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Candidate Susceptibility Genes for Powdery and Downy Mildew in Watermelon and Squash

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Abstract

Powdery mildew (PM) caused by *Podosphaera xanthii* and downy mildew (DM) caused by *Pseudoperonospora cubensis* are two of the most economically important diseases for watermelon (*Citrullus lanatus*) and squash (*Cucurbita pepo*, *C. maxima* and *C. moschata*). Traditional breeding for resistance to PM and DM is resource intensive, often requiring decades' long phenotyping and selection processes. As an alternative, durable and broad-spectrum resistance to PM and DM can be obtained through loss-of-function of susceptibility genes in elite breeding material. Susceptibility genes for PM [Mildew-Locus-O (MLO) and Powdery Mildew Resistance (PMR)] and DM [Downy Mildew Resistance (DMR)] have been functionally proven in model plant species. Previous studies have reported candidate MLO genes for *C. lanatus* and *C. pepo*, but none for *C. maxima* and *C. moschata*. On the contrary, no PMR or DMR candidate genes have been identified for *C. lanatus* or any of the *Cucurbita* species. The current study used bioinformatics approaches based on sequence similarity, phylogenetic relationships and presence of conserved domains to predict candidate MLO genes in *C. maxima* and *C. moschata* and PMR and DMR genes in *C. lanatus*, *C. pepo*, *C. maxima* and *C. moschata*. Four MLO homologs in *C. maxima* and five in *C. moschata* clustered within Clade V, a clade containing all MLO susceptibility genes in dicots, and had highly conserved transmembrane domains and C-terminal PM interaction motif. Sixty-three candidate PMR genes were identified among the four species, 16 of which had close similarity to functionally proven PMR homologs in model species. Similarly, 37 candidate DMR genes were identified 12 among which clustered with functionally proven DMR homologs in model species. Functional analysis of the genes identified in the current study will reveal their role in pathogenesis and assess their potential for manipulation through gene editing methods to generate novel resistant plant genotypes.

Keywords: Powdery mildew; Downy mildew; MLO; DMR; PMR; Watermelon; Squash

Introduction

Watermelon (*Citrullus lanatus*) and squash (*Cucurbita pepo*, *C. maxima* and *C. moschata*) are economically important cucurbits in the U.S. with a combined annual value of approximately 0.75 billion dollars [1]. Powdery mildew (PM) caused by *Podosphaera xanthii* and downy mildew (DM) caused by *Pseudoperonospora cubensis* are economically important diseases for the two vegetable crops [2] and can cause significant yield and quality losses to growers if not properly managed. Strict preventative fungicide spray regimens are often required to keep plants healthy and free from disease [3]. Although effective, persistent use of fungicides poses toxicity hazards to humans and non-target organisms, as well as a risk for fungicide-resistance [4-6]. Genetic resistance is the most preferred management option for the two diseases. However, traditional breeding for PM and DM resistance is resource intensive, often requiring decades' long phenotyping and selection processes.

Knockout of susceptibility genes, also known as S-genes, may be a rapid and effective method for conferring resistance in cultivated plant species. Inactivation of S-genes through gene-knockout, gene-knockdown or virus induced gene-silencing has been proven a viable option for generating resistance genotypes against important diseases such as rice blast in rice (*Oryza sativa*) [7], citrus canker in citrus (*Citrus paradisi*) [8] and powdery mildew in grapevine (*Vitis vinifera*) [9], tomato and pepper [10]. Currently, two classes of PM S-genes are known, Mildew-Locus-O (MLO) genes and Powdery Mildew Resistance (PMR), both of which are loss-of-function genes [11-12]. Since the first discovery of MLO genes in barley in 1942, many MLO-like genes have been described and annotated in several model species including *Arabidopsis thaliana* and *Solanum lycopersicum* [11-12]. Phylogenetic analysis of MLO proteins reveal as many as 6 distinct clades, denoted clades I through VI, and Clade V harbors all the proven MLO susceptibility genes for PM in dicots [11]. MLO genes are characterized by 7 transmembrane helices, similar to metazoan and fungal G-protein coupled receptors [13]. All the MLO susceptibility genes in clade V share highly conserved domains within transmembrane portions of the protein and harbor a four amino acid long motif at the C-terminus that has been associated with PM interaction [14]. Functional clade V MLO proteins negatively regulate defense pathways at the site of PM inoculation, thus allowing the pathogen to induce pathogenesis [11]. Therefore, a loss of function in MLO genes consequently induce resistance to PM [10,15]. PMR genes on the other hand are far less studied and most are putative, but some have been shown to play a role in PM susceptibility in *Arabidopsis* (AtPMR4, AtPMR5, and AtPMR6) and tomato (SlPMR4) [16-19]. PMR genes are involved in cell wall biology where they mediate structure formation and pectin accumulation [18]. In *Arabidopsis*, loss-of-function in AtPMR4, which encodes callose synthase, resulted in PM resistant genotypes [16].

Two S-genes for downy mildew, DMR1 and DMR6, have been described in *Arabidopsis* and tomato [12,19-21]. dmr1 and dmr6 mutants induce resistance to DM by accumulating elevated levels of homoserine [21] and 2OG-Fe(II) oxygenase [20], respectively.

In *cucurbits*, MLO candidate genes have been identified in cucumber (*Cucumis sativus* [12], watermelon (*C. lanatus*), melon (*Cucumis melo*) and squash (*C. pepo*) [22]. However, no MLO candidate genes have been identified for *C. maxima* or *C. moschata*. Similarly, information on candidate genes for PMR and DMR in *C. lanatus*, *C. pepo*, *C. maxima* and *C. moschata* is currently lacking. Identification of MLO, PMR and DMR genes across the Cucurbitaceae family is an indispensable prerequisite for fundamental studies into the functional role of candidate genes in pathogenesis, and subsequently, identification of potential targets for genetic manipulation to generate novel resistant plant genotypes. Therefore, the objective of the current study was to use bioinformatics approaches based on sequence similarity, phylogenetic relationships and presence of conserved domains to predict candidate MLO genes in *C. maxima* and *C. moschata* and PMR and DMR genes in *C. lanatus*, *C. pepo*, *C. maxima* and *C. moschata*.

Materials and Methods

Identification of MLO, PMR and DMR homologs and their chromosomal locations

Amino acids sequences for MLO genes in dicots *Arabidopsis* (*Arabidopsis thaliana*, AtMLO1-AtMLO15), *Capsicum annuum* (CaMLO2), *Solanum lycopersicum* (SlMLO1), *Pisum sativus* (PsMLO1), *Medicago truncatula* (MtMLO1), *Lotus japonicas* (LjMLO1), *Vitis vinifera* (VvMLO1) and *Cucumis sativus* (CsaMLO1, CsaMLO8 and CsaMLO11)], and monocots [*Hordeum vulgare* (HvMLO), *Triticum aestivum* (TaMLO_A1b and TaMLO_B1) and *Oryza sativa* (OsMLO2 and OsMLO3)], PMR genes in *Arabidopsis* (AtPMR4, AtPMR5 and AtPMR6), tomato (SlPMR4) and cucumber (CsaPMR4-2 and CsaPMR4-9), as well as DMR genes in *Arabidopsis* (AtDMR1 and AtDMR6), tomato (SlDMR1) and cucumber (CsaDMR6-1 and CsaDMR6-2), were collected from the NCBI protein database and the cucurbit genomics database.

