Expression Profiling of MLO Family Genes under Podosphaera xanthii Infection and Exogenous Application of Phytohormones in Cucumis melo L.

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Powdery mildew disease caused by Podosphaera xanthii is a major concern for Cucumis melo production worldwide. Knowledge on genetic behavior of the related genes and their modulating phytohormones often offer the most efficient approach to develop resistance against different diseases. Mildew Resistance Locus O (MLO) genes encode proteins with seven transmembrane domains that have significant function in plant resistance to powdery mildew fungus. We collected 14 MLO genes from 'Melonomics' database. Multiple sequence analysis of MLO proteins revealed the existence of both evolutionary conserved cysteine and proline residues. Moreover, natural genetic variation in conserved amino acids and their replacement by other amino acids are also observed. Real-time quantitative PCR expression analysis was conducted for the leaf samples of P. xanthii infected and phytohormones (methyl jasmonate and salicylic acid) treated plants in melon 'SCNU1154' line. Upon P. xanthii infection using 7 different races, the melon line showed variable disease reactions with respect to spread of infection symptoms and disease severity. Three out of 14 CmMLO genes were up-regulated and 7 were down-regulated in leaf samples in response to all races. The up- or down-regulation of the other 4 CmMLO genes was race-specific. The expression of 14 CmMLO genes under methyl jasmonate and salicylic acid application was also variable. Eleven CmMLO genes were up-regulated under salicylic acid treatment, and 7 were up-regulated under methyl jasmonate treatments in C. melo L. Taken together, these stress-responsive CmMLO genes might be useful resources for the development of powdery mildew disease resistant C. melo L.

Key words: Cucumis melo, Mildew Resistance Locus O, phytohormone treatment, Podosphaera xanthii, powdery mildew

Introduction

Melon (Cucumis melo L.), a cucurbitaceous fruit, is ninth among horticultural crops in terms of worldwide total production [14]. Powdery mildew (PM), one of the world’s most widespread diseases caused by plant pathogenic fungi, affects around 10,000 species of angiosperms [16]. Both field and greenhouse cultivated cucurbit crops are threatened by these fungi, which decrease the quantity as well as the quality of crop yields [20]. Currently, frequent fungicide applications are the main management practices used to address PM; however, the use of PM resistant plant is the most desirable control strategy [35].

Mildew Resistance Locus O (MLO) is a disease resistance gene first discovered in barley in 1997 [4]. This gene family encodes plasma membrane localized seven transmembrane domain (TM) proteins with C-terminal calmodulin binding domain (CaMBD) and an extracellular N-terminus [4, 9, 21]. MLO proteins with the 20 amino acid CaMBD in barley enhance the susceptibility of plant to PM diseases [21-22]. Interaction of MLO proteins with PM fungi modulates the plant defense signaling mechanism resulting in PM susceptibility [38]. The invariable cysteine and proline residues in extracellular loops or in TM domains are necessary for MLO protein function and/or durability [11]. In addition, second and third cytoplasmic loops in MLO proteins are important for PM susceptibility [40]. Moreover, histological reports show that MLO mediated resistance involves the formation of cell wall appositions just before penetration of the PM fungus to the host cell [1, 7] and this resistance is also partially related to the actin cytoskeleton [30].

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The MLO genes are observed both in higher plants and in certain mosses [9, 10]. In spite of different developmental expression of MLO proteins they are also highly induced by biotic and exogenous application of plant defense signaling compounds, such as, methyl jasmonate and salicylic acid etc. [5, 12, 21, 23, 37]. Besides plant defense roles, the homozgyous MLO genes related to some biological functions like spontaneous death of mesophyll cells resulting in increased leaf senescence [24, 37, 46]. In mlo resistant and MLO susceptible barley lines an epidermal oxidative burst has been reported caused by the Blumeria graminis f. sp. hordei. This burst is more prominent in the resistant mlo mutants plants compared to the MLO susceptible (wild-type MLO) plants [19, 37].

