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A natural insertion in melon's *MLO1* gene homologue leads to partial resistance to powdery mildew

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Abstract

Powdery and downy mildew (PM and DM) are two major diseases that severely affect cucurbit crop cultivation. Research for durable resistance is the key for an efficient disease-free crop production. Recessive resistance which is based on loss of functional susceptibility (S) genes offers an important lead in the evolutionary arms race between plant and pathogens. Using re-sequencing data from 100 melon genomes, allele-mining on known S genes for both mildews were performed. We identified accessions carrying loss-of-function mutations in a melon homologue of an already known S gene, *TCP14,* for DM in *Arabidopsis*, as well as of one Clade V *MLO* gene that has been previously associated with PM susceptibility in cucumber, *CmMLO1*. Using a segregation population, we showed that a two-nucleotide insertion in the coding sequence of the *CmMLO1* gene co-segregated with partial resistance to *Podosphaera xanthii*, which is the causal agent of PM in cucurbits. The findings further confirm earlier speculations regarding the general role of this specific gene in PM susceptibility. At the same time, the mutated allele could be an extra asset utilized in melon breeding programs for the improvement of plant fitness and resilience under high PM pressure field conditions.

Keywords: melon, *Cucumis melo*, powdery mildew, *Podosphaera xanthii,* recessive resistance, *MLO*

INTRODUCTION

Cucurbit crops are susceptible to a variety of fungal, oomycete and viral diseases that can cause significant yield losses, decreased crop quality, and reduced economic returns for growers. Powdery and downy mildews, which can infect a wide variety of both monocot and dicot plant species, are the two main devastating foliar diseases in cucurbit crops.

Cucurbit downy mildew (CDM) is a plant foliar disease that is caused by the oomycete *Pseudoperonospora cubensis* (Palti and Cohen, 1980). Infected plants exhibit chlorotic and angular lesions on the adaxial side of the leaves. Chlorotic lesions become necrotic and eventually necrosis expands in the whole leaf and in extension to stems and the whole plant (Savory et al., 2011). *P. cubensis* is classified to 10 pathotypes depending on the ability to infect different cucurbit crops (Cohen et al., 2003; Cohen et al., 2015; Thomas et al., 2017). Infectivity of the pathogen is at large extent dependent on its ability to secrete RXLR type effectors (Tian et al., 2011) into the cytoplasm of host cells during infection process in order to exploit host machinery for its establishment and proliferation. Resistance to CDM can be achieved by modifying plant susceptibility (S) genes that encode such host targets. There is a wide array of plant genes that are reported to be S genes for downy mildew (DM) in various DM-host plant pathosystems. For example, the *DMR6* gene coding for a protein that converts salicylic acid (SA) a major defense signaling hormone to 2,3-dihydroxybenzoic acid (2,3-DHBA). Loss of function of this gene results into DM resistance in multiple plant species e.g., *Arabidopsis*, tomato, grapevine, barley (van Damme et al., 2008; van Schie and Takken, 2014; Thomazella et al., 2021; Pirrello et al., 2022). Other well-known S-genes to DM from *A. thaliana* are a) *AGD5*

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coding for GTPase-activating protein at the trans-Golgi network (Stefano et al., 2010); b) *MKP1* coding for Mitogen-Activated Protein Kinase Phosphatase (Escudero et al., 2019) as well as *NUP133* (Ried et al., 2019) coding for nucleoporin protein. Loss of function of these genes leads to resistance to DM in *Arabidopsis*. In cucurbits, *TCP14* encoding a leaf-specific transcription factor has been shown to contribute to susceptibility to foliar diseases like CDM (Zheng et al., 2019).

Cucurbit powdery mildew (CPM) is a plant disease caused by the biotrophic ascomycete fungus *Podosphaera xanthii* and one of the most common and economically important diseases in cucurbit crops (Rabelo et al., 2017). While three distinct fungal species are able to infect cucurbits, i.e., *Leveillula taurica, Golovinomyces orontii* and *Podosphaera xanthii*, the latter is the most prevalent one (Glawe, 2008; Lebeda et al., 2016). Plants infected with *P. xanthii* exhibit reduced yield, primarily caused by the reduction of photosynthetic activity since the pathogen can cover the complete leaf surface. Losses come also from damages in stems, leaves and fruits which reduce plant fitness and the overall quality of production (Kristkova et al., 2009). Disease symptoms of CPM are easy to identify since plants are often covered with characteristic white powdered fungal growth (Figure 1). In addition to the leaves, the stems or petioles can be covered with spores; CPM infection can spread and eventually cover the whole plant.