Each dicot MLO protein was used in a blast search against the *C. maxima* and *C. moschata* genomes, while PMR and DMR proteins were searched against *C. lanatus*, *C. pepo*, *C. maxima* and *C. moschata* genomes (http://cucurbitgenomics.org/). For *C. lanatus*, *C. maxima* and *C. moschata*, a blastp search was used and the top five results were saved after removal of duplicates. However, since blastp function is not available for *C. pepo*, a tblastn search was performed against the unigene database (v1.0). The chromosomal distribution of MLO, PMR and DMR protein homologs across the *C. lanatus*, *C. pepo*, *C. maxima* and *C. moschata* genomes was visualized using Mapchart [23].

Sequence alignment, phylogenetic analysis and clade annotation

All MLO (monocots and dicots), PMR and DMR protein sequences from model species were aligned with protein sequences extracted from *C. lanatus*, *C. pepo*, *C. maxima* and *C. moschata* genomes using the ClustalW alignment program in MEGA6 software [24]. The neighbor-joining clustering method was used to generate bootstrap consensus phylogenetic trees using 100 replicates. For MLO genes, clades were annotated as described by Devoto et al. [25].

Conserved domain analysis of MLO proteins

All MLO-like sequences from *C. maxima* and *C. moschata* that clustered in clade V were aligned to functionally proven MLO proteins in dicots to confirm presence of conserved domains in the transmembrane protein (TM1 - TM7), and the PM interaction C-terminus (D/E-F-S/T-F) using ClustalO alignment with boxshade [14,26]. To build a MLO consensus sequence, an amino acid was regarded as conserved if at least 7 out of 8 of the functionally proven MLO proteins harbored it, or an amino acid with similar chemical properties as determined by the Rasmol color scheme [12,27]. The number of amino acids deviating from the conserved sequence in each of the candidate proteins as well as in all the clade V proteins was counted to determine degree of similarity.

Results and Discussion

MLO homologs, clade annotation and conserved domains

The search for MLO homologs revealed five unique proteins each in *C. maxima* (CmaMLO1, CmaMLO2, CmaMLO3, CmaMLO4 and CmaMLO5) and *C. moschata* (CmaMLO1, CmoMLO2, CmoMLO3, CmoMLO4 and CmoMLO5) (Table 1).

Homolog	Designation in genome database
Cucurbita maxima	
CmaMLO1	CmaCh18G008880.1
CmaMLO2	CmaCh02G008830.1
CmaMLO3	CmaCh04G013590.1
CmaMLO4	CmaCh13G001880.1
CmaMLO5	CmaCh20G004770.1
Cucurbita moschata	
CmoMLO1	CmoCh18G008900.1
CmoMLO2	CmoCh02G008830.1
CmoMLO3	CmoCh04G014330.1
CmoMLO4	CmoCh20G005110.1
CmoMLO5	CmoCh13G001880.1

Table 1: Mildew-locus-O homologs and their designation in the *Cucurbita maxima* and *Cucurbita moschata* genome databases.

All the ten homologs clustered in Clade V, a clade containing all MLO susceptibility genes in dicots (Figure 1). A similar study by Lovieno et al. [22] identified 14 MLO homologs in *C. lanatus* and 18 in *C. pepo*, three of which clustered in Clade V in the former and latter, respectively. In cucumber, Schouten et al. [12] identified 14 MLO-like proteins, 3 of which placed within clade V. Examination of the transmembrane portions of the MLO proteins as well as the PM interaction motif at the C-terminus (D/E-F-S/T-F) revealed a total of 119 conserved amino acids in the consensus sequence. Alignment of the consensus sequence to the functionally proven MLO proteins in clade V revealed high similarity, ranging from 94.8% to 100% (Table 2 and Figure 2). High level of similarity among clade V proteins has been noted in other plant species including cucumber [12], apricots (*Prunus*

armeniaca), peaches (*Prunus persica*), apples (*Malus domestica*) and strawberry (*Fragaria xananassa*) [11]. Although CmaMLO1 homolog in *C. maxima* clustered in Clade V, it is likely not a PM susceptibility gene since it lacks the C-terminus PM interaction motif, and is annotated as an ubiquitin-conjugating enzyme on the genome database (http://cucurbitgenomics.org/).

Homolog	Number of deviating amino acids	Similarity to consensus sequence (%)
AtMLO2	2	98.3
AtMLO6	3	97.5
AtMLO12	2	98.3
PsMLO1	2	98.3
MtMLO1	2	98.3
LjMLO1	6	94.9
SlMLO1	0	100
CaMLO2	0	100
CmaMLO2	2	98.7
CmaMLO3	7	95.5
CmaMLO4	1	99.4
CmaMLO5	1	99.4
CmoMLO1	3	98.1
CmoMLO2	2	98.7
CmoMLO3	8	94.8
CmoMLO4	1	99.4
CmoMLO5	1	99.4

Table 2: Similarity in amino acid sequence within the transmembrane proteins and the C-terminus motif portions of clade V MLO proteins.

PMR and DMR phylogenetic analysis

Sixteen PMR-like homologs were identified in watermelon, five each for PMR4 and PMR5, and six for PMR6 (Table 3). Of these, one homolog each for PMR4 (ClaPMR4-1) and PMR5 (ClaPMR5-1), and two homologs for PMR6 (ClaPMR6-2 and ClaPMR6-3) clustered with PMR genes in *Arabidopsis*, cucumber and tomato (Figure 3A-C). For *C. pepo* fifteen PMR-like homologs were identified, five each for PMR4, PMR5 and PMR6 (Table 3). However, only CpePMR4-4 and CpePMR5-3 were closest in similarity to PMR genes in model species (Figure 3A- C). Thirty-two PMR-like homologs for *C. maxima* and *C. moschata* were identified, ten among which clustered with PMR genes in *Arabidopsis*, cucumber and tomato (Table 3). These candidate genes included four PMR4 (CmaPMR4-1, CmaPMR4-2, CmoPMR4-1 and CmoPMR4-2), two PMR5 (CmaPMR5-1 and CmoPMR5-1) and four PMR6 (CmaPMR6-4, CmaPMR6-5, CmoPMR6- 2 and CmoPMR6-4) homologs (Figure 3A-C). In cucumber, Schouten et al. [12] found 10 PMR-like homologs for PMR4, 1 for PMR5 and 13 for PMR6, none of which was differentially expressed following challenge with PM pathogen.