Vesicle associated and actin dependent plant defense systems at PM fungus penetration sites are negatively regulated by functional MLO proteins [34]. The targeting of such functional MLO proteins by PM fungi is believed to be the reason for pathogenesis. After PM fungus inoculation, MLO susceptible genes responded with up-regulation at early stages in barley [37], tomato [2], grape [12, 45] and pepper [47]. Furthermore, over-expression of a functional MLO allele causes MLO super-susceptibility to the PM pathogen [22, 46]. Conversely, in barley, stable broad-spectrum resistance is conferred by recessive mutations of MLO proteins [4]. A similar durable broad-spectrum resistance to powdery mildew due to the loss of MLO function has been found to be conferred by mutant SIMLO1 cloned from tomato [2]. In Arabidopsis, a large reduction of disease susceptibility to Golovinomyces orontii was observed in a mutant AtMLO2 gene [7]. In addition, triple mutation of AtMLO2, AtMLO6 and AtMLO12 were also necessary to obtain absolute resistance to PM [7].

The medium size gene family MLO are variably distributed and 39, 15, 17, 12, 21, 13 and 38 MLO genes were noticed in soybean, Arabidopsis, grape, rice, apple, cucumber, and cotton, respectively [8, 10, 13, 26, 36, 42, 44]. MLO genes have been thoroughly studied both in monocots and dicots. Despite a number of studies, there are very little results available related to gene expression level of MLO genes under the infection of different races of PM fungus, Podosphaera xanthii. Moreover, behavior of those genes under the exogenous application of different phytohormones that modulate PM resistance signaling of the melon plants are also not much existing in the literature. However, the complete genome sequence database of melon 'Melonomics' offers a scope for comprehensive investigation of MLO family genes [15].

In this study, we identified candidate CmMLO genes related to powdery mildew resistance through expression analysis under pathogenic infections with seven different races of Podosphaera xanthii. Besides that, the expression of MLO genes have also been explored under two different plant defense signaling molecules, methyl jasmonate (MeJA) and salicylic acid (SA). In previous studies, MLO genes were induced under the exogenous application of different phytohormones in Arabidopsis [5], barley [37], and grapes [12], pepper [23], rice [21], cotton [44]. Results of this study would be helpful for PM race-specific resistance variety development in cucurbitaceae family.

Materials and Methods

Sequence analysis of MLO proteins in C. melo L.

Sequences for melon MLO proteins were retrieved from the Melon Genome Database (https://melonomics.net/) using keyword ‘MLO’. Multiple protein sequence alignments were constructed with the 14 CmMLO proteins using Gene Doc (www.psc.edu/biomed/genedoc) [31].

Plant materials

The melon variety C. melo 'SCNU1154' line susceptible to all identified races of PM fungus was selected for this study. Overnight soaked seeds were sown in sterilized soil mixture for germination. Seeds were germinated at 5 days after sowing. Seedlings were grown in the plastic pots in a culture room at 22°C temperature, 16:8 h light/dark with a photon flux density of 140 μmolm⁻²s⁻¹. The relative humidity was kept between 65% and 75% in the culture room.

Biotic stress treatments

Six week old seedlings were treated with seven different races of P. xanthii. The seven races viz., KPH19, KPH01, BN968, DH487, BN625, SN102 and BN103, of P. xanthii were initially cultured on detached leaves of SCNU1154 melon line in petri dishes containing fungal growth media (mixture of 5 g agar powder + 20 g D-mannitol + 10 g saccharose in 1,000 ml distilled water) in the growth chamber at 22°C with 16:8 h day: night photoperiodic cycle and a photon flux density of 140 μmolm⁻²s⁻¹. The relative humidity was 80% during the culture period. To induce biotic stress, active fungal inocula were dusted manually using the sterilized pencil
samples were snap-frozen immediately in liquid nitrogen in both the infected and non-infected plants. The collected samples were immediately frozen and then stored 0 hr, 30 min, 1 hr, 3 hr, 6 hr, 12 hr, 24 hr and 48 hr. The harvested samples were immediately frozen and then stored at -80°C until subsequent analysis.

