XVI. EUCARPIA

Capsicum and Eggplant Meeting

KECSKEMÉT • HUNGARY • 12-14.SEPT.2016

in memoriam
Dr. Alain Palloix

PROCEEDINGS

Editors:

Katalin Ertsey-Peregi
Zsuzsanna Füstös
Gábor Palotás
Gábor Csilléry

P5-06

Genetic mapping of the *Powdery Mildew Resistance* (*PMR1*) gene in pepper (*Capsicum annuum* L.)

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Abstract

Powdery mildew disease caused by *Leveillula taurica* is a serious fungal threat to greenhouse pepper production. In contrast to most epiphytic powdery mildew species, *L. taurica* is an endophytic fungus which colonizes in the mesophyll tissues of the leaf. In the genus *Capsicum*, several studies have been conducted to identify resistance sources to *L. taurica*. In the previous studies, five quantitative trait loci (QTLs) for powdery mildew resistance have been identified. An F_2:F_3 population derived from self-pollination of the *Capsicum annuum* commercial cultivar 'PM Singang' resistant to *Leveillula taurica* was used for genetic analysis of powdery mildew resistance. Resistance of the F_2:F_3 families were tested under the natural environmental conditions.White powder observed on infected leaves was used as a disease scale to determine resistance of plants. A total of 86 F_2: F_3 families were evaluated for resistance. The results showed that 16 F_2 plants were homozygous resistant, 50 F_2 were heterozygous resistant, and 20 F_2 were susceptible. The segregation ratio fitted to a single dominant resistance gene model and we named the resistance *Powdery Mildew Resistance 1* (*PMR1*). We developed two closely linked markers to the *PMR1* gene andrevealed that this gene is located on the chromosome 4. These developed markers will be used to fine mapping the *PMR1* locus and identify underlying resistance gene.

Keywords: powdery mildew, *Capsicum annuum*, *PMR1*, *Leveillula taurica*

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PROGRAM

PROGRAM

Sunday, 11 September

16:00	**Registration and Poster set up**
19:00-21:30	**Welcome reception**

Monday, 12 September

7:30-	**Registration**
8:30-9:30	**Opening Session** **Chair:** Sergio Lanteri **Lajos Helyes, Katalin Ertsey-Peregi** Welcome on behalf of the Hungarian Local Organizers **Jaime Prohens** Welcome by the EUCARPIA Representative **Véronique Lefebvre** Alain Palloix commemorative lecture **Miklós Fári** **Szent-Györgyi Memorial Lecture** Father of vitamin C, Albert Szent-Györgyi (1893-1986) and his time. Science, creativity and society behind a Nobel Prize winner (1937)
9:30-10:45	**Session 1 – Breeding Strategies** **Chairs:** Paul Bosland, Giuseppe L. Rotino **9:30-9:45** **Jaime Prohens** Utilization of crop wild relatives in eggplant pre-breeding for adaptation to climate change **9:45-10:00** **Gábor Palotás** New hot interspecific hybrid variety between *Capsicum annuum* L. and *Capsicum chinense* Jacq.

10:00-10:15
Sanjeet Kumar
Male sterility research in peppers at AVRDC – The World Vegetable Center

10:15- 10:30
Dario Danojevic
Sweet pepper breeding against bacterial spot *(Xanthomonas euvesicatoria)* in Serbia

10:30-10:45
Miklós Fári
What kind of root should a pepper plant have?

10:45-11:10 **Coffee break and Poster viewing** *sponsored by:*

11:10-11:55 **Session 1 – Breeding Strategies (ctd.)**
Chairs: Paul Bosland, Giuseppe L. Rotino

11:10-11:25
Roeland E. Voorrips
Aphid resistance in a *Capsicum* collection

11:25-11:40
Pál Salamon
Symptoms caused by *Tomato spotted wilt virus* (TSWV) in Pepper (*Capsicum* spp.) and marker assisted selection of TSWV resistant pepper lines for hybrid constructions

11:40-11:55
István Tóbiás
Evergreen question: Whether *Tobamoviruses* are transmitted via pepper seeds or not?