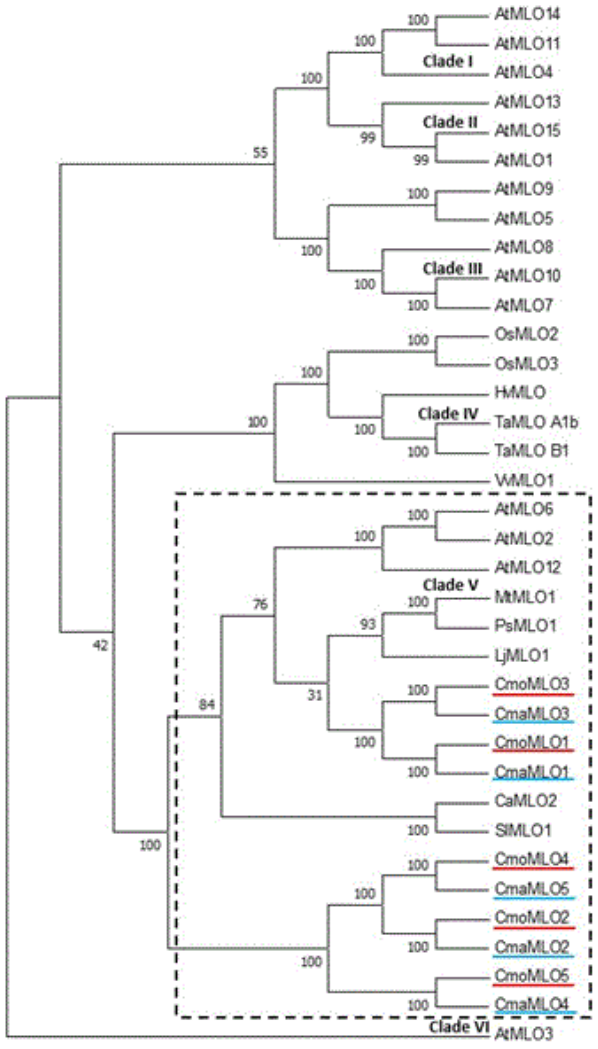

Figure 1: Mildew-locus-O phylogenetic analysis. Phylogenetic relationships and clade annotation of MLO-like proteins in *Cucurbita moschata* and *Cucurbita maxima* and other functionally proven MLO proteins. Clade IV and V represent MLO proteins in monocots and dicots (within dotted box), respectively. Proteins underlined in red (*C. moschata*) and blue (*C. maxima*) represent those closest in similarity to functionally proven MLO proteins in dicots.

Thirty-seven DMR homologs were identified across the four species, seven for DMR-1 and thirty for DMR-6 (Table 4). For DMR-1, one candidate gene each for *C. lanatus* (ClaDMR1), *C. pepo* (CpeDMR1-2), *C. maxima* (CmaDMR1-2), and *C. moschata* (CmoDMR1-2) clustered with DMR-1 proteins in *Arabidopsis*, cucumber and tomato (Figure 4A-B). Similarly, two candidate DMR-6 genes each for *C. lanatus* (ClaDMR6-1 and ClaDMR6-2), *C. maxima* (CmaDMR6-1 and CmaDMR6-2), and *C. moschata* (CmoDMR6-1and CmoDMR6-2), and one for *C. pepo* (CpeDMR6-9) clustered with DMR genes in *Arabidopsis* and cucumber (Figure 4A-B). These results

are similar to those found in cucumber where one DMR1 genes and two DMR6 genes were found [12].

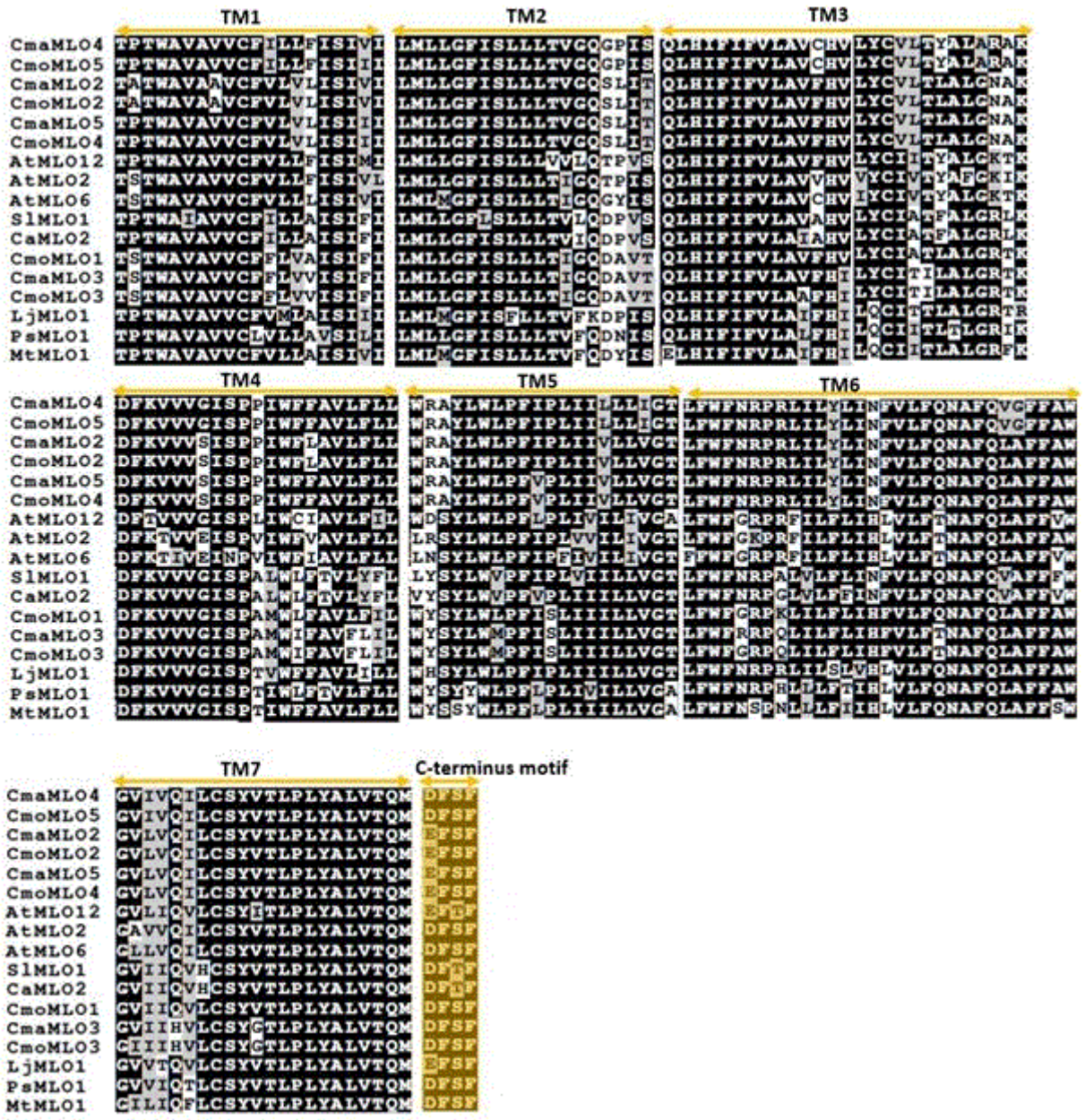

Figure 2: Alignment of conserved sequences. ClustalO alignment with boxshade of transmembrane proteins and C-terminus motif in *Cucurbita moschata* and *Cucurbita maxima* homologs and functionally proven MLO homologs in clade V. Orange shading indicates the C-terminus motif involved in powdery mildew interaction.