Imposition of phytohormone stress treatments

Seedlings with three true leaf stages were used for two phytohormone stress treatments. The seedlings were exogenously sprayed with 100 μM solutions of SA, and MeJA at their adaxial position. The treated plants were covered by polyvinyl bags upto 48 hr, leaving perforations for the exchange of air until the development of typical symptoms. To maintain a high humidity level, the inoculated plants were wrapped with polythene leaving perforations for the exchange of air until the development of typical symptoms. Control plants were also similarly brushed with a sterilized pencil brush without any fungal inoculum. Sampling was carried out at 18 d post inoculation from the local 5th leaf in both the infected and non-infected plants. The collected samples were snap-frozen immediately in liquid nitrogen and kept at -80°C for RNA isolation.

RNA extraction

Total RNA was extracted from control and treated frozen samples using an RNeasy mini kit (Qiagen, Hilden, Germany) after it was treated with RNase-free DNase (Promega, Madison, USA) to eliminate the contaminants of genomic DNA. The cDNA synthesis was subsequently performed by using the Superscript® III First-Strand synthesis kit (Invitrogen, California, USA) following the kit’s manual.

Quantitative PCR expression analysis

Real-time quantitative PCR (qPCR) was carried out using 1 μL of 50 ng cDNA in a 20 μL reaction volume comprising 2× qPCR BIO SyGreen Mix Lo-Rox SYBR® Green Super-mix with ROX (PCR Biosystems Ltd., London, UK). Specific primers for all genes were used for real-time PCR whereas Cm-Actin primers from C. melo (Gene Bank Acc. AY859055) [6] were used as internal control (Table 1). The real-time PCR conditions were subjected to the following conditions: pre-incubation at 95°C for 5 min, followed by 3-step amplifications at 95°C for 15 s, 58°C for 15 s, and 72°C for 20 s for 40 cycles. The melting temperature was set at 95°C for 10 s, 65°C for 60 s, and 97°C for 1 s as a default setting. The fluorescence was measured at the last step of each cycle and three replications were used per sample. Amplification, detection and data analysis were performed by using LightCycler®96 (Roche, Mannheim, Germany). The calculation of relative gene expression was conducted on the basis of the 2^{-ΔΔCt} method [27].

Statistical analyses

Analysis of variance was conducted following a generalized linear model by using Minitab statistical software version 15 (Minitab Inc., State College, PA, USA) to find out significant variation between sampling points under each condition. Analysis of variance was conducted following a general-

Table 1. Specific primer sequences used for real-time quantitative PCR amplification of MLO genes of Cucumis melo L.

| Sl. No. | Gene name | Forward primer (5'-3') | Reverse primer (5'-3') | Melt. Temp. (°C) | Product size (bp) |
|--------|-----------|------------------------|------------------------|----------------|-----------------|
| 01     | CmMLO1    | TTGACCGAACATTACTTCC    | ACAATAACCACGGTGTAAG    | 58             | 180             |
| 02     | CmMLO2    | CACCTCAGTTGGGCTTAG     | GGGTGTGAGTTACGGTCA     | 61             | 198             |
| 03     | CmMLO3    | GCAATTGTCACCACCGTCCG  | GCTCCAACCGTGTTACGAAC   | 58             | 182             |
| 04     | CmMLO4    | CATCACGAGGATACCACTT   | CAGCAGGTATATTTCAAC    | 58             | 153             |
| 05     | CmMLO5    | GCGTGGAGGCTTCTCITG    | CCAGCCACCTTTACGGTGTC   | 58             | 181             |
| 06     | CmMLO6    | AGCCCTACCATCTACCAAC   | TAAAGGACATAGAAACAG    | 61             | 189             |
| 07     | CmMLO7    | TCGAAGGCTCAAGAAATCAT  | CAGTGGAGAATAGGTGTC     | 58             | 185             |
| 08     | CmMLO8    | GCAGCTTCGCTCGAGATGGG  | GTCGTCGTCTCTCAGTGACATC| 58             | 177             |
| 09     | CmMLO9    | GCAGCTTACGAGCTTGCC    | GATAAGGTGACAAGTGCA     | 58             | 152             |
| 10     | CmMLO10   | TCCGTTGAACTGCGAGTTT   | CAACAAAAATAGTGACAG     | 58             | 162             |
| 11     | CmMLO11   | GCATCGTGGACAACTGTCCGC | CTGACCCAGCACTAGCCG     | 58             | 176             |
| 12     | CmMLO12   | GCCACCAACAGGTGTTATATC | GATGATGGACAGTGTTCTC    | 61             | 179             |
| 13     | CmMLO13   | GCCCTCCCCATGTTCT      | CACCCGTACTCTTTCACC     | 58             | 189             |
| 14     | CmMLO14   | CGTGGTCCCTCGGTGAT    | AAGGAGAGGGGTGTTTAG     | 58             | 194             |
| 15     | Cm-Actin  | TGCCCAAGGTCTATCCAGC   | CATAGTTCAACCACCTAGGAC  | 58             | 147             |
Results