12:00-12:45 **Session 2 – Growing and Seed Production**
Chair: Zsuzsanna Füstös

12:00-12:15
Katalin Ertsey-Peregi
Sweet pepper (*Capsicum annuum* L.) growing on a basis of thermal water with respect of protection the natural environment

12:15- 12:30
John Damicone
Biology and management of bacterial spot of pepper in Oklahoma, United States

12:30- 12:45
András Kovács
Short evaluation of eggplant production and variety usage in Romania

12:45-14:30 **Lunch**

14:30-14:50 **Gathering in the lobby for the technical visit**

15:00-19:30 **Technical visits**
- Pepper and eggplant trials (ZKI)
- Pepper processing plant (UNIVER)

Open field trip

19:30-22:30 **Traditional folklore dinner**

Tuesday, 13 September

8:30-10:00 **Session 3 – Genetic Resources**
Chairs: Jaime Prohens, Marie-Christine Daunay

8:30-8:45
Marie-Christine Daunay
Eggplant resistance to bacterial wilt and to *Fusarium* wilt: Is there a link?

8:45-9:00
Zsuzsanna Füstös
Study of morphological characteristics of eggplant *(Solanum melongena* L.*)* varieties

9:00-9:15
Claudio Dal Zovo
Wild *Capsicum* in the area of the Amboró National Park in Bolivia

9:15-9:30
John Samuels
Solanum insanum L. (Solanaceae): Linnaean species or introgressed hybrid?

9:30-9:45
Olga Babak
Development of DNA-markers to fruit quality genes of sweet pepper *(Capsicum annuum* L.*)*

9:45-10:00
Rosana Rodrigues
A breeding program for resistance to anthracnose in sweet and chili pepper

10:00-10:15
François Villeneuve
Screening of solanaceous wild relatives for graft affinity with eggplant *(Solanum melongena* L.*)*

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10:15-10:40 **Coffee break and Poster viewing** *sponsored by:*

10:40-11:40 **Session 3 – Genetic Resources (ctd.)**
Chairs: Jaime Prohens, Marie-Christine Daunay

10:40-10:55
Awang Maharijaya
Antixenosis and antibiosis based resistance of chilli pepper to melon aphid

10:55-11:10
Orarat Mongkolporn
Genetic diversity of Thai native chili using diversity arrays technology

11:10-11:25
Lucie Tamisier
Quantitative trait loci in pepper genome control the effective population size of two RNA viruses at inoculation

11:25-11:40
Helena Stavělíková
Germplasm of pepper *(Capsicum annuum* L.*)* in Czech Republic

11:40-12:55 **Session 4 – Physiology and Nutritional Value**
Chair: Lajos Helyes

11:40-11:55
Zsuzsanna Füstös
The nutrition value and storage of eggplant *(Solanum melongena* L.*)* varieties

11:55-12:10
I. Kutalmis Kutsal
Effects of mychorriza on pepper plant growth parameters and nutrient uptake under salinity stress

12:10-12:25
Kietsuda Luengwilai
Does anthracnose resistance associate with cuticle characteristics and spore attachment?

12:25-12:40
Ozlem Altuntas
Effects of michorriza on alleviating salt stress of *Capsicum annuum* L. by ion regulation

12:40-12:55
Lajos Helyes
Correlation between carotenoid components of chili pepper fruits and VIS/NIR reflectance

13:00-14:45 **Lunch and Poster viewing**

14:45-16:15 **Session 5 – Molecular Genetics and Biotechnologies**
Chairs: Sergio Lanteri, Anikó Gémes Juhász

14:45-15:00
Ezio Portis
A high quality eggplant *(Solanum melongena* L.*)* genome sequence

15:00-15:15
Zoltán Kristóf
Ultrastructural study of *in vitro* and *in situ* pepper embryo development

15:15-15:30
Giuseppe L. Rotino
MicroRNA156/7-Mediated control of anthocyanin pigment accumulation in eggplant fruit skin

15:30-15:45
Rodrigo A. VALVERDE
Interactions between *Bell pepper endornavirus*, bell pepper, and acute plant viruses

15:45-16:00
Santiago Vilanova
The transcriptomes of *Solanum incanum* and *S. aethiopicum* provide information of relevance for common eggplant breeding

16:00-16:15
Sylvia E. Salgon
Genetic mapping of broad-spectrum QTLs and strain-specific major QTL for resistance to *Ralstonia solanacearum* in eggplant using GBS

16:15-16:30
Yoshiyuki Tanaka
Multiple mutated putative aminotransferase alleles contribute to low pungency and capsinoid biosynthesis in *Capsicum chinense*