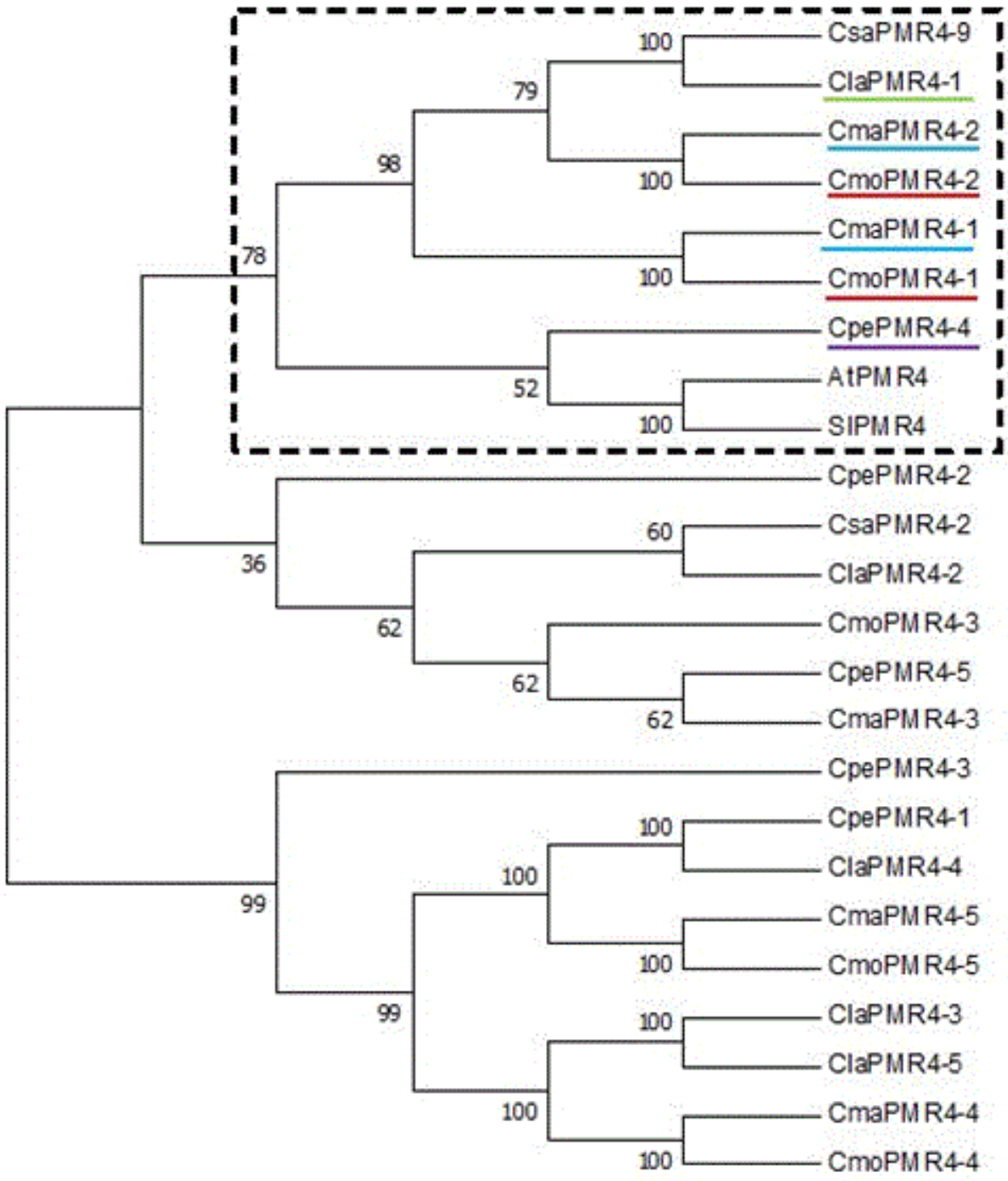

Figure 3A: Powdery Mildew Resistance phylogenetic analysis. Phylogenetic tree for PMR4 in *Arabidopsis* (AtPMR4, AtPMR5 and AtPMR6), tomato (SlPMR4) and cucumber (CsaPMR4-2, CsaPMR4-9, CsaPMR5, CsaPMR6-5, CsaPMR6-12 and CsaPMR6-13) and PMR-like proteins in *Citrullus lanatus*, *Cucurbita pepo*, *Cucurbita maxima* and *Cucurbita moschata*. Underlined proteins represent those closest in similarity to functionally proven PMR genes (within dotted box).

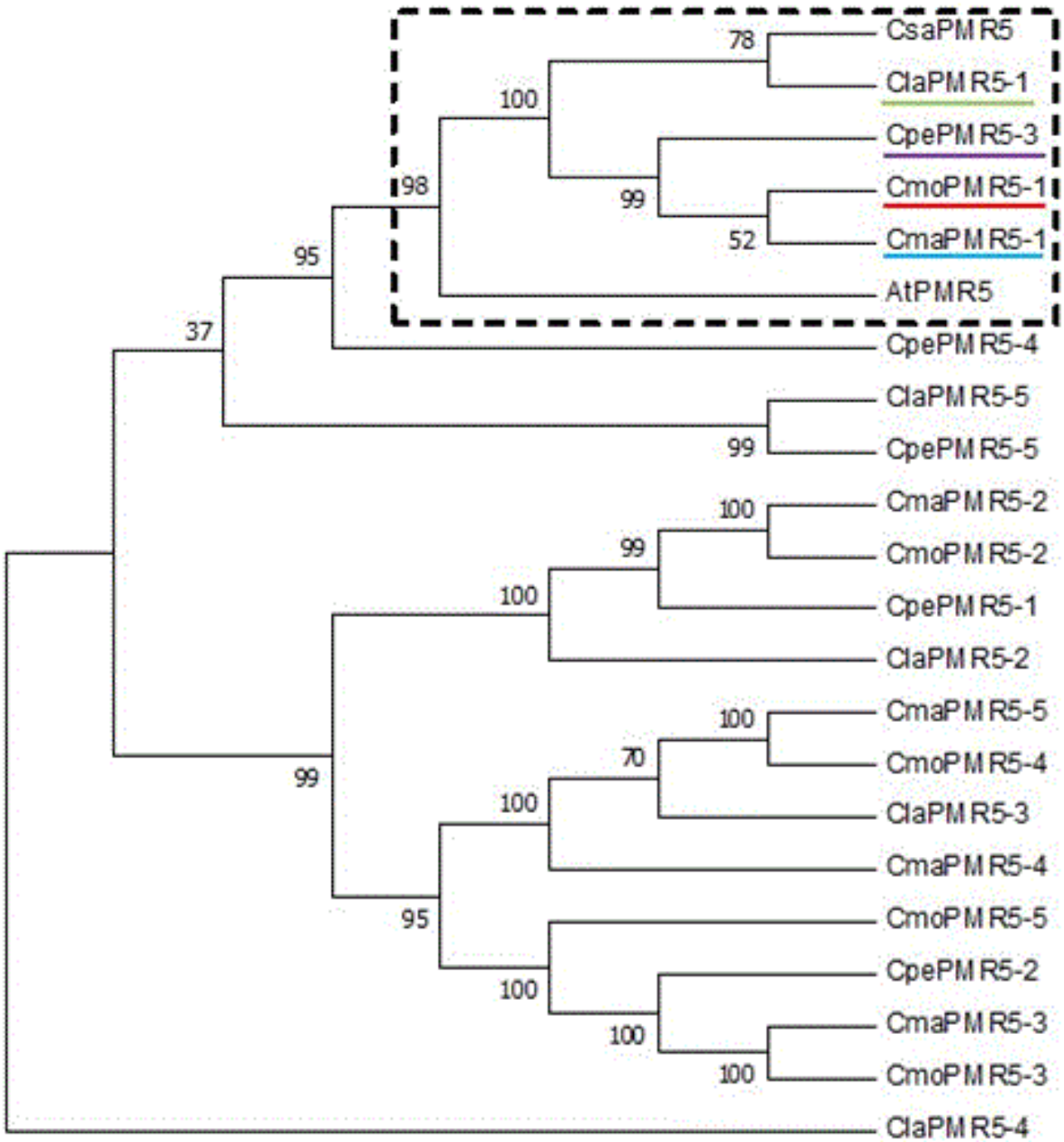

Figure 3B: Powdery Mildew Resistance phylogenetic analysis. Phylogenetic tree for PMR5 in *Arabidopsis* (AtPMR4, AtPMR5 and AtPMR6), tomato (SlPMR4) and cucumber (CsaPMR4-2, CsaPMR4-9, CsaPMR5, CsaPMR6-5, CsaPMR6-12 and CsaPMR6-13) and PMR-like proteins in *Citrullus lanatus*, *Cucurbita pepo*, *Cucurbita maxima* and *Cucurbita moschata*. Underlined proteins represent those closest in similarity to functionally proven PMR genes (within dotted box).