Sequence analysis of MLO proteins in *C. melo* L.

We recovered 14 CmMLO sequences from the Melon Genome database ‘Melonomics’ using keyword ‘MLO’. All 14 CmMLO proteins showed variable numbers of TM domains (Fig. 1). We found CmMLO2, CmMLO3 and CmMLO15 contained evolutionarily conserved cysteine (Cys) and proline (Pro) residues in TM2 and TM5 domains, as well as in extracellular loops 1 and 3 (Fig. 1). However, we observed natural mutations of these conserved residues in CmMLO5, CmMLO7, CmMLO8, CmMLO9 and CmMLO10 (red boxes in Fig. 1). In CmMLO7, CmMLO8, CmMLO9 and CmMLO10 proteins, we also observed genetic variation of the conserved glutamine (Q) in TM2 domain, as well as replacements of lysine (K) by arginine (R), phenylalanine (F) by tyrosine (Y), and aspartate (D) by glutamate (E) in intracellular loop 2, and of methionine (M) by isoleucine (I) in intracellular loop 3 (red boxes in Fig. 1). In intracellular loop 3, proline is replaced by alanine (Ala) and glycine (Gly) in CmMLO4 and CmMLO6, respectively (red box in Fig. 1). Glutamine is replaced by serine and methionine in the TM6 domain of CmMLO4 and CmMLO5, respectively, whereas glutamine is mutated to leucine (Leu) in the TM7 domain of CmMLO4 and CmMLO6. A conserved cysteine in intracellular loop 2 is also deleted in CmMLO1 and mutated in CmMLO10, CmMLO11, and CmMLO14 (red box in Fig. 1). Moreover, NCBI BLAST results between functionally sus-

Fig. 1. Sequence alignment of CmMLO proteins. The alignment was generated by CLUSTALW using default parameters. The positions of the seven transmembrane regions (TM1-TM7) inferred from the experimentally determined topology of HvMLO [9] and the approximate position of the calmodulin binding domain (CaMBD) [22] are indicated. Asterisks (*) indicate conserved proteins and narrow coloured boxes indicate variation within conserved sequences.
ceptible cucumber CsaMLO8 proteins and the 14 CmMLO proteins of C. melo revealed a high degree of similarity, especially with CmMLO2, CmMLO3 and CmMLO13 (data not shown).

**Powdery mildew disease progress by different races**

Inoculated leaf showed the sharp visible disease progress by different races of PM fungus compared to control at 18 d post inoculation (Fig. 2). Disease intensity was not similar in response to different PM races (Fig. 2). The diseases progressed comparatively quick under DH487, BN625, SN102 and BN103 races. The disease area was gradually enlarged and scattered by DH487, BN625, and SN102 races (Fig. 2). On the other hand, disease area was concentrated in case of BN103 race (Fig. 2). The minimum disease development was recorded by BN968 race (Fig. 2).

