csa5G623490* that encode, identified from *Cucumis hystris* introgression lines, and a single recessive gene (*Csa5G622830*), which encodes GATA transcription factors, have been considered candidates for controlling PM resistance [25]. From PI 197088 and *C. sativus* cv. Anaxo, a single-recessive gene (*CsaMLO8*, *Csa5G623470*) is considered a strong candidate gene for PM-susceptibility [16,17,26,27].

To date, *CsaMLO8* is the only cucumber PM-resistant gene whose function has been identified. Our previous recent study identified *CsGy5G015660*, which encodes a putative leucine-rich repeat receptor-like serine/threonine kinase (*CsLRR-RPK2*), as a strong PM-resistant candidate gene derived from a Korean inbred line [28]. In the current study, we applied PM-resistant molecular markers derived from two PM-resistant candidate genes (*CsaMLO8* and *CsLRR-RPK2*) to 100 Korean inbred lines to evaluate the accuracy of marker-assisted selection. Furthermore, we attempted to understand the origin of PM resistance in Korean cucumber inbred lines.

2. Materials and Methods

2.1. Plant Materials and Cultivation

In the present study, we used 100 commercial Korean cucumber inbred lines, including sixty-two PM resistant lines. The F₁ progenies were generated by crossing different PM-susceptible (GHDHW, HD, LC, CPA, RV, FPDN and MDSS) and PM-resistant parental lines (CA/794, 2K79, 64E2K9, 2JKJ, 249, 253XNH, K9AKJ, 64E3K9, 045911., KJK9, JCDS and 02789AGN). Detailed information is shown in Table 1. In this study, twelve different PM-resistant resources were used as PM-resistant donor plants (Table 1). Consecutive self-pollinations from these progenies were performed to obtain the F₃–F₉ generation and pure lines. The seeds used in this study were kindly supplied by DONGOH SEED (Ansung, Korea). All seeds used in this study were germinated indoors in the spring of 2020 and then transplanted to DONGOH SEED greenhouse. All plants were irrigated, fertilized, and treated with pesticides according to the standard cultivation protocol of DONGOH SEED.

Table 1. List of PM resistant and PM-susceptible lines used for making inbred lines.

Crossing Combination		Types of PM Resistance Source
Resistance Source	Susceptible Source	
CA/794 (♀)	GHDHW (♂)	A
2K79 (♀)	HD (♂)	B
64E2K9 (♀)	HD (♂)	C
2JKJ (♀)	LC (♂)	D
249 (♂)	LC (♀)	E
253XNH (♂)	CPA (♀)	F
K9AKJ (♀)	CPA (♂)	G
64E3K9 (♀)	RV (♂)	H
045911 (♀)	RV (♂)	I
KJK9 (♀)	CPA (♂)	J
JCDS (♂)	FPDN (♀)	K
02789AGN (♂)	MDS (♀)	L

Note: (♀) and (♂) represent the maternal and paternal lines used for crossing.

2.2. Evaluation of PM Response

Disease symptoms caused by natural infection of *P. xanthii* under greenhouse conditions became apparent 60 days after transplantation. When PM disease symptoms were clearly distinguishable, phenotypic scoring of PM resistance was conducted at 2-week intervals until disease symptoms had spread to all parts of the susceptible plants. The plants were considered to be PM-susceptible when the white spores of *P. xanthii* covered more than 10% of the overall cucumber leaves. Others were considered to be PM resistant. Ten individuals from each cucumber inbred line were used for the evaluation of PM response. Finally, 100 Korean cucumber inbred lines were clearly distinguishable in the PM-resistant and PM-susceptible groups. The PM resistance and PM susceptibility are presented in Figure 1 and Table 2.

Figure 1. Typical phenotypic responses of PM infection. (a,b) Represent PM-susceptible and PM resistant phenotypes, respectively.

Table 2. PM phenotypic evaluation and genotyping analysis results of 100 cucumber inbred lines.