More than 200 *Cucumis melo* CPM resistant accessions to different PM races have been identified over the years with 25 dominant loci clustered mostly in chromosomes 2 and 5 while seven resistance QTLs have also been reported (Cui et al., 2022). Apart from dominant resistance two recessive resistance loci have been reported in melon wild species; a loss of function *MLO* (mildew resistance locus O) gene as well as a locus designated as *pm-s* (Cui et al., 2022) in PI 313970. Loss of function of *MLO* genes can result in resistance to PM in various crops of different plant species. Natural mutations in *MLO* were initially found in Ethiopian barley in the middle of the previous century and was exploited by barley breeding for more than 40 years exhibiting exceptional durability (Kusch and Panstruga, 2017). Since then, natural loss of function of MLO genes for PM resistance has been reported in germplasm of several crops (Bai et al., 2008; Berg et al., 2015;) and were also induced artificially (Pessina et al., 2016;). *MLO* is a family of plant genes coding for proteins that consist of seven transmembrane domains and a calmodulin binding domain (Jones et al., 2017). Ca^{2+} influx is a key component for plant defense signaling during pathogen invasion and it is monitored by calmodulin binding proteins such as MLO. *MLO* gene families are categorized in seven distinct clades based on phylogenetic analysis. *MLO* genes from clades IV (for monocot species) and V (for dicot species) participate in PM susceptibility (Berg et al., 2017), and shown to be negative regulators of immunity and their expression is induced by PM infection (Kim et al., 2002).

Research on *Cucurbitaceae* has revealed several *MLO* gene homologues, including the 16 *MLO* homologues that are identified in melon (*Cucumis melo*), 14 in watermelon (*Citrulus lanatus*) and cucumber (*Cucumis sativus*) and 18 in pumpkin (*Cucurbita pepo*) (Iovieno et al., 2015; Zhou et al., 2013). Despite the large numbers of *MLO* genes in the different cucurbit genomes only a few of them belong to the clade V which is responsible for PM susceptibility. In cucumber, three clade V MLO gene homologues were previously reported namely *CsaMLO1* (*Csa1M085890*), *CsaMLO8* (*Csa5M623470*) and *CsaMLO11* (*Csa6M292430*) (Berg et al., 2017). A transposable element insertion that was detected in the *CsaMLO8* gene of the wild cucumber accession PI 215589 was associated with hypocotyl resistance to *P. xanthii,* highlighting the role of this gene in CPM infection (Berg et al., 2015). The same loss of function mutation on *CsaMLO8* was also detected in multiple other CPM resistant cucumber accessions exhibiting a conservation in the plant's germplasm (Nie et al., 2015a, b). In melon, natural mutations in one of the three Clade V MLO gene homologues, *MELO3C012438* were found in CPM resistant wild melon species (Hong et al., 2015).

In addition to MLO, other gene categories have been identified to be required for susceptibility to PM and loss of function of these genes has been shown to lead to PM resistance in various crops. *PMR4* codes for a callose synthase protein that reinforces plant cell walls by depositing callose at the site of the pathogen penetration, making them more

resilient to infection (Santillán Martínez et al., 2020; Nishimura et al., 2003). Since a loss of function in such a gene would be paradoxical to lead to resistance researchers showed that callose deposition also negatively affects salicylic acid levels, which is a major defense signaling hormone of the plants. Additionally, *PMR5* which codes for an acetylation protein and *PMR6* which codes for pectate lyase are also correlated to PM susceptibility and mutants of both genes in *A. thaliana* exhibit resistance to PM (Vogel et al., 2002, 2004). Eventually, two more well-known PM S genes in *A. thaliana* that are also involved in SA regulation are the enhanced disease resistance genes *EDR2* and *EDR4* (Wu et al., 2015; Nie et al., 2012).