**qPCR expression study after biotic stresses**

Three genes, namely CmMLO2, CmMLO3 and CmMLO13, were generally up-regulated in response to all seven races viz., KPH19, KPH01, BN968, DH487, BN625, SN102 and BN103 of PM fungus *P. xanthii* compared to control (Fig. 3). CmMLO3 and CmMLO13 displayed maximal expression in response to race SN102 and were approximately 3.0 and 2.5 fold up-regulated, respectively (Fig. 3). Seven other genes, namely CmMLO4, CmMLO5, CmMLO6, CmMLO7, CmMLO8, CmMLO9 and CmMLO10, were generally down-regulated in response to all seven races of *P. xanthii* com-

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Fig. 2. A representation of disease symptoms infected by different races of powdery mildew fungus (*Podosphaera xanthii*) viz., BN968, DH487, BN625, SN102, and BN103 in *C. melo* 'SCNU1154' plants at 18 days post inoculation. The white circles indicate the inoculated 5th leaf number from the ground level.
pared to control (Fig. 3). The approximate relative expressions ranged between 0.1 to 0.8 fold among the down-regulated genes (Fig. 3). The relative expression levels of the remaining four genes (CmMLO1, CmMLO11, CmMLO12 and CmMLO14) were race specific. CmMLO1 was slightly up-regulated in response to two races namely DH487 and BN625 but that was markedly down-regulated in response to another two races viz., BN968 and BN103 (Fig. 3). The down-regulation of CmMLO11 was observed in response to five races viz., KPH19, KPH01, BN968, DH487 and BN103 whereas this gene was up-regulated against only one race SN102 and showed almost equal expression against BN625 race (Fig. 3). For CmMLO12, very low expression was noted in response to all races except DH487 (Fig. 3). CmMLO14 showed com-

![Races of Podosphaera xanthii](chart.png)

Fig. 3. Real-time quantitative PCR relative expression analysis of CmMLO genes after infection with seven races of Powdery mildew fungus *Podosphaera xanthii* in *C. melo* 'SCNU1154' plants. The error bars represent the standard error of the means of three independent replicates.
paratively higher relative expression in response to four races viz., KPH19, KPH01, DH487, and SN102 and comparatively lower relative expression in response to three other races viz., BN968, BN625, and BN103 (Fig. 3).

Expression analysis after phytohormone stress treatments

We also performed the relative expression level of all 14 CmMLO genes under the exogenous application of two signaling molecules viz. SA and MeJA. In case of SA treatment, we observed gradual up-regulation and down-regulation among eleven CmMLO genes compared to control (Fig. 4). CmMLO1, CmMLO3, CmMLO4, CmMLO5, CmMLO6, CmMLO7, CmMLO10 and CmMLO11 gradually increased in expression until 12 hr after stress treatment and declined thereafter (Fig. 4). CmMLO2, CmMLO13 and CmMLO14 had the highest level of expression at 3 hr after treatment (Fig. 4). The approximate relative expression of up-regulated genes ranged between 2.0 and 12 fold (Fig. 4). The other three genes, CmMLO8, CmMLO9, and CmMLO12 exhibited no remarkable responses in stressed plants compared to controls (Fig. 4). In response to MeJA treatment, seven CmMLO genes were up-regulated and down-regulated compared to control (Fig. 5). CmMLO4 and CmMLO7 showed low expression at an early stage but gradually reached a peak at 6 hr and afterwards declined (Fig. 5). The approximate relative expression of these two genes ranged between 2.7 and 5.1 fold up-regulation (Fig. 5). CmMLO5 and CmMLO6 had also low expression level at an early stage but progressively touched a peak at 12 hr and subsequently declined (Fig. 5). The app-
proximate relative expression level of these two genes ranged between 5.5 and 6.5 fold up-regulation compared to control (Fig. 5). Although CmMLO1, CmMLO3 and CmMLO11 showed irregular expression at early stages but exhibited maximum up-regulation at 24 hr and then declined (Fig. 5). The approximate relative expression of these two genes ranged between 3 and 7 fold up-regulation (Fig. 5). The remaining seven genes, CmMLO2, CmMLO8, CmMLO9, CmMLO10, CmMLO12, CmMLO13 and CmMLO14 displayed no noteworthy responses in treated plants compared to control (Fig. 5).