Line No.	Generation ^a	Resistance Source ^h	Phenotypic Evaluation (PE) ^b	<i>CsaMLO8</i> ^c	PE vs. <i>CsaMLO8</i> ^d	<i>CsLRR-RPK2</i> ^e	PE vs. <i>CsLRR-RPK2</i> ^f	<i>CsaMLO8</i> vs. <i>CsLRR-RPK2</i> ^g
DS 1	8	-	S	S	True	S	True	True
DS 2	9	-	S	S	True	S	True	True
DS 3	9	-	S	S	True	S	True	True
DS 4	9	-	S	S	True	S	True	True
DS 5	9	-	S	S	True	S	True	True
DS 6	5	-	S	S	True	S	True	True
DS 7	5	-	S	S	True	S	True	True
DS 8	5	-	S	S	True	S	True	True
DS 9	5	-	S	S	True	S	True	True
DS 10	5	-	S	S	True	S	True	True
DS 11	5	-	S	S	True	S	True	True
DS 12	5	-	S	S	True	S	True	True
DS 13	5	-	S	S	True	S	True	True
DS 14	5	-	S	S	True	S	True	True
DS 15	9	-	S	S	True	S	True	True
DS 16	9	-	S	S	True	S	True	True
DS 17	9	-	S	S	True	S	True	True
DS 18	9	-	S	S	True	S	True	True
DS 19	5	-	S	S	True	S	True	True
DS 20	5	-	S	S	True	S	True	True
DS 21	5	-	S	S	True	S	True	True
DS 22	5	-	S	S	True	S	True	True
DS 23	5	-	S	S	True	S	True	True

Table 2. Cont.

Line No.	Generation ^a	Resistance Source ^h	Phenotypic Evaluation (PE) ^b	<i>CsaMLO8</i> ^c	PE vs. <i>CsaMLO8</i> ^d	<i>CsLRR-RPK2</i> ^e	PE vs. <i>CsLRR-RPK2</i> ^f	<i>CsaMLO8</i> vs. <i>CsLRR-RPK2</i> ^g
DS 24	5	-	S	S	True	S	True	True
DS 25	5	-	S	S	True	S	True	True
DS 26	5	-	S	S	True	S	True	True
DS 27	7	-	S	S	True	S	True	True
DS 28	7	-	S	S	True	S	True	True
DS 29	7	-	S	S	True	S	True	True
DS 30	P	-	S	S	True	S	True	True
DS 31	8	-	S	S	True	S	True	True
DS 32	8	-	S	S	True	S	True	True
DS 33	8	-	S	S	True	S	True	True
DS 34	8	-	S	S	True	S	True	True
DS 35	9	-	S	S	True	S	True	True
DS 68	7	A	R	S	False	R	True	False
DS 72	6	B	R	R	True	R	True	True
DS 86	6	C	R	R	True	R	True	True
DS 88	6	C	R	R	True	S	False	False
DS 92	6	D	R	R	True	R	True	True
DS 101	5	D	R	S	False	R	True	False
DS 102	5	D	R	S	False	R	True	False
DS 103	5	D	R	S	False	R	True	False
DS 106	5	D	R	R	True	R	True	True
DS 111	5	D	R	R	True	R	True	True
DS 112	5	D	R	R	True	R	True	True
DS 114	5	D	R	R	True	S	False	False
DS 115	5	D	R	R	True	R	True	True
DS 116	5	D	R	R	True	R	True	True
DS 117	5	D	R	R	True	R	True	True
DS 119	5	D	R	R	True	R	True	True
DS 121	5	C	R	R	True	S	False	False
DS 122	5	C	R	R	True	R	True	True
DS 123	5	D	R	R	True	R	True	True
DS 124	5	H	R	R	True	R	True	True
DS 125	5	H	R	R	True	R	True	True
DS 126	5	H	R	R	True	S	False	False
DS 128	4	E	R	R	True	R	True	True
DS 129	4	E	R	R	True	R	True	True
DS 130	4	E	R	R	True	R	True	True
DS 131	4	E	R	R	True	R	True	True
DS 133	4	E	R	R	True	R	True	True
DS 195	6	F	R	S	False	R	True	False
DS 196	6	F	R	S	False	R	True	False
DS 197	4	G	R	S	False	R	True	False
DS 198	4	G	R	R	True	R	True	True
DS 200	4	H	R	R	True	R	True	True
DS 201	4	H	R	R	True	S	False	False
DS 202	4	H	R	R	True	S	False	False
DS 204	4	H	R	R	True	S	False	False
DS 205	4	H	R	S	False	R	True	False
DS 206	4	H	R	S	False	R	True	False
DS 208	4	H	R	R	True	R	True	True
DS 209	4	H	R	S	False	R	True	False
DS 211	4	C	R	R	True	R	True	True
DS 212	4	C	R	S	False	R	True	False
DS 213	4	C	R	R	True	R	True	True
DS 214	4	C	R	R	True	R	True	True
DS 215	4	C	R	S	False	S	False	True
DS 216	4	C	R	R	True	R	True	True
DS 217	4	C	R	R	True	S	False	False
DS 219	4	C	R	S	False	S	False	True
DS 221	3	I	R	R	True	S	False	False
DS 223	3	I	R	S	False	S	False	True
DS 225	3	I	R	S	False	S	False	True
DS 229	3	J	R	S	False	R	True	False
DS 234	3	J	R	S	False	R	True	False
DS 235	3	J	R	S	False	R	True	False
DS 241	3	C	R	S	False	S	False	True