In the current research we investigated the presence of natural loss-of-function mutations in known candidate susceptibility gene homologues of 100 re-sequenced melon genomes available at the Center for Genetic Resources, the Netherlands (CGN) (Demirci et al., 2021). The aim of the research was to provide the breeding industry with promising insights on natural durable recessive resistance sources against PM and DM.

MATERIALS AND METHODS

Investigation of known S-gene homologues from *Arabidopsis* in melon

Coding sequences of several *S*-genes reported in *Arabidopsis* and retrieved from NCBI were aligned against melon genome version 3.6 (Cucurbit Genomics Database) in order to determine the candidate homologues in melon based on the top hits. The candidate genes were selected based on certain criteria such as: a) mutant S-gene alleles confer full resistance to PM or/and DM; b) no orthologous genes present in the genome; c) low fitness cost upon loss of function. Melon homologues of MLO clade V genes were retrieved after blasting the known Clade V cucumber protein homologues against melon genome version 4.0 proteins (Cucurbits Genomic Database). The top hits for each homologue were aligned and a maximum likelihood phylogenetic tree was constructed to further confirm the homologuey using MEGA X software alignment tools.

CGN resequencing data examination for high impact mutation detection in candidate susceptibility genes

For all accessions Illumina paired end reads were mapped to the *Cucumis melo* v 3.6.1 reference genome using bwa mem v0.7.17-r1188 and then sorted and indexed using SAMtools v1.8. For each gene of interest, the alignments overlapping the locus were extracted using SAMtools. Duplicate reads were then marked using Picard MarkDuplicates v2.22.1 with options:

ASSUME_SORT_ORDER=coordinate,
OPTICAL_DUPLICATE_PIXEL_DISTANCE=2500
VALIDATION_STRINGENCY=SILENT,
MAX_SEQUENCES_FOR_DISK_READ_ENDS_MAP=50000 (DEFAULT),
MAX_FILE_HANDLES_FOR_READ_ENDS_MAP=8000 (DEFAULT),
SORTING_COLLECTION_SIZE_RATIO=0.25,
TAG_DUPLICATE_SET_MEMBERS=false,
REMOVE_SEQUENCING_DUPLICATES=false, TAGGING_POLICY=DontTag
DUPLEX_UMI=false, REMOVE_DUPLICATES=false, ASSUME_SORTED=false,
DUPLICATE_SCORING_STRATEGY=SUM_OF_BASE_QUALITIES,
PROGRAM_RECORD_ID=MarkDuplicates,
PROGRAM_GROUP_NAME=MarkDuplicates,
READ_NAME_REGEX=<optimized capture of last three ':' separated fields
as numeric values> MAX_OPTICAL_DUPLICATE_SET_SIZE=300000,
VERBOSITY=INFO, QUIET=false COMPRESSION_LEVEL=5,
MAX_RECORDS_IN_RAM=500000, CREATE_INDEX=false
CREATE_MD5_FILE=false, GA4GH_CLIENT_SECRETS=client_secrets.json
USE_JDK_DEFLATER=false USE_JDK_INFLATER=false. fi.

Using the GATK v4.1.3.0 HaplotypeCaller variants were called using default settings.

Subsequently, SNPeff v4.3t was used to determine variant effects and to add ANN, LOF and NMD tags to the vcf INFO field.

Detection of the CGN140842 mutation and generation of the F_2 population

Seeds of CGN140842 were sown in a Unifarm greenhouse climate chamber at 25°C, relative humidity 75%, and 16 h-photoperiod. Following DNA isolation from cotyledons using the DNA extraction method described before (Siskos et al., 2023) the genomic locus flanking the insertion was amplified by PCR using primers CmMELO1F: 5'-GAAGCTATC GACTTCACTCTTGTA-3', CmMELO1R 5'-TTATGATCTATCAGGGTGCTTGTAC-3'. PCR products were sent for Sanger sequencing in order to determine the presence and the zygosity of the mutation in the seed batch. Plants carrying the mutation in homozygous state were crossed with SAKATA X0790 Piel de Sapo PM susceptible melon to produce the F_1 and by subsequent selfing, the F_2 populations.