Discussion

We conducted qPCR expression analysis of all 14 CmMLO genes under seven races of P. xanthii infection and two exogenous phytohormone treated samples to explore transcript accumulation level. The disease development was sharply visible at 18 d post inoculation and interestingly the disease intensity and pattern were not similar against different PM races (Fig. 2). In literature, as evolutionary variation is observed in cucurbit powdery mildew pathogen [28], pathogenicity may vary in different pathotypes and races [25, 29]. We identified three up-regulated genes, CmMLO2, CmMLO3 and CmMLO13, however, behavior of these three genes towards resistance or susceptibility is subject of further investigation. Previous results indicated that the higher the up-regulation of the gene under P. xanthii infection was often associated with susceptibility reaction of pathogen [2, 3, 7, 12, 36, 37, 42].

The functions of MLO proteins in molecular biology still remain elusive. The vesicle fusion events are controlled by MLO at the plasma membrane of host cells [12]. PM fungus may use MLO proteins as an ‘invasion door’ for the establishment of successful penetration before haustorium formation [34] by modulating plant antifungal defense reactions [13]. In addition, the TM domain and extracellular loops of CmMLO2, CmMLO3 and CmMLO13 contained evolutionarily conserved cysteine and proline residues, which could be related to susceptibility to PM fungus (Fig. 1). It was also reported that transmembrane domains containing conserved proline residues may be related to helix-helix interactions of polytopic membrane proteins (‘helix packing’) required for MLO function [32].

In our study, CmMLO4, CmMLO5, CmMLO6, CmMLO7, CmMLO8, CmMLO9 and CmMLO10 genes showed marked down-regulation in response to all seven races viz., KPH19, KPH01, BN968, DH487, BN625, SN102 and BN103 of P. xan-
investigation. PM race-specific MLO resistance in melon awaits further investigation. (Fig. 1) CmMLO12, and that different cysteine mutations in extracellular loop 1, had reduced protein accumulation in the host cells compared to barley with wild-type MLO [11]. In our study, along with natural variation of evolutionarily conserved cysteine and proline residues in the TM domain and extracellular loop [11], we also found some new amino acid substitutions e.g., glutamine (Q), lysine (K), phenylalanine (F), aspartate (D) and methionine (M) in the intracellular loop (Fig. 1 a), which might facilitate PM resistance conferred by CmMLO genes. As the second and third cytoplasmic loops of MLO proteins play a critical role in powdery mildew susceptibility [40], any mutation of these positions might be involved in PM resistance.

Interestingly, we observed that CmMLO1, CmMLO11, CmMLO12, and CmMLO14 genes showed race-specific responsiveness to P. xanthii (Fig. 3). This race-specific expression could be due to interaction of cognate genes involving race-specific resistant (R) genes in the host and a corresponding fungal avirulent (Avr) gene [17, 41]. The current concept of plant fungus interactions suggests that PM fungus might produce microbial effector molecules of unknown characteristics that would interact only with resistant genes of the host cells for hypersensitive reactions. The absence of resistant genes possibly leads to a compatible disease reaction [33]. This result suggested that the effector proteins produced by the different races of P. xanthii were variable and that different MLO genes were required for their effective recognition. We also observed that the cysteine residues were not conserved in intracellular loop 2 of CmMLO11, CmMLO12, and CmMLO14 and were deleted in CmMLO1 (Fig. 1). The role of deleted/mutated cysteine residues in PM race-specific MLO resistance in melon awaits further investigation.

We also investigated two plant defense signaling molecules, SA and MeJA, in our present study. Under SA treatment, eleven CmMLO genes including three PM susceptible genes (CmMLO2, CmMLO3 and CmMLO13) showed up-regulation compared to control plants (Fig. 4). As exogenous application of SA induces some plant defense genes against biotrophic pathogens in Arabidopsis [43], we may speculate that SA treatments might change the responses of those three genes to PM fungus in melon. This hypothesis requires further validation by applying exogenous SA after P. xanthii infection. Previous study exhibited that exogenous application of defense signaling compound SA induced PM fungus (Erisyphe necator) responsive genes (VeMLO3, VeMLO4, VeMLO9 and VeMLO17) in grapes [12]. Exogenous application of SA also increased transcript accumulation of OsMLO genes in rice [21].