16:30-16.50 **Coffee break** *sponsored by:*

16:50-19:30 **Optional cultural program**

20:00-23:00 **Gala dinner**

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Wednesday, 14 September

8:30-10:30 **Poster session**
Chair: Katalin Ertsey-Peregi

10:30-10:45 **Coffee break** *sponsored by:* Dél-Kerti SZENTES

10:45-11:45 **Session 5 – Molecular Genetics and Biotechnologies (ctd.)**
Chairs: Sergio Lanteri, Anikó Gémes Juhász

10:45-11:00
Laura Toppino
QTLs mapping for *Fusarium oxysporum* and *Verticillium dahliae* resistance in eggplant *(Solanum melongena* L.*)*

11:00-11:15
Pasquale Tripodi
Genotyping by sequencing for population structure and genome-wide association analysis for fruit shape and size in pepper (*C. annuum* L.) germplasm

11:15-11:30
Jana Leide
Cutin deficiency of bell pepper *(Capsicum annuum* L.*)* results in gluey berries

11:30-11:45
Hatira Taskin
Comparison of pepper genotypes originated from Turkey and the other countries for anther culture response

11:45-12:15 **Conclusion and closing**

12:15-14:00 **Lunch**

14:30- **Optional post-conference tours**

LIST OF POSTERS

Session 1 – Breeding Strategies

Poster number	Presenting author	Title of poster
P1-01	**András Andrásfalvy**	István Túri – The innovative pepper breeder
P1-02	**Andrea Moór**	Lambert Angeli, pioneering breeder of the first white, sweet variety of bell pepper was born a hundred years ago
P1-03	**Zoltán Timár**	The life and work of a paprika breeder Ferenc Márkus
~~P1-04~~ WITHDRAWN	~~**Saadet Büyükalaca**~~	~~Investigation of obtaining fertile *S. melongena x S. torvum* hybrid populations~~
P1-05	**Ros Caridad**	Could quantitative resistance increase the durability of major genes conferring nematode resistance in pepper?
P1-06	**Dilek Kandemir**	Determination of reaction of *Solanum aethiopium* and *Solanum incanum* genotypes against *Fusarium oxysporum f. sp. melongenae*
P1-07	**Gábor Palotás**	20 years of non-hypersensitive, non-specific, recessive resistance in pepper – review
P1-08	**Mariola Plazas**	Screening for drought tolerance in eggplant relatives and interspecific hybrids
P1-09	**Claudia Ribeiro**	Breeding Calabrian pepper lines (*Capsicum annuum* L.) for Brazilian agriculture from *sui generis* introduction of germplasm
P1-10	**Claudia Ribeiro**	Synthesis of a base population of Habanero chile pepper and initial assessment of derived F_3 lines *(Capsicum chinense)*
P1-11	**Attila Rózsás**	Conservation, landscape and home garden varieties in South part of Hungary
P1-12	**Zsolt Sági**	Higher quality traits – breeding strategies in pepper
P1-13	**Csaba Sebesi**	High quality apple peppers for the canning industry
P1-14	**Olga Timina**	Breeding use of *Capsicum annuum* L. mutant genepool
P1-15	**Péter Varró**	Breeding of a high yielding white waxy hibrid ZKI 113485 for the Mediterranean region
P1-16	**Lajos Zatykó**	The role of general and specific combining abilities in pepper hybrid breeding

Genetic Mapping of the *Powdery Mildew Resistance* (*PMR1*) Gene in Pepper (*Capsicum annuum*)

Jinkwan Jo[1], Jelli Venkatesh[1], Koeun Han[1], Yeaseong Ha[1], Ayoung Jung[1], and Byoung-Cheorl Kang[1]*

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Abstract

Pepper powdery mildew caused by *Leveillula taurica* is considered to be the major fungal disease of peppers (*Capsicum annuum*) grown in greenhouse. Previous studies have demonstrated that powdery mildew resistance has a complex mode of inheritance. In the present study, a novel powdery mildew resistant gene, *PMR1* introgressed in a resistant breeding line, 'VK515 R' was investigated using the $F_{2:3}$ families derived from a cross between the resistant parent 'VK515 R' and the susceptible parent 'VK515 S'. Genetic analysis of the 'VK515' $F_{2:3}$ families showed a single dominant inheritance of the resistance. Genetic mapping showed that the *PMR1* locus is located on pepper chromosome 0 in a 4 Mb-region between CZ2_11628 and HRM4.1.6 markers. No recombinants were observed in 4 Mb-region, one SCAR marker and five SNP markers were found to be 0 cM apart from the *PMR1* locus, which could be due to the suppressed recombination, as revealed by the GBS analysis. In addition, comparison of species-specific InDel markers as well as GBS derived SNP markers indicated that *C. baccatum* as a possible source of alien introgression of powdery mildew resistance in 'VK515 R'. The molecular markers developed herein especially helpful in marker-assisted selection in pepper breeding programs against powdery mildew resistance.