Functional analysis of natural mutation present in CGN140842 – genotyping, disease assays and scoring

The CGN140842 F_2 population was grown at Unifarm climate chamber with conditions that were mentioned above. Genotyping of ~100 F_2 plants was performed in order to detect the segregation ratio of the mutant mlo1 allele in the population with PCR and Sanger sequencing using the primers mentioned above. Following genotyping, 28 plants from each F_2 population genotype (MLO1/MLO1, MLO1/mlo1, mlo1/mlo1), five plants of CGN140842 parent (resistant control) and five 'Rampicante Zuccherino' commercial melon line plants (susceptible control), were grown in individual plastic pots and maintained according to standard procedures. Drip irrigation was used in order to prevent spread of PM in water. A completely randomized block design was used to arrange the plants in the chamber, minimizing environmental effects. As soon as the plants reached the first true leaf stage PM inoculation took place by spraying with SAKATA PM race Px3.5 spore suspension. The inoculum was prepared by washing 10 pm infected leaves in 150 mL of water until a concentration of 4×10^4 conidia mL^{-1} was reached. Subsequent dilution to a final volume of 1.5 L created the final sprayed inoculum suspension. Scoring of disease progress took place 7, 10 and 14 dpi while a 0-5 scale was used with 0 representing asymptomatic plants and 5 fully symptomatic ones. A single factor ANOVA statistical test was used to determine significant differences in symptom scores between the different genotypes considering a p value <0.05.

RESULTS

Re-sequencing data of 100 melon genomes reveal high impact mutations in the coding sequence of a known *Arabidopsis* S-gene for DM

The melon homologues of candidate S-genes (Table 1) for PM and DM already identified in *Arabidopsis* (and other plants) were examined utilizing resequencing data of 100 melon CGN re-sequenced genomes for the presence of high impact mutations in their coding sequence. Naturally occurring high impact mutations in those genes would ideally result into non-functional proteins. For DM S-genes homologues no high impact mutations were detected in the coding sequences of *AGD5* (MELO3C018195), *NUP133* (MELO3C024202) and *MKP1* (MELO3C023830) in any of the 100 melon genomes that were examined. The same applied for all the *Arabidopsis* S-genes homologues for PM namely *IDD4* (MELO3C022568), *PMR4* (MELO3C013621), *EDR2* (MELO3C013803), *EDR4* (MELO3C020021) as well as *IAN9* (MELO3C005230) that was previously reported as a S-gene for hemi-biotrophs. The only *Arabidopsis* S-gene homologue that did exhibit several high impact mutations in multiple CGN accessions was *TCP14* (MELO3C025629). More specifically, insertions of one or multiple nucleotides were detected in accessions 140862, 140866, and deletions of one or more nucleotides were detected in 140805, 14058, 140872, 24602, 140862, 140866, 140874 and 140806 (Table 1). The effect of these changes in the resistance against CDM were not examined in any of the aforementioned accessions in the current research, due to difficulty of production of reproducible DM inoculum for melon.

Table 1. Melon homologues of known *Arabidopsis* S-genes for powdery mildew and downy mildew. High impact mutations detected in coding sequences of CGN melon accessions are shown along with their respective position in genome (melon genome version 3.6).

Pathogen	Gene name	High impact change	Position (nt, genome v.3.6)	CGN accession
Downy mildew	*AGD5*	-	-	-
	NUP133	-	-	-
	TCP14	GGGAGTTCAGTAATCAACGCC CGGCGAAATCAGCAAAACCGG GAGTAAGAACCTGTGGTGAT ->G	4,807,424	140805, 14058, 140872, 24602, 140862, 140866
		TGG –>T	4,807,430	140862, 140866
		TAATCAACGCCCGGC ->T	4,807,434	140862, 140866
		CA ->C	4,807,454	140862, 140866
		T ->TC	4,807,479	140862, 140866
		GTGAT ->G	4,807,481	140806, 140862, 140866
		CGATT ->C	4,807,535	140874
		GT ->G	4,807,564	140806
		T->TGGGAGAGGCCGCATTGGACG AAATTGAACAAGAAACAAC	4,807,566	140866
	MKP1	-	-	-
Powdery mildew	*IDD4*	-	-	-
	PMR4	-	-	-
	EDR2	-	-	-
	EDR4	-	-	-
(Hemi)-biotrophs	*IAN9*	-	-	-

Re-sequencing data of 100 melon accessions reveal a high impact mutation in MELO3C005044 of CGN accession 140842

Apart from known *Arabidopsis* S-genes for PM and DM, melon homologues of loci associated with susceptibility in cucurbits were examined in the 100 melon genomes. The melon homologue (MELO3C012426) of Csa5M622830.1, which is a gene participating in a QTL for recessive resistance against CPM and CDM in cucumber, was examined for high impact mutations in the 100 melon genomes. No high impact mutations were detected whatsoever in any of the accessions examined, while multiple moderate impact changes were detected in various accessions.