Table 2. Cont.

Line No.	Generation ^a	Resistance Source ^h	Phenotypic Evaluation (PE) ^b	<i>CsaMLO8</i> ^c	PE vs. <i>CsaMLO8</i> ^d	<i>CsLRR-RPK2</i> ^e	PE vs. <i>CsLRR-RPK2</i> ^f	<i>CsaMLO8</i> vs. <i>CsLRR-RPK2</i> ^g
DS 340	9	K	R	S	False	R	True	False
DS 341	9	K	R	S	False	R	True	False
DS 389	4	L	R	S	False	R	True	False
DS 394	4	L	R	S	False	S	False	True
DS 396	4	L	R	S	False	R	True	False
DS 398	4	L	R	S	False	R	True	False
DS 417	3	M	S	R	False	S	True	False
DS 420	3	M	S	R	False	S	True	False
DS 424	3	L	R	S	False	S	False	True
DS 425	3	L	R	S	False	R	True	False
DS 429	3	M	S	R	False	S	True	False

Note: ^a Represents the generations of progeny by consecutive crossings of self-pollination (F₃ to F₉ generations and pure line (P), respectively). ^b Represents the degree of PM disease symptoms. ^c and ^e represent the genotyping analysis results using the *CsaMLO8*-InDel and *CsLRR-RPK2*-CAPS markers, respectively. ^d and ^f show the consistency between the phenotypic and genotyping analysis results. True and False represent the consistency and inconsistency between the phenotypic evaluation and genotyping analysis results, respectively. ^g True or False represents the consistency and inconsistency between the two genotyping analysis results. Underlined and bold represent the seven PM-resistant inbred lines, which did not originate from introgression of either *CsaMLO8* or *CsLRR-RPK2* alleles. ^h Represents the original sources of the PM resistance. The same character indicates that the same PM-resistant germplasm was used as the PM-resistant donor for the breeding of inbred lines. The PM resistance sources A, B, C, D, E, F, G, H, I, J, K, and L are shown in Table 1.

2.3. Genomic DNA Isolation and Molecular Marker Design

Fresh young leaves of 100 Korean cucumber germplasms were used to isolate genomic DNA (gDNA). The DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was used to isolate gDNA. The quality and quantity of the gDNAs were calculated using a Nanodrop ND-2000 spectrophotometer, following the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA, USA). Geneious Pro 9.1.8 (Biomatters, Auckland, New Zealand) was used to (I) design InDel and cleave amplified polymorphic sequences (CAPS) markers for *CsaMLO8* and *CsLRR-RPK2*, respectively; and (II) identify appropriate restriction enzymes and restriction sites for the *CsLRR-RPK2* gene. *HinfI* enzyme (New England BioLabs, Ipswich, MA, USA) was selected and used as a restriction enzyme for the *CsLRR-RPK2*-CAPS marker. The sequences of primers for both markers are shown in Table 3, while the gene organization is presented in Figure 2. In this study, Gy14 (version 2.0) and Chinese Long (version 2.0) genome sequences were used as the cucumber reference sequences.