Objectives

- To develop molecular markers linked to *PMR1* in *Capsicum*
- To conduct fine mapping of the *PMR1* gene
- To investigate the origin of the *PMR1* gene

Materials and Methods

Plant materials

- Resistant parent *C. annuum* 'VK-515 R' (Samsung Seeds Co., Ltd., Korea)
- Susceptible patent *C. annuum* 'VK-515 S' (Samsung Seeds Co., Ltd.)
- Resistant control is the commercial cultivar *C. annuum* 'PM Singang' (Nongwoo Bio Co., Ltd.)
- Susceptible control is the commercial cultivar *C. annuum* 'Bukang' (Hungnong Seed Co.)
- 'VK515' 102 $F_{2:3}$ families were derived from a 'VK515 R' × 'VK515 S' cross

Inoculum preparation and disease infection

- Infected 'Bukang' and 'VK515 S' plants were kept around $F_{2:3}$ plants grown in plastic trays (50 cell trays) at one-tray intervals.
- Presence or absence of white fungal hyphae observed on infected leaves 60 days after sowing was used as a measure of disease infection.

Chromosomal localization

- Genotyping was performed with the Fluidigm® EP1™ system (Fluidigm, USA), (Kang et al, 2014)

Genotyping-by-sequencing (GBS)

- Disestion: *Pst*I and *Mse*I
- Sequencing: Illumina Hiseq 2000
- CLC genomics workbench

Genetic mapping of *PMR1* locus

- CarthaGene Software and MapChart 2.3 software

Comparative map analysis of the *PMR1* locus using GBS derived SNP markers

- Genome: *C. annuum* L_Zunla-1, *C. chinense* v.1.2, C. *baccatum* v.1.2 (Prof. Choi of SNU)

Phygenetic analysis

- DARwin 6.0.9

Results and Discussion

Inheritance analysis of the *PMR1* gene

Among 102 'VK515' $F_{2:3}$ families, 24 families (a total of 451 plants) were resistant homozygous, 48 families (a total of 898 plants) were segregating, and 30 families (a total of 582 plants) were susceptible homozygous, which showed a good fit to 1:2:1 ratio (χ^2 = 1.06; P = 0.59), suggesting that resistant to powdery mildew is controlled by a single dominant gene, *PMR*1 in 'VK515 R' (Table 1).

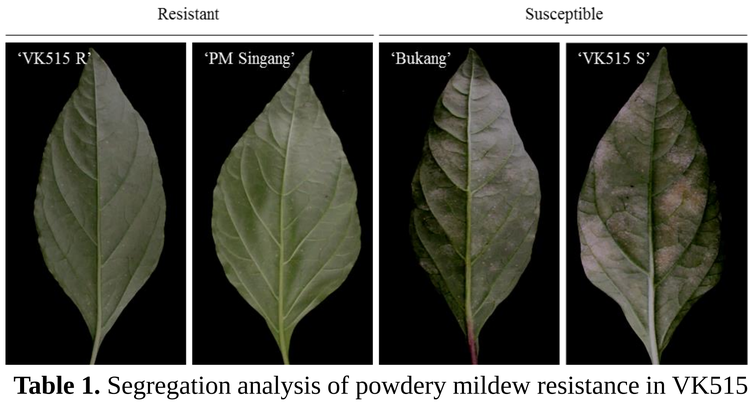

Fig. 1 Phenotype analysis of powdery mildew resistance in pepper lines. Comparison of phenotypes of resistant, susceptible parental lines, and commercial pepper cultivar infected with *L. taurica*.

Table 1. Segregation analysis of powdery mildew resistance in VK515 families

Population	Number of plants/families	Phenotype			Expected ratio	χ^2	P-value
		R	H	S			
'VK515 R'	20	20					
'VK515 S'	20			20			
'VK515' F_1	20	20					
'VK515' $F_{2:3}$ families	102 (1931)	24 (451)	48 (898)	30 (582)	1 : 2 : 1	1.06	0.59

R, resistant; H, heterozygous; S, susceptible; Number of $F_{2:3}$ 'VK515' plants used were indicated in parenthesis.