The Cucumber Clade V MLO protein sequences of CsaMLO1 (Csa1M085890), CsaMLO8 (Csa5M623470) and CsaMLO11 (Csa6M292430) already known to contribute to PM susceptibility in cucumber (Berg et al., 2017), were aligned against the melon proteome in order to detect the respective melon homologue proteins that would share similar functions. The three Clade V melon homologues that were chosen based on protein alignment and phylogeny were: a) MELO3C005044 (CsaMLO1 homologue), b) MELOC012438 (CsaMLO8 homologue), and c) MELO3C025761 (CsaMLO11 homologue) (Figure 1). No high impact mutations were detected for MELO3C012438 and MELO3C025761 in any of the melon that examined. One high impact (frameshift) mutation (TA insertion, ch11: 4,012,907 nt, melon genome v.3.6.1) that could functionally inhibit MELO3C005044 was detected in *C. melo* subsp. *agrestis* accession CGN140842. Seedlings of CGN140842 were genotyped and the presence of the mutation was confirmed through Sanger sequencing in one or both alleles.

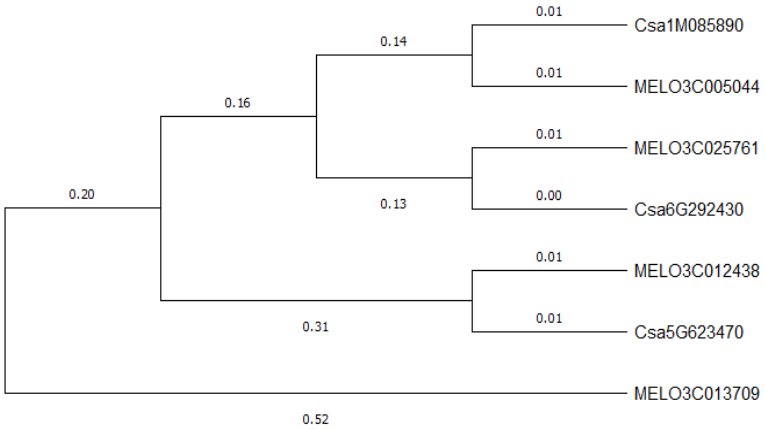

Figure 1. Protein phylogeny (Maximum Likelihood Tree) between known cucumber (Csa-) clade V MLO proteins Csa1M08590 (CsaMLO1), Csa5M623470 (CsaMLO8), Csa6M292430 (CsaMLO11) and melon (MELO-) MLO candidate orthologs.

The mutation of *MLO* gene MELO3C005044 co-segregated with reduced CPM susceptibility in melon

In order to test the effect of the insertion in the *MLO* clade V gene MELO3C005044 on CPM susceptibility we generated F_1 (CGN 140842 × X0790 Piel de Sapo) and F_2 populations. Genotyping approximately 100 plants of the F_2 population using markers flanking the mutation genomic locus revealed a 1 MLO1/MLO1: 2 MLO1/mlo1: 1 mlo1/mlo1 segregation ratio. We subsequently challenged the F_2 population and the respective controls with *P. xanthii* and scored the progress of the disease 7-, 10- and 14-days post inoculation (dpi) (Figure 2). Symptoms started to be visible around 3-4 dpi with small white powdery and light chlorotic spots on the upper side of the leaves, first in the *MLO1/mlo1* and susceptible control plants (SC), with mlo1/mlo1 plants and the parental CGN plants exhibiting similar symptoms shortly after (Figure 2). At 7 dpi CPM was progressing on the same pace between the F_2 plants independent of the mutation presence. The parental CGN plants remained less symptomatic compared to the F_2 plants. However, the difference was significant only compared to the MLO1/mlo1, MLO1/MLO1 and the SC ones ($p<0.05$). At 10 dpi symptoms progressed with colonies becoming bigger in size and increasing in numbers in most of the genotypes. The plants of the F_2 started to segregate considering the disease severity with the mlo1/mlo1 exhibiting significantly less symptoms than the MLO1/MLO1 and the MLO1/mlo1.