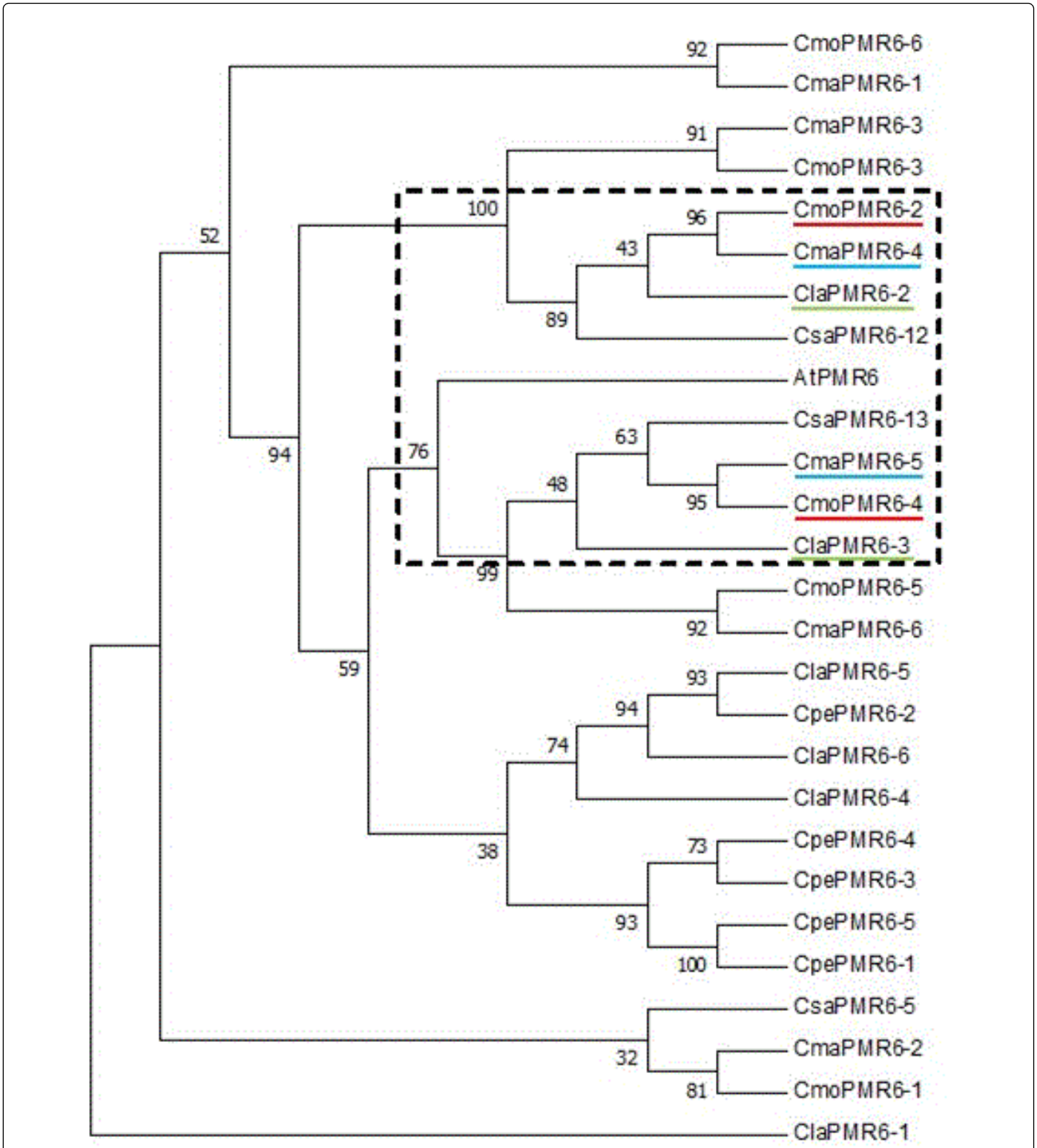

Figure 3C: Powdery Mildew Resistance phylogenetic analysis. Phylogenetic tree for PMR6 proteins in *Arabidopsis* (AtPMR4, AtPMR5 and AtPMR6), tomato (SlPMR4) and cucumber (CsaPMR4-2, CsaPMR4-9, CsaPMR5, CsaPMR6-5, CsaPMR6-12 and CsaPMR6-13) and PMR-like proteins in *Citrullus lanatus*, *Cucurbita pepo*, *Cucurbita maxima* and *Cucurbita moschata*. Underlined proteins represent those closest in similarity to functionally proven PMR genes (within dotted box).

	Homolog	Gene	Homolog	Gene
PMR4	*Citrullus lanatus*		*Cucurbita maxima*	
	ClaPMR4-1	Cla013561	CmaPMR4-1	CmaCh02G000990.1
	ClaPMR4-2	Cla019505	CmaPMR4-2	CmaCh20G002000.1
	ClaPMR4-3	Cla007811	CmaPMR4-3	CmaCh13G006450.1
	ClaPMR4-4	Cla004782	CmaPMR4-4	CmaCh02G006270.1
	ClaPMR4-5	Cla007809	CmaPMR4-5	CmaCh17G009290.1
	Cucurbita pepo		*Cucurbita moschata*	
	CpePMR4-1	PU037453	CmoPMR4-1	CmoCh02G001000.1
	CpePMR4-2	PU029642	CmoPMR4-2	CmoCh20G002200.1
	CpePMR4-3	PU034142	CmoPMR4-3	CmoCh13G006640.1
	CpePMR4-4	PU119647	CmoPMR4-4	CmoCh02G006310.1
	CpePMR4-5	PU043411	CmoPMR4-5	CmoCh17G009010.1
PMR5	*Citrullus lanatus*		*Cucurbita maxima*	
	ClaPMR5-1	Cla008154	CmaPMR5-1	CmaCh01G006760.1
	ClaPMR5-2	Cla013528	CmaPMR5-2	CmaCh20G002230.1
	ClaPMR5-3	Cla009685	CmaPMR5-3	CmaCh13G006130.1
	ClaPMR5-4	Cla005543	CmaPMR5-4	CmaCh07G001940.1
	ClaPMR5-5	Cla005905	CmaPMR5-5	CmaCh03G013170.1
	Cucurbita pepo		*Cucurbita moschata*	
	CpePMR5-1	PU037441	CmoPMR5-1	CmoCh01G007050.1
	CpePMR5-2	PU059522	CmoPMR5-2	CmoCh20G002430.1
	CpePMR5-3	PU055704	CmoPMR5-3	CmoCh13G006370.1
	CpePMR5-4	PU050925	CmoPMR5-4	CmoCh03G013170.1
	CpePMR5-5	PU041638	CmoPMR5-5	CmoCh18G003850.1
PMR6	*Citrullus lanatus*		*Cucurbita maxima*	
	ClaPMR6-1	Cla013736	CmaPMR6-1	CmaCh04G025490.1
	ClaPMR6-2	Cla020532	CmaPMR6-2	CmaCh15G005000.1
	ClaPMR6-3	Cla018329	CmaPMR6-3	CmaCh18G009110.1
	ClaPMR6-4	Cla002573	CmaPMR6-4	CmaCh04G013920.1
	ClaPMR6-5	Cla002940	CmaPMR6-5	CmaCh07G008920.1
	ClaPMR6-6	Cla021445	CmaPMR6-6	CmaCh03G012030.1
	Cucurbita pepo		*Cucurbita moschata*	
	CpePMR6-1	PU055220	CmoPMR6-1	CmoCh15G005080.1
	CpePMR6-2	PU055149	CmoPMR6-2	CmoCh04G014650.1
	CpePMR6-3	PU047808	CmoPMR6-3	CmoCh18G009150.1