Marker development and genetic mapping of the *PMR1* locus

Genotyping was performed with the Fluidigm® EP1™ system and *PMR1* locus were localized on chromosome 0. Therefore, more markers were tried to be added around the *PMR1* locus using genomic information of *Capsicum*.

A total of six polymorphic markers (ZL1_10691, CZ2_11628, ZL1_1826, KS16052G01, HRM2_A4, and HRM4.1.6) were developed and mapped in 102 'VK515' $F_{2:3}$ families (Fig. 2). Based on genetic analysis, the *PMR1* locus was delimited to a 1 cM-region between CZ2_11628 and HRM4.1.6 markers on chromosome 0 (Fig. 2). Among the six markers used, three markers, ZL1_1826, KS16052G01, and HRM2_A4, were co-segregated with powdery mildew resistant phenotype and found to be at a genetic distance of 0 cM from the *PMR1* locus (Fig. 2).

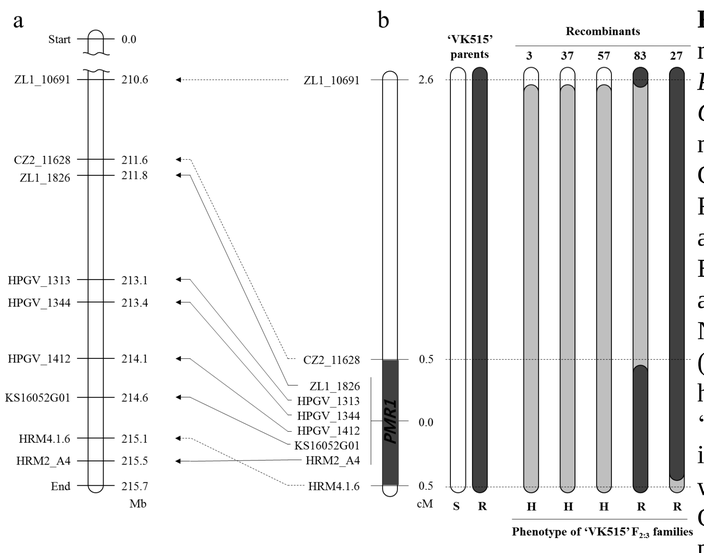

Fig. 2 Comparative genetic linkage and physical maps of the powdery mildew resistance gene *PMR1*. **a** Physical location of linked scaffolds in *C. chinense* v.1.2. **b** Physical location of linked markers in Zunla-1 genome chromosome 0 .**c** Genetic map of 'VK515' $F_{2:3}$ families. Recombinant heterozygous resistant plants 3, 37, and 57; homozygous resistant plants 27 and 83. Eight SNP markers linked to the *PMR1* locus are indicated next to pepper chromosome 0. Numbers on the right indicate genetic distances (cM). Black and white rectangles indicate the homozygous intervals of 'VK515 R' and 'VK515 S' chromosome 0, and gray rectangles indicate heterozygous intervals. The *PMR1* gene was delimited to a 4 Mb-region between the CZ2_11628 and HRM4.1.6 markers on the pepper 'L_Zunla-1' chromosome 0

Investigation origin of the *PMR1*

To know the origin of the *PMR1* gene, flanking markers (ZL1_1826 and HRM4.1.6) sequence were compared among *Capsicum* species. ZL1_1826 and Chr4.1.6 (HRM4.1.6) marker sequences with species-specific InDels indicated that the *PMR1* locus from 'VK515 R' are closely related to both *C. baccatum* and *C. chinense* than 'VK515 S' or *C. annuum* (Fig. 3a). And the GBS data were aligned with *C. annuum* 'L_Zunla-1', *C. chinense*, and *C. baccatum* genomic sequences. Based on GBS data 22 SNPs (Table 2) were detected in the *PMR1* locus. Phylogenetic analysis of the GBS data (Fig. 3b) from the *PMR1* locus revealed clustering of 'VK515 S', *C. annuum*, and *C. chinense*, whereas 'VK515 R' and *C. baccatum* shares a common node suggesting that the *PMR1* locus in the 'VK515 R' is closely related to *C. baccatum*. These results indicate that the *PMR1* locus might be introgressed from the *C. baccatum*.