The powdery spots that were detected in the CGN parental plants at 7 dpi became smaller and fainter and the spread of the mycelium on other tissues was limited. A similar effect was observed in some mlo1/mlo1 F_2 plants, but the effect was not consistent for all replicates. At 14 dpi the difference between the MLO1/MLO1 and the MLO1/mlo1 F_2 plants followed the same trend as 7 dpi with both genotypes exhibiting similar disease levels. The significant difference of these plants with the homozygous mlo1/mlo1 plants remained whereas the CGN parental plants remained in similar disease levels as 7 dpi.

DISCUSSION

Powdery and downy mildew constitute two of the most common pathogens in cucurbit crop cultivation. There is an everlasting need for generating resistant cultivars either through introgression of resistance (R) genes from wild relative species or by impaired S-genes. Research on recessive resistance, which is a durable type of resistance based on non-functional S-genes, has produced a few promising results over the years (van Schie and Takken,

2014). At the same time, there is a large pool of knowledge on genes for PM and DM susceptibility from studies in various other crops and especially *A. thaliana*. In the current research, we exploited existing knowledge on S-genes of other crops in order to apply it in the investigation of resistance against PM and DM in cucurbits and specifically in melon. For this reason, we used the 100 re-sequenced CGN melon genomes for allele-mining on nine S-genes for PM and DM reported in literature for high impact mutations that impair their function.