	Homolog	Gene	Homolog	Gene
	CpePMR6-4	PU119980	CmoPMR6-4	CmoCh07G009250.1
	CpePMR6-5	PU049955	CmoPMR6-5	CmoCh03G012010.1
			CmoPMR6-6	CmoCh04G026670.1

Table 3: Powdery Mildew Resistance homologs and their designation in the *Citrullus lanatus*, *Cucurbita pepo*, *Cucurbita maxima* and *Cucurbita moschata* genome databases.

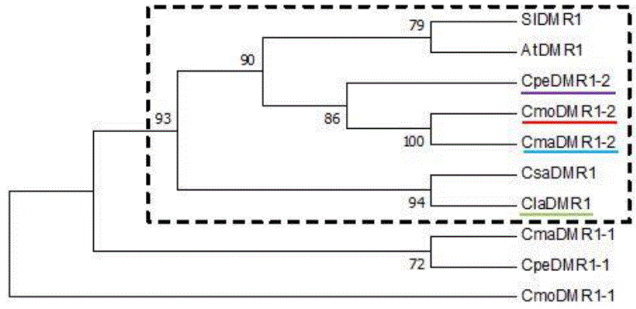

Figure 4A: Downy Mildew Resistance phylogenetic analysis. Phylogenetic tree for DMR1 protein in *Arabidopsis* (AtDMR1 and AtDMR6) and AtPMR6), tomato (SlDMR1) and cucumber (CsaDMR1, CsaDMR6-1 and CsaDMR6-2) and DMR-like proteins in *Citrullus lanatus*, *Cucurbita pepo*, *Cucurbita maxima* and *Cucurbita moschata*. Underlined proteins represent those closest in similarity to functionally proven DMR genes (within dotted box).

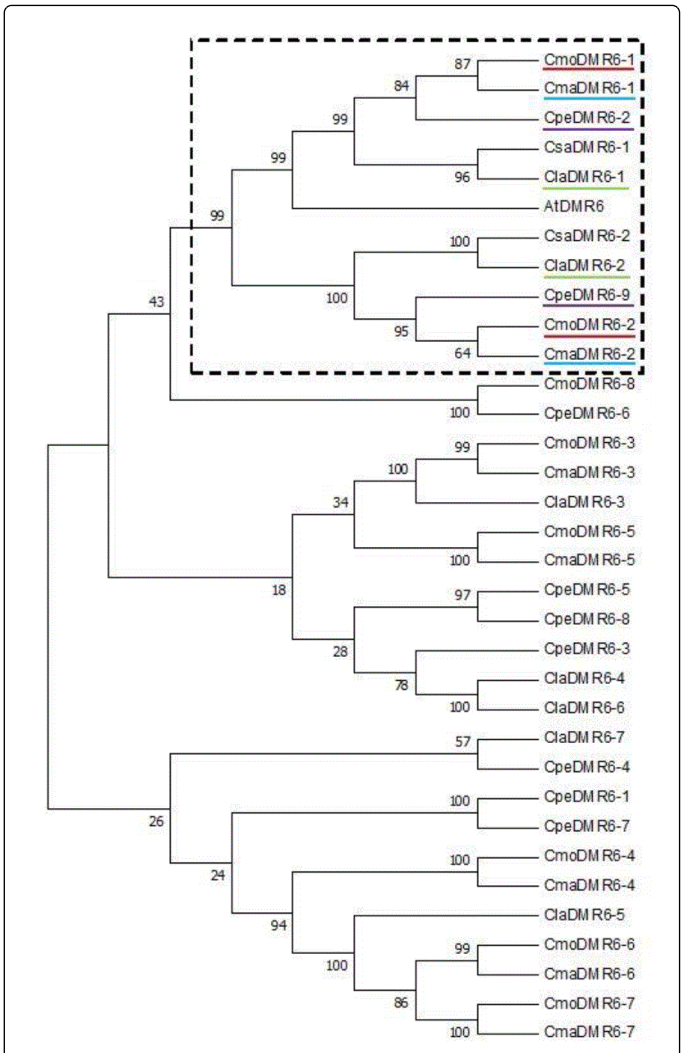

Figure 4B: Downy Mildew Resistance phylogenetic analysis. Phylogenetic tree for DMR6 proteins in *Arabidopsis* (AtDMR1 and AtDMR6) and AtPMR6), tomato (SlDMR1) and cucumber (CsaDMR1, CsaDMR6-1 and CsaDMR6-2) and DMR-like proteins in *Citrullus lanatus*, *Cucurbita pepo*, *Cucurbita maxima* and *Cucurbita moschata*. Underlined proteins represent those closest in similarity to functionally proven DMR genes (within dotted box).

Chromosomal distribution of MLO, PMR and DMR homologs

The candidate genes identified in this study were distributed across the *C. lanatus*, *C. maxima* and *C. moschata* genomes (Figure 5A). In watermelon, all the 11 chromosomes had at least one candidate susceptibility gene and clustering of MLO [previously identified by Lovieno et al. [22] and PMR genes was noted on chromosome 3 in an interval (0.5 Mb to 5.9 Mb) known for high nucleotide divergence and enrichment of disease response genes [28] (Figure 5A). Other gene clusters for PMR and DMR homologs were identified on Chromosome 2. In *C. maxima* and *C. moschata*, candidate susceptibility genes were found in all chromosomes except chromosomes 9,10,11 and 12. For the two species, most candidate homologs were located at similar positions on respective chromosomes (Figure 5B-C), thus suggesting high levels of synteny between *C. maxima* and *C. moschata*.

Significance of MLO, PMR and DMR candidate genes

The current study identified multiple MLO, PMR and DMR candidate gene in *C. lanatus*, *C. pepo*, *C. maxima* and *C. moschata* which should be investigated to elucidate their role in PM and DM pathogenesis. Approaches such as gene knock out, gene knock down or gene expression analysis will further narrow down the candidate gene list to just a few that can be manipulated through gene-editing methods to create novel PM or DM resistant genotypes.