Table 3. Information on *CsLRR-RPK2*-CAPS and *CsaMLO8*-InDel markers.

Name of Gene	Marker Types	Restriction Enzyme	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
<i>CsLRR-RPK2</i>	CAPS	<i>HinfI</i>	GCAACAAGTTCAATGGACCAC	GAATCTCTCCAGTCAAATTGTTTC
<i>CsaMLO8</i>	InDel	-	TATGGCTGCCTTTCATCTCCT	TCCAAGCAAAGAAGGCAAGC

Figure 2. Schematic structure of two PM resistance alleles (*CsLRR-RPK2* and *CsaMLO8*). Exon–intron structures of *CsaMLO8* and *CsLRR-RPK2* genes are represented by black and white boxes, respectively. The gray arrows represent the forward and reverse primers of *CsaMLO8*-InDel and *CsLRR-RPK2*-CAPS markers, respectively. The gray triangle in the 11th exon of *CsaMLO8* represents the position of 1449 bp insertion. The white bar in the first exon of *CsLRR-RPK2* represents the SNP (T/C). The digits, 1–5498 and 1–3126 represent the genomic DNA length of *CsaMLO8* and *CsLRR-RPK2*, respectively.

2.4. Genotyping of Cucumber Inbred Lines

For the genotyping of *CsaMLO8* and *CsLRR-RPK2* alleles, we developed InDel and CAPS markers, respectively. *CsaMLO8*-InDel markers were composed of a combination of a forward primer (5'-TATGGCTGCCTTTCATCTCCT-3') and reverse primer (5'-TCCAAGCAAAGAAGGCAAGC-3'). DNA samples were amplified using *CsaMLO8*-InDel markers and followed by a polymerase chain reaction (PCR) reaction program: 94 °C for 5 min, 30 cycles of 94 °C for 20 s, 65 °C for 20 s, and 72 °C for 90 s, with a final cycle at 72 °C for 5 min. *CsLRR-RPK2*-CAPS markers were composed of a combination of forward primer (5'-GCAACAAGTTCAATGGACCAC-3') and reverse primer (5'-GAATCTCTCCAGTCAAATTGTTTCC-3'). DNA samples were amplified using *CsLRR-RPK2*-CAPS markers and the PCR program was as follows: 94 °C for 5 min, 35 cycles of 94 °C for 20 s, 60 °C for 10 s, and 72 °C for 20 s, with a final cycle at 72 °C for 5 min. For the PCR, Maxime PCR PreMix (iNtRON Biotechnology, Seoul, Korea) was used. PCR amplifications were performed in a Bio-Rad T100™ thermocycler (BioRad, CA, USA). *HinfI*, a restriction enzyme, was added to the PCR amplicons and incubated for 1 hour at 37 °C. The final reaction products were run on a 2% agarose gel with 0.5× Tris-borate-EDTA (TBE) buffer and visualized by staining with RedSafe (iNtRON Biotechnology, Seoul, Korea).

3. Results and Discussion

The evaluation of PM resistance and genotyping analysis using the markers for *CsaMLO8* and *CsLRR-RPK2* alleles in 100 Korean cucumber inbred lines is shown in Table 2 and Figure 3. Of the 100 cucumber inbred lines, 62 had a PM-resistant phenotype. In contrast, 38 inbred lines had a PM-susceptible phenotype. The two PM resistance candidate genes used in this study were *CsaMLO8* (*Csa5G623470*) and *CsLRR-RPK2* (*CsGy5G015660*). The *CsLRR-RPK2*-CAPS marker amplified the flanking region of the SNP (T/C) (Figures 2 and 3a). The SNP of the PM-susceptible line produced double bands (224 bp and 100 bp) after *HinfI* digestion, whereas the PM-resistant line showed an absence of restriction sites in the amplicon (Figure 3b). The *CsLRR-RPK2*-CAPS marker was successfully applied to detect the PM-resistant and PM-susceptible alleles using the ten phenotypically identified (five PM-resistant and five PM-susceptible lines) inbred lines (Figure 3c). The *CsaMLO8*-InDel marker detected 1449 bp insertion in PM-resistant lines (Figures 2 and 3d). Ten phenotypically identified inbred lines, consisting of five PM-resistant and five PM-susceptible lines, were also verified for the successful application of *CsaMLO8*-InDel (Figure 3d).