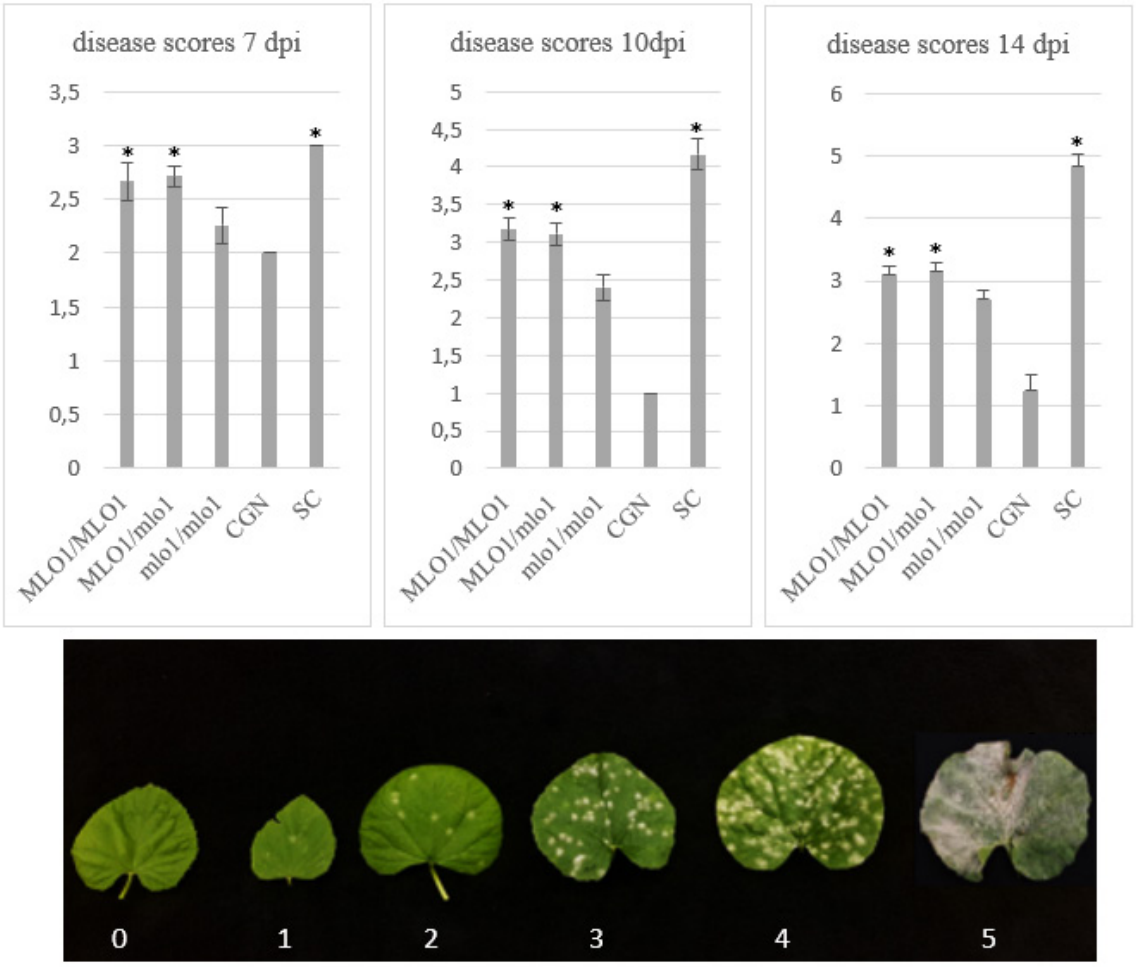

Figure 2. Disease index of different genotypes challenged with powdery mildew at 7 and 10 dpi. A 0-5 scale of disease index was used with 0 representing absence of symptoms and 5 fully symptomatic leaves. Asterisks indicate significant differences in disease scores between plants that carry two mutant MLO1 alleles (CGN, mlo1/mlo) and plants that carry only one or no mutant alleles (MLO1/MLO1, MLO1/mlo1, SC) ($p<0.05$).

One out of the nine candidate S-genes examined, *TCP14* (MELO3C025629), showed multiple high impact mutations in its coding sequence in multiple melon accessions. The mutations consisted of large deletions and insertions that could render the gene non-functional. There are approximately 21 *TCP* genes in *Arabidopsis* that participate in various physiological processes such as leaf and flower development, seed development and hormone signaling (Martín-Trillo and Cubas, 2010; Kieffer et al., 2011; Palatnik et al., 2003; Ori et al., 2007). *TCP14* is a transcription factor that participates in immune and growth regulatory networks and is targeted by oomycete and bacterial pathogen effectors (Stam et al., 2021). In tomato it regulates the expression of defense genes however its activity is inhibited by binding

to pathogen effectors leading to transcriptional changes that hamper immunity activation and favor the pathogen (Stam et al., 2021). In cucumber transcriptional analysis has revealed that the gene was negatively regulating the expression of a native R gene upon CDM infection (Zheng et al., 2019). Considering the specific targeting of this gene by different DM pathogens in distinct plant species we speculate that there is an evolutionary conservation of the *TCP14* function in DM pathogenicity. Therefore, any change in this gene could inhibit the interaction of the transcription factor with the pathogen effectors and in extent pathogenicity. The high impact mutations found in TCP14 coding sequences of many melon accessions are self-evidently not lethal for the plants probably due to the large abundance of similar genes from the same class that are present in the genome and are likely to complement its function. Due to the above it would be interesting to challenge the accessions mentioned above carrying mutations in the TCP14 gene with CDM to examine the effect on susceptibility.

There are several known and candidate susceptibility loci identified and studied in cucurbits including the candidate S-gene *Csa5M622830.1* which is a transcription factor in chromosome 5 of cucumber. A SNP in the 3' untranslated region (3'UTR) of the gene was correlated with resistance against CPM in cucumber and could also contribute to CDM resistance, however, the effect of the melon homologue is unknown. The absence of any high impact natural mutations in the coding sequences of 100 melon genomes raises doubts on whether this gene could result into viable melon plants upon knock out. An effector could potentially bind to the 3' untranslated region in order to alter the transcriptional regulation of the gene promoting pathogenicity without completely inhibiting its function for the plant. In that case the protein could still be accumulated in lower levels enough for the viability of the plant in the case it is a necessary physiological component for the latter. Therefore, the absence of changes in the coding sequence of this gene were not unexpected. Finally, it would be interesting to examine changes in such regulatory areas as 3' or 5' UTRs and cis regulatory elements of this gene that could be binding hubs for pathogen effectors, something that was out of the scope of this research.