	Homolog	Gene	Homolog	Gene
DMR1	*Citrullus lanatus*		*Cucurbita maxima*	
	ClaDMR1	Cla015737	CmaDMR1-1	CmaCh19G008030.1
	Cucurbita pepo		CmaDMR1-2	CmaCh06G015720.1
	CpeDMR1-1	PU030048	*Cucurbita moschata*	
	CpeDMR1-2	PU000104	CmoDMR1-1	CmoCh19G008250.1
			CmoDMR1-2	CmoCh06G015660.1
DMR6	*Citrullus lanatus*		*Cucurbita maxima*	
	ClaDMR6-1	Cla010665	CmaDMR6-1	CmaCh16G000150.1
	ClaDMR6-2	Cla004982	CmaDMR6-2	CmaCh15G013330.1
	ClaDMR6-3	Cla014824	CmaDMR6-3	CmaCh08G002970.1
	ClaDMR6-4	Cla004353	CmaDMR6-4	CmaCh16G002270.1
	ClaDMR6-5	Cla017758	CmaDMR6-5	CmaCh05G000430.1
	ClaDMR6-6	Cla017993	CmaDMR6-6	CmaCh14G002480.1
	ClaDMR6-7	Cla001290	CmaDMR6-7	CmaCh06G004160.1
	Cucurbita pepo		*Cucurbita moschata*	
	CpeDMR6-1	PU055463	CmoDMR6-1	CmoCh16G000180.1
	CpeDMR6-3	PU057959	CmoDMR6-2	CmoCh15G013950.1
	CpeDMR6-4	PU036758	CmoDMR6-3	CmoCh08G002950.1
	CpeDMR6-5	PU029235	CmoDMR6-4	CmoCh16G002480.1
	CpeDMR6-6	PU041846	CmoDMR6-5	CmoCh05G000500.1
	CpeDMR6-7	PU019772	CmoDMR6-6	CmoCh14G002380.1
	CpeDMR6-8	PU052773	CmoDMR6-7	CmoCh06G004140.1
	CpeDMR6-9	PU135070	CmoDMR6-8	CmoCh16G012830.1

Table 4: Downy Mildew Resistance homologs and their designation in the *Citrullus lanatus*, *Cucurbita pepo*, *Cucurbita maxima* and *Cucurbita moschata* genome databases.

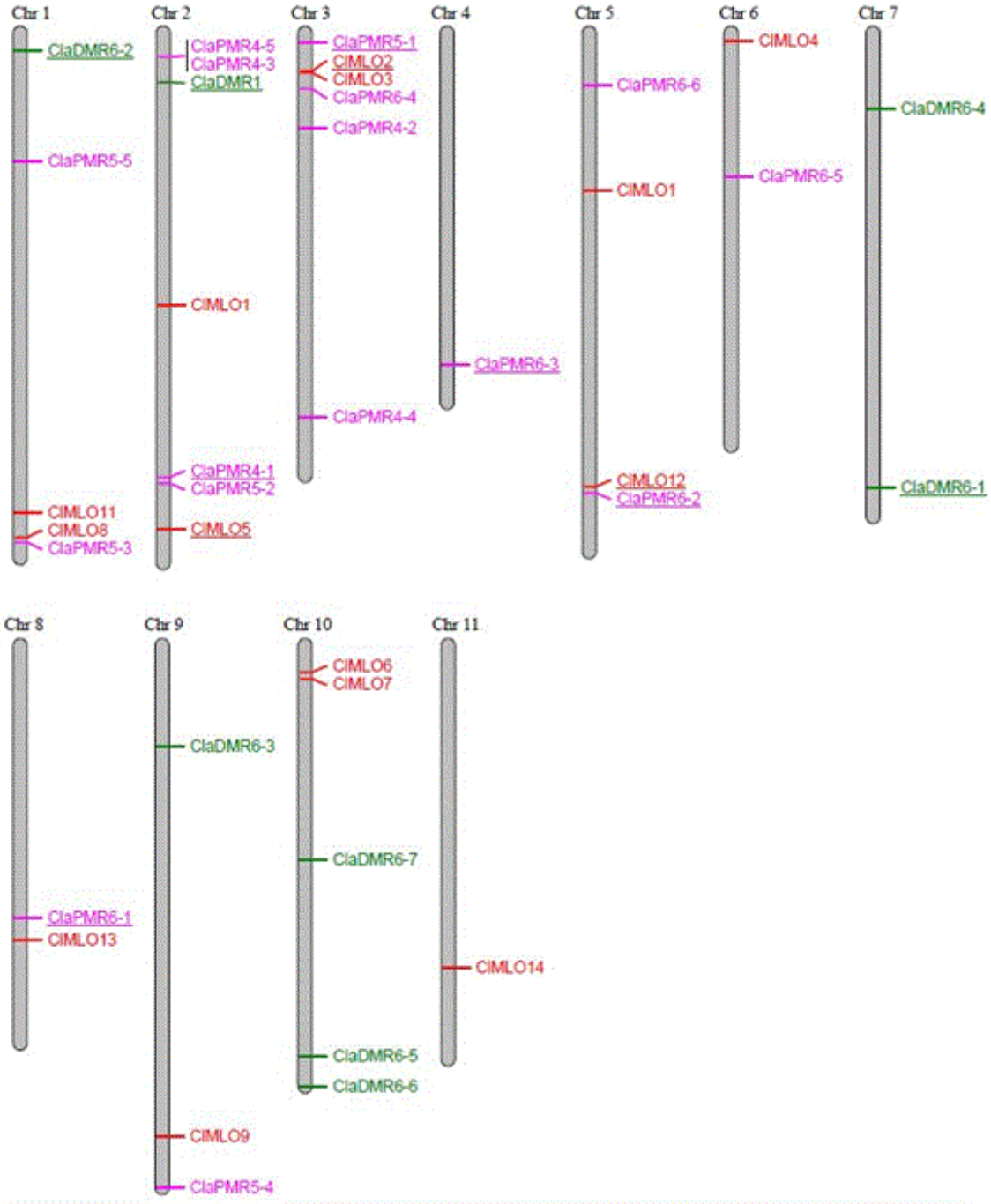

Figure 5A: Physical positions of candidate genes. Distribution of candidate Mildew-locus-O (MLO), Powdery Mildew Resistance (PMR) and Downy Mildew Resistance (DMR) homologs across the genomes for *Citrullus lanatus*. *Citrullus lanatus* map includes 14 MLO homologs previously identified by Lovieno et al. [22]. Red, purple and green font represent MLO, PMR and DMR homologs, respectively. Underlined proteins represent those closest in similarity to functionally proven homologs in other species. Chromosomes (Chr) without any candidate genes are not included.