To compare the involvement of *CsaMLO8* and *CsLRR-RPK2* alleles in the PM-resistant phenotype, we applied *CsaMLO8*-InDel and *CsLRR-RPK2*-CAPS markers on 100 inbred lines. Genotyping analysis using the *CsaMLO8*-InDel marker revealed that 30 inbred lines were inconsistent with the phenotypic evaluation results (Table 2). In particular, most of the inconsistency was observed in PM-resistant lines; 44% (27/62) of PM-resistant inbred lines had PM-susceptible *CsaMLO8* alleles. Only 11% (3/28) of the PM-susceptible inbred lines (DS 417, DS 420, and DS 429) had the PM-resistant *CsaMLO8* allele. However, genotyping analysis using the *CsLRR-RPK2*-CAPS marker revealed fewer inconsistent results. In detail, 16 inbred lines showed inconsistency between *CsLRR-RPK2*-based genotyping and phenotypic evaluation results. Interestingly, all of the inconsistent results were obtained from the PM-resistant inbred lines. This indicates that analysis of the *CsLRR-RPK2* allele is useful for the elimination of PM-susceptible lines during PM resistance breeding programs. More interestingly, the seven PM-resistant inbred lines (DS 215, DS 219, DS 223, DS 225, DS 241, DS 394, and DS 424) based on phenotypic evaluation showed none of the PM resistance alleles of *CsaMLO8* and *CsLRR-RPK2*. This indicates that the PM resistance of these seven inbred lines did not originate from *CsaMLO8* or *CsLRR-RPK2*, but originated from different PM resistance sources. Further studies should be conducted, using these PM-resistant inbred lines, to identify PM-resistant QTL(s) and/or PM-resistant gene(s) to reveal the origin of a new PM-resistant source(s).

Figure 3. Genotyping analysis of parental and inbred lines using *CsLRR-RPK2*-CAPS and *CsaMLO8*-InDel markers. (a) represents the bands amplified by *CsLRR-RPK2*-CAPS primers. (b) Represents the band patterns after application of *HinfI* enzyme. (c) Represents the application of *CsLRR-RPK2*-CAPS marker to the 10 inbred lines (5 PM-resistant and 5 PM-susceptible inbred lines). (d) Represents the bands of parental lines amplified by *CsaMLO8*-InDel primers and application to the 10 inbred lines (5 PM-resistant and 5 PM-susceptible inbred lines). M, CA794, 2K79, GHDHW, and HD represent the molecular markers (100 bp and 1 kb), PM resistant (CA/794 and 2K79), and PM-susceptible (GHDHW and HD) parental lines, respectively. DS1, DS2, DS3, DS4, DS5 and DS72, DS86, DS92, DS106, and DS111 represent the inbred lines of PM-susceptible and PM-resistant lines, respectively. The digits next to the black arrows represent the size of the PCR band.

Furthermore, we classified the sources of PM resistance used in this study (Tables 1 and 2). We detected three different types of PM resistant sources from 12 PM-resistant germplasms used for the generation of 62 PM resistance inbred lines. Firstly, a PM resistance source originated from the *CsLRR-RPK2* gene, represented as A, J, and K (Table 2; Supplementary Figure S1). Secondly, a PM resistance source originated from both the *CsLRR-RPK2* and *CsaMLO8* genes, represented as B, D, E, F, G, and H (Table 2; Supplementary Figure S1). Thirdly, a PM resistance source originated from an unidentified PM resistance source and also contained both resistance genes (*CsLRR-RPK2* and *CsaMLO8*), represented as C, I, and L (Table 1). These results reveal that divergent sources (at least 1–3 PM-resistant genes were introduced) of PM resistance have been introduced in PM-resistant cucumber breeding programs in Korea. Further extensive study and comparison of phenotypic evaluation and genotyping analysis using increased numbers of PM-resistant sources and their progenies should be conducted, because a limited number of PM-resistant sources and progenies were used in this study.