Three genes that were previously connected to PM susceptibility in cucumber are the Clade V MLO genes Csa1M085890 (CsaMLO1), Csa5M623470 (CsaMLO8) and Csa6M292430 (CsaMLO11). Due to the abundance of MLO genes in melon we opted for aligning the amino acid sequences of cucumber proteins against melon proteome to detect the most proximate functional homologues. From the three gene homologues detected in melon only MELO3C005044 (CmMLO1) was found to carry a high impact mutation in its coding sequence while CmMLO8 and CmMLO11 carried only moderate impact mutations. The two-nucleotide insertion detected in CmMLO1 results into a frameshift mutation and therefore probably a non-functional protein. Similarly, *CsaMLO8* has been previously reported to carry a transposon insertion in 31 cucumber accessions resulting into hypocotyl PM resistance (Berg et al., 2015). The loss of function of *CsaMLO8* mediated PM resistance caused by transposon insertion was related to cell wall apposition formation and cell death (Sun et al., 2023). Interestingly, contrary to cucumber, in melon, no high impact mutation was found in the CmMLO8 homologue which was unexpected taken into account the abundance of the mutated allele in cucumber germplasm. Moreover, the absence of high impact mutations in CmMLO11 in all melon accessions aligns with the respective absence of naturally occurring mutations in its cucumber homologue too (Tek et al., 2022).

CmMLO1 is located in chromosome 12 of melon at the position chr12: 4012296 .. 4019430 (DHL92, v3.6.1). Melon chromosome 12 includes several reported QTLs for PM resistance (Fukino et al., 2008; Cao et al., 2021; Li et al., 2017; Toporek et al., 2021) but no QTL contains CmMLO1. The resistance conferred by the insertion mutation in CmMLO1 coding sequence was found to be partial, under optimal conditions for the disease. It has been reported that *CsaMLO1* and *CsaMLO11* play a minor role in PM susceptibility compared to CsaMLO8 (Berg et al., 2017). Our findings on the melon homologue of *CsaMLO1* are in alignment with the previous. Additionally, recent research in cucumber has shown that *CsaMLO1*:CRISPR/Cas9 knockout cucumber lines exhibit quite higher penetration rates than *CsaMLO8* knockout lines independent whether this mutation is present in combination with *CsaMLO1* and/or *CsaMLO11* loss of function mutations (Tek et al., 2022). In the same research

it was shown that the resistance conferred by *CsaMLO8* is pre-invasive whereas the *CsaMLO1/CsaMLO11* resistance is post-invasive. The previous is another point of agreement with our findings since the differences in disease scores between mlo1/mlo1 melon (CGN parent or F_2) is increasing with the progress of the infection. The PM colonies in our mutant melon are initially similar in size and density as in the SC plants implying that the penetration is occurring but is not completely obstructed, whereas with the progress of time colonies become fainter, smaller and more localized. Despite the previous, it is noteworthy that CsaMLO1 is the only one of the Clade V genes upregulated 8 h post inoculation with PM indicating an early participation of the gene in pathogenicity to PM (Schouten et al., 2014). Eventually, it would be insightful to perform histological examination on the infected leaves of mlo1/mlo1 in order to examine any potential formation of localized cell wall reinforcement at the attempted penetration sites (papillae) which is characteristic for MLO-based resistance. This examination could shed more light on the nature and the timing of *CmMLO1*'s contribution to PM pathogenicity.

CONCLUSIONS

Resistance against PM and DM in melon was investigated by examining the presence of naturally occurring high impact mutations in the coding sequences of known candidate S-genes in 100 melon genomes available at CGN. Out of the nine melon homologues of known *Arabidopsis* S- genes one gene associated with DM susceptibility was found to carry various insertions and deletions in its coding sequence in multiple melon accessions. It would be interesting to challenge these accessions with DM in order to examine whether the mutations segregate with resistance to the pathogen. Melon homologues of known Clade V cucumber MLO genes associated with *P. xanthii* susceptibility were examined for loss of function mutations over 100 melon accessions. One gene, *MELO3C005044*, which is the homologue of *CsaMLO1* was found to carry a 2-nucleotide insertion in its coding sequence. Partial resistance to PM was associated with the mutation in this gene. The locus can be exploited by melon breeders in order to improve the performance of commercial melon lines under PM pressure field conditions.

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