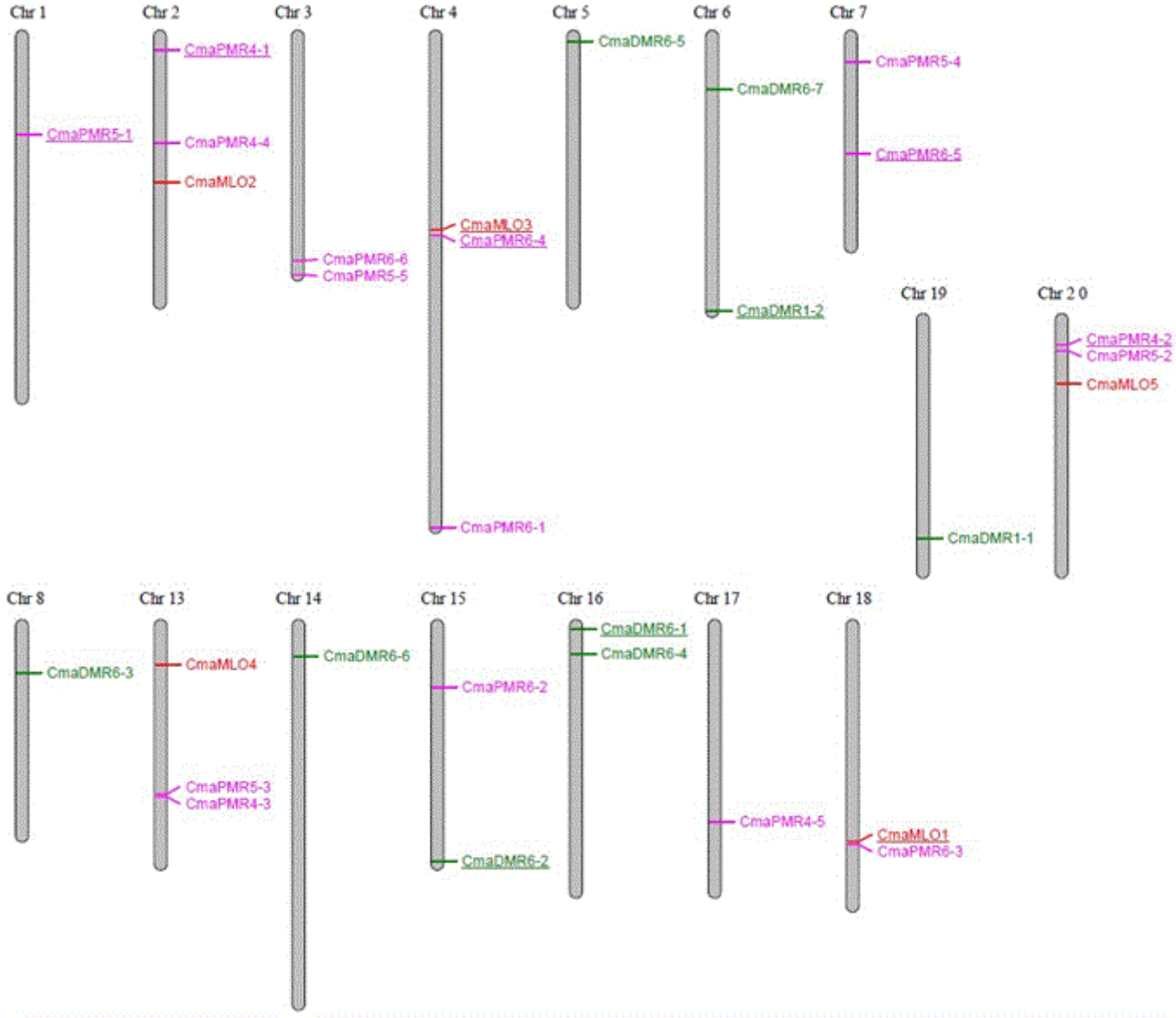

Figure 5B: Physical positions of candidate genes. Distribution of candidate Mildew-locus-O (MLO), Powdery Mildew Resistance (PMR) and Downy Mildew Resistance (DMR) homologs across the genomes for *Cucurbita maxima*.

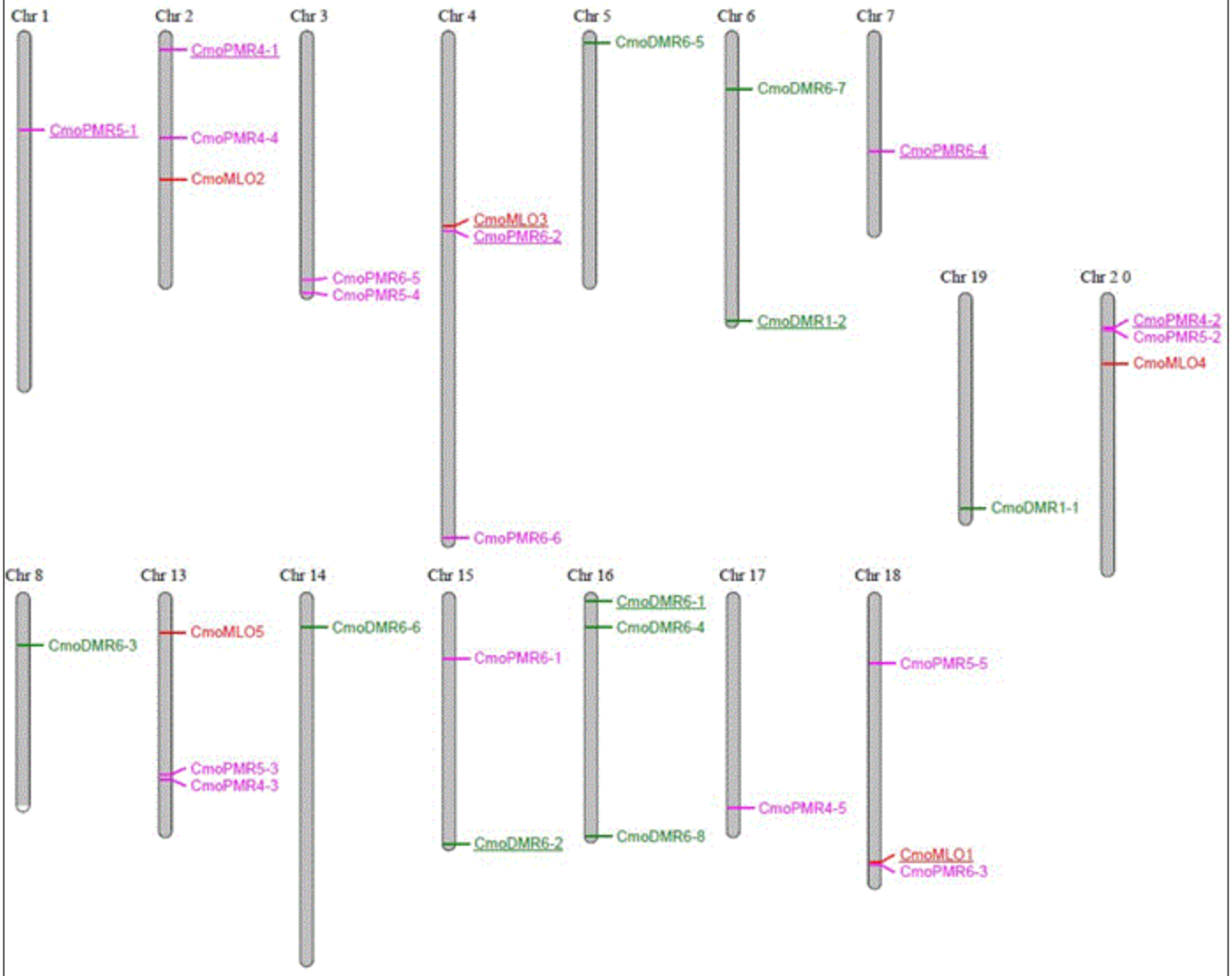

Figure 5C: Physical positions of candidate genes. Distribution of candidate Mildew-locus-O (MLO), Powdery Mildew Resistance (PMR) and Downy Mildew Resistance (DMR) homologs across the genomes for *Cucurbita moschata*.

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