Comparison of genotyping analysis results between *CsaMLO8* and *CsLRR-RPK2* showed 32% (32 inbred lines) inconsistency (Table 1). The three PM-susceptible lines (417, 420, and 429) showed PM-resistant *CsaMLO8* alleles, but not PM-resistant *CsLRR-RPK2* alleles. Twenty-nine PM-resistant inbred lines showed either PM-susceptible alleles of *CsaMLO8* (21 lines) or *CsLRR-RPK2* (8 lines). This indicates that PM resistance could be acquired by the introgression of either one of the alleles of *CsaMLO8* and/or *CsLRR-RPK2*. Although current phenotypic evaluation analysis results did not strongly support

the additive effect of both alleles of *CsaMLO8* and *CsLRR-RPK2* on the number of PM resistance, we observed a moderate correlation between the degree of PM-resistant alleles and the PM-resistant phenotype (data not shown). This fact should be verified by further extensive phenotypic evaluation of divergent PM-resistant inbred lines.

When we compared the allele types of *CsaMLO8* and *CsLRR-RPK2*, 68 inbred lines showed alleles of both PM resistance and PM susceptibility. However, 32 inbred lines showed different allele types between *CsaMLO8* and *CsLRR-RPK2*. This fact can be explained in different ways. Considering the high proportion (more than two-fold) of the same alleles of *CsaMLO8* and *CsLRR-RPK2*, it may be thought that both alleles could easily co-migrate during meiosis. However, both genes (*CsLRR-RPK2* and *CsaMLO8*) are located 8.8 Mb apart on Chromosome 5. When we consider the genetic recombination (bin) of Chromosome 5 as 0.37 cM, equivalent to 228 kb [29], it is unlikely to co-migrate during meiosis. In addition, it has been proposed that three segregation distorted regions (SDR) could exist on Chromosome 1, 4, and 6 [29]. The SDRs also cannot explain the co-migration of both allele pairs, because the Chromosome 5 is not likely to contain SDRs. Considering the fact that most inconsistencies were observed before the F₄ generation, each allele of *CsLRR-RPK2* and *CsaMLO8* probably followed independent meiotic recombination events. Race identification of PM has been well identified in melons [30–36]. For example, 12 races of PM (race 1, 2, 3, 4, 5, 6, 7, N3, N4, A, O, and S) have recently been identified in melons [33]. However, race identification of PM in cucumbers has not been well identified. In Korea, Race 1 of PM has been proposed to be dominant. Therefore, we do not have a detailed population structure of the races of PM in cucumbers. This suggests that each allele of *CsLRR-RPK2* and *CsaMLO8* may be responsible for different races of PM in cucumbers. Breeders should continuously select the cucumber plants that show strong resistance to PM. Therefore, consecutive selections of PM-resistant lines were followed after self-pollination. The consecutive selection of PM-resistant lines from generation to generation may accumulate PM-resistant allele pairs (*CsaMLO8* and *CsLRR-RPK2*) in PM-resistant lines. This could be a more plausible explanation than the co-migration of both allele pairs during meiotic division.

4. Conclusions

In this study, we applied PM-resistant molecular markers derived from two PM-resistant candidate genes (*CsaMLO8* and *CsLRR-RPK2*) to 100 Korean inbred lines to evaluate the accuracy of marker-assisted selection and to understand the origin of PM resistance in breeding inbred lines in Korea. Our results provided reliable evidence that confirms two PM-resistant candidate genes and efficiently utilized these genes for the detection of PM-resistant resources and inbred lines. Furthermore, the *CsLRR-RPK2*-CAPS marker in comparison to the *CsaMLO8*-InDel marker showed a strong correlation with the PM-resistant phenotype. In addition, our results revealed that unidentified PM-resistant sources used in breeding programs in Korea are not related to either PM-resistant candidate gene (*CsaMLO8* and *CsLRR-RPK2*).

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11112191/s1>, Figure S1: *CsLRR-RPK2*-CAPS and *CsaMLO8*-InDel markers-based genotyping analysis results.

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