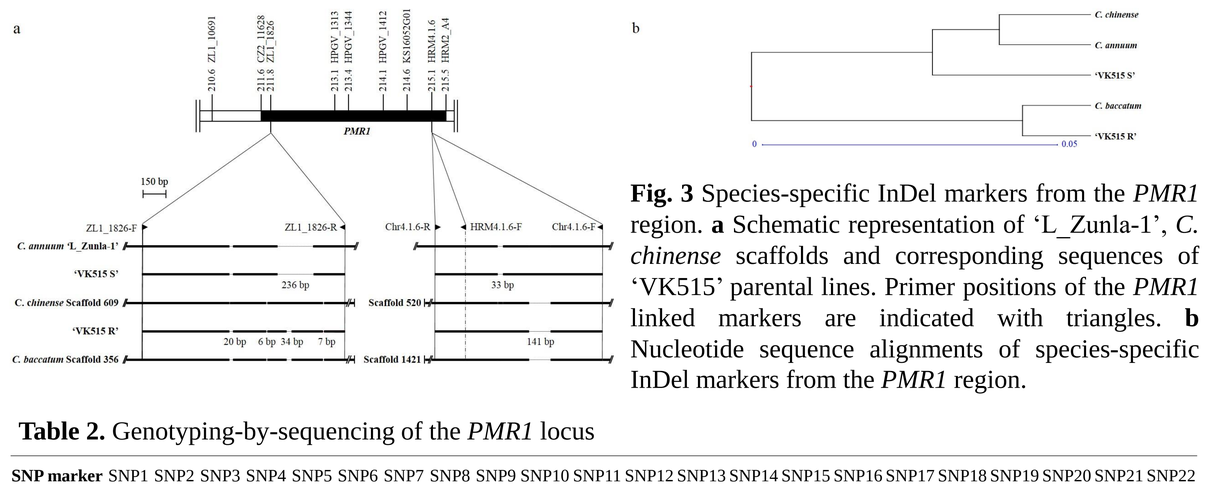

Fig. 3 Species-specific InDel markers from the *PMR1* region. **a** Schematic representation of 'L_Zunla-1', *C. chinense* scaffolds and corresponding sequences of 'VK515' parental lines. Primer positions of the *PMR1* linked markers are indicated with triangles. **b** Nucleotide sequence alignments of species-specific InDel markers from the *PMR1* region.

Table 2. Genotyping-by-sequencing of the *PMR1* locus

SNP marker	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10	SNP11	SNP12	SNP13	SNP14	SNP15	SNP16	SNP17	SNP18	SNP19	SNP20	SNP21	SNP22
C. chinense	G	G	G	A	G	C	G	C	G	G	G	C	T	T	C	A	A	T	T	G	G	G
C. annuum	A	G	G	A	G	C	T	C	G	G	G	C	T	T	C	A	A	T	T	G	G	G
'VK515 S'	A	G	G	A	G	C	G	C	G	G	G	C	T	C	C	A	A	T	T	G	C	A
C. baccatum	G	G	A	G	A	T	T	T	A	A	T	A	A	T	T	G	G	C	C	A	G	G
'VK515 R'	G	A	A	G	A	T	T	T	A	A	T	A	A	T	T	G	G	C	C	A	G	G

Conclusions

Phenotype and genetic analysis of powdery mildew resistance in pepper cv. 'VK15 R' identified a single dominant gene for powdery mildew resistance. We have mapped the *PMR1* locus to a 0 cM genetic interval on chromosome 0, co-segregating with six molecular markers. The molecular markers that we developed herein will be especially useful in MAS and pyramiding of powdery mildew resistance genes into an elite cultivar as they are tightly linked to the *PMR1* locus and the availability of efficient SNP marker detection platforms. Further fine mapping and candidate gene analyses are needed to reveal the relationship between the predicted receptor kinase/NBS-LRR genes and the *PMR1* gene.

References

- Kang JH, Yang HB, Jeong HS, Choe P, Kwon JK, Kang BC (2014) Single nucleotide polymorphism marker discovery from transcriptome sequencing for marker-assisted backcrossing in *Capsicum*. Kor J Hortic Sci 32:535-543.
- Kim DH, Park JH, Lee JS, Han KS, Han YK, Hwang JH (2009) Effect of temperature, relative humidity on germination and development of powdery mildew (*Leveillula taurica*) on pepper and its inoculation method. *Res Plant Dis* 15:187-192.

Acknowledgement

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