We identified seven CmMLO genes that showed up-regulation in response to exogenous application of MeJA compared to control plant (Fig. 5). We also observed that out of three genes those were up-regulated under P. xanthii infections, only CmMLO3 was highly induced whereas two other up-regulated genes were not induced by MeJA treatment (Fig. 5). Jasmonic acid plays a key role in plant defense, programmed cell death and leaf senescence [39]. Signaling molecules MeJA also induced OsMLO gene expression in rice [21]. However, in this study MeJA did not induce high expression of CmMLO genes as compared to SA treatments probably because this fungus is biotrophic (Fig. 4 and 5) [18]. However, the effectiveness of exogenous SA over MeJA suggests that MLO genes might follow SA mediated signaling. However, this speculation is a subject of further investigations. In pepper, CaMLO2 is highly induced by exogenous application of SA whereas it is not induced by JA treatments [23]. From all of these results, we may infer that responses of CmMLO genes would be different to these two signaling molecules.

Conclusions

We identified 14 CmMLO genes from Melonomics database. Sequence analysis revealed an invariable number of TM domains among all CmMLO genes. We systemically studied the expression level of all 14 CmMLO genes in response to seven races of P. xanthii fungus and two different phytohormone stress conditions. Up-regulation of three genes with conserved cysteine and proline residues whether related to PM susceptibility or resistance is matter of further investigation. The seven down-regulated genes under PM infection might bear site-specific variation, but that spec-
ulation deserves further investigation. We identified four race-specific candidates CmMLO genes responded under PM stress, suggesting the nature of effector proteins produced by those genes might be different. Moreover, 11 and 7 CmMLO genes were induced by the exogenous application of signaling molecules SA and MeJA, respectively, suggesting possible roles of these two molecules in PM disease control. Taken together, it can be concluded that data presented here will be potential resources for developing stress resistant variety of *C. melo* L. by applying molecular breeding techniques against biotic stress.

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초록 : 멜론 황가루병균 및 식물 호르몬 처리하에서 MLO 유전자군의 발현검정

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멜론 황가루병(Podosphaera xanthii)은 멜론 생산량에 영향을 미치는 중요한 병해 중 하나로 알려져 있다. 작물 육중에 있어서 황가루병을 포함한 병해항성 계통 육성은 병해항성 관련 유전자의 유전양식 및 그 유전자를 조절하는 식물호르몬에 대한 정보는 매우 중요하다. 식물에 있어 황가루병균 저항성에 관여한다고 알려진 Mildew Resistance Locus O (MLO) 유전자를 멜론 database인 ‘Melonomics’로부터 14개 동정하여 CmMLO1~14 (Cucumis melo MLO)로 표기하였다. 동정된 14개의 CmMLO 유전자들의 아미노산 서열을 비교한 결과, 9개의 CmMLO 유전자들은 황가루병균에 대한 감수성 관련 아미노산 cysteine과 proline이 잘 보존되고 있었지만, 나머지 CmMLO 유전자들은 다른 아미노산 서열을 가지고 있었다. 멜론 황가루병균의 7 race에 대하여 이병성을 나타내는 멜론 계통 ‘SCNU1154’에 멜론 황가루병균(P. xanthii)을 접종하고, 식물호르몬(methyl jasmonate와 salicylic acid) 처리한 후 qPCR을 통해 CmMLO 유전자의 상대적인 발현량을 분석한 결과, 멜론 황가루병 7 race에 대하여 14개의 CmMLO 유전자들 중 3개의 유전자들에서 발현이 증가하였고, 7개의 유전자들은 발현이 감소하였으며, 4개의 CmMLO 유전자들은 race 특이적으로 발현량이 증가 혹은 감소하였다. 또한 14개의 CmMLO 유전자의 발현량은 methyl jasmonate와 salicylic acid를 처리하였을 때 다양한 발현 양상을 나타내었다. 11개의 CmMLO 유전자들은 salicylic acid 처리하였을 때 발현량이 증가하였으며, 7개의 유전자들은 methyl jasmonate 처리하였을 때 발현량이 증가하였다. 이와 같이, 스트레스에 반응을 보이는 CmMLO 유전자들은 멜론 황가루병 저항성 계통 육성을 위한 유용한 정보가 될 것으로 기